Research Laboratories

GARDASIL®
(Human Papillomavirus [Types 6, 11, 16, 18] Recombinant Vaccine)

Supplemental Biologics Licensing Application for Use in Anal Cancer Prevention

Vaccines and Related Biological Products Advisory Committee (VRBPAC) Briefing Document

Presented to VRBPAC on
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List of Abbreviations

Abbreviation	Definition
AAHS	Amorphous aluminum hydroxyphosphate sulfate
AE	Adverse experience
AIN	Anal intraepithelial neoplasia
AIS	Adenocarcinoma in situ
ANOVA	Analysis of variance
ASC-US	Atypical squamous cells of undetermined significance
CDC	Centers for Disease Control
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
cLIA	Competitive Luminex immunoassay
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EGL	External genital lesion
FAS	Full Analysis Set
FDA	Food and Drug Administration
GHN	Generally HPV naïve
GMT	Geometric mean titer
HIV	Human Immunodeficiency Virus
HM	Heterosexual men
HPV	Human Papillomavirus
HRA	High resolution anoscopy
HSIL	High-grade squamous intraepithelial lesion
LSIL	Low-grade squamous intraepithelial lesion
MedDRA	Medical dictionary for regulatory affairs
MRL	Merck Research Laboratories
MSM	Men having sex with men
NWAES	New Worldwide Adverse Experience System
Pap test	Papanicolaou test
PCR	Polymerase chain reaction
PIN	Penile/perianal/perineal intraepithelial neoplasia
PPE	Per Protocol Efficacy
pRB	Retinoblastoma protein
PSUR	Periodic Safety Update Report
SAP	Statistical Analysis Plan
sBLA	Supplemental Biologics Licensing Application
SEER	Surveillance, Epidemiology and End Results
SIL	Squamous intraepithelial lesion

List of Abbreviations (Cont.)

Abbreviation	Definition
SRC	Safety Review Committee
STD	Sexually transmitted disease
VaIN	Vaginal intraepithelial neoplasia
VIN	Vulvar intraepithelial neoplasia
VLP	Virus-like particles
VRBPAC	Vaccines and Related Biological Products Advisory Committee
VRC	Vaccination report card
VSD	Vaccine Safety Datalink
VAERS	Vaccine Adverse Events Reporting System
WHO	World Health Organization

1. Summary

Human papillomaviruses (HPV) cause a significant burden of benign and malignant anogenital disease in men and women. The HPV-related anogenital cancers all develop through a common pathogenetic process of neoplastic change that begins with the necessary event of HPV infection. After cervical cancer, the anogenital malignancy with the strongest overall causal relationship to HPV is anal cancer, which affects both men and women. Like the other HPV-related cancers, anal cancer is primarily related to high-risk HPV types 16 and 18 and is preceded by high-grade intraepithelial neoplasia (high-grade anal intraepithelial neoplasia, high-grade AIN, or AIN 2/3), which is widely accepted as its precursor lesion. The incidence of anal cancer in the United States is increasing in men and women, with a higher incidence rate in women compared to men. Despite the growing number of individuals affected by anal cancer, there is currently no preventive measure and no standard screening for anal cancer in the general population. Prevention of anal cancer is thus an unmet medical need. The Protocol 020 AIN efficacy study was designed to evaluate the potential of GARDASIL® to address this unmet need.

The original GARDASIL® (human papillomavirus [Types 6, 11, 16, 18] recombinant vaccine) studies in young women provided the first demonstration that by preventing persistent cervical HPV infection with a high degree of type-specific efficacy, the vaccine prevents high-grade cervical intraepithelial neoplasia (CIN 2/3), the precursor of cervical cancer caused by those types. GARDASIL® was also shown to prevent the external genital lesions of vulvar and vaginal intraepithelial neoplasia, the precursors of HPV-related vulvar and vaginal cancers, as well as genital warts. The consistently high vaccine efficacy observed in women was then extended to young men, in whom type-specific prevention of persistent genital infection was shown to prevent the development of genital warts, with favorable, although limited data on prevention of penile intraepithelial lesion, the precursor of HPV-related penile cancer.

On the basis of these studies, GARDASIL® is currently indicated in the United States for prevention of HPV 16/18-related cervical, vulvar and vaginal cancer and HPV 6/11/16/18-related genital intraepithelial neoplasia in women 9 to 26 years of age, and for prevention of genital warts in both men and women 9 to 26 years of age. The favorable safety profile of GARDASIL® in both males and females continues to be supported by clinical trials data, postlicensure studies, and surveillance systems.

The study of anal intraepithelial neoplasia (AIN) (the AIN Substudy) was an efficacy trial within Protocol 020, the GARDASIL® efficacy study in men. Protocol 020 was a Phase III randomized, placebo controlled, international, multicenter study of the efficacy of GARDASIL® against external genital lesions (EGL) in men aged 16-26 years. The substudy was designed to investigate the impact of administration of a 3-dose regimen of GARDASIL® on the combined incidence of HPV 6, 11, 16, or 18-related AIN or anal cancer in men who have sex with men (MSM) who were negative to the relevant HPV type. The AIN Substudy enrolled 602 subjects at selected sites with expertise in High

Resolution Anoscopy (HRA), and all subjects were randomized in a 1:1 ratio to receive either GARDASIL® or placebo at Day 1, Month 2, and Month 6. All subjects participating in the AIN Substudy were also enrolled in the main efficacy study of Protocol 020, and contributed to the EGL efficacy and safety data of the overall trial.

The AIN study was performed in an MSM population due to the high rates of anal HPV infection and disease that are a result of the preponderance of behavior-associated risk factors in this subpopulation. This approach of studying a population with a high incidence of the target endpoint is common in clinical trials, and allowed for a demonstration of vaccine efficacy within reasonable study timelines. Demonstration of vaccine efficacy against AIN in MSM should, however, reflect efficacy in the general population of men and women, for several reasons that will be presented in detail in this document. First, anal infection with HPV, in particular HPV type 16, is common and can lead to cancer in heterosexual men and women, as well as MSM. In addition, the predominant HPV types that cause anal cancer are the same in men and women. The pathogenesis, histopathologic, and clinical presentation of HPV-related anal disease are identical across genders and populations (women, heterosexual men [HM], MSM). Furthermore, the anatomy and histology of the anal canal is identical in males and females, and there are no gender-specific characteristics of HPV-related lesions that differentiate AIN and anal cancers in men and women. In fact, AIN and anal cancer are indistinguishable between the genders. Finally, based on the clinical trials experience to date, there is no evidence that the efficacy of GARDASIL® is gender- or sexual-behavior dependent.

Analyses of efficacy data from the AIN study have now demonstrated that GARDASIL® is efficacious in the prevention of the predefined endpoint of HPV 6/11/16/18-related AIN or anal cancer. Efficacy of 77.5% (multiplicity–adjusted 95% CI 39.6, 93.3) was observed. GARDASIL® was also shown to reduce the incidence of HPV 6/11/16/18-related high-grade AIN (AIN 2 or worse) by 74.9% (95% CI 8.8, 95.4). These efficacy findings are further supported by the favorable case split of vaccine and placebo group HPV 16/18-related high-grade AIN (1 vs. 8 cases respectively). When considered in conjunction with the greater than 90% observed type-specific efficacy against persistent HPV 16 and 18 anal infection, and corresponding findings in other anogenital precancers, this analysis provides compelling support for the efficacy of GARDASIL® against HPV 16/18-related high-grade AIN, the precursor of anal cancer. Analyses in the intention to treat populations also supported the prophylactic efficacy findings, providing further evidence of the potential public health benefit of HPV vaccination against anal cancer.

On the basis of these new data, a Supplemental Biologics Licensing Application (sBLA) was filed by Merck on February 26, 2010 for the use of GARDASIL® in the prevention of anal disease and cancer. When considered in light of the evidence reviewed in the sBLA, which 1) establishes that anal cancer is caused by HPV, primarily HPV 16 and 18; 2) supports the identical pathogenetic process of HPV-related carcinogenesis in the anal canals of men and women; 3) demonstrates the rising burden of anal cancer in men and

women; 4) shows that anal HPV infection is common in all men and women regardless of sexual practices; and 5) supports high-grade AIN as the precursor of HPV-related anal cancer in men and women, the efficacy data presented in the application establish GARDASIL® as having significant potential to reduce the burden of HPV 6/11/16/18-related AIN and HPV 16/18-related anal cancer in all individuals.

Taken together, the totality of the GARDASIL® clinical trials data supports the consistently high efficacy of the vaccine against persistent HPV infection and related premalignant lesions, irrespective of gender or location in the anogenital tract.

This briefing document provides:

- A brief summary of the overall clinical development program for GARDASIL®
- A summary of the role of HPV in anal cancer and the natural history of anal HPV infection
- The evidence for high-grade AIN as the obligate precursor of HPV-related anal cancer
- An overview of the unmet medical need related to anal cancer in men and women
- Rationale for an anal cancer indication in males and females 9 to 26 years of age
- A summary of the key efficacy data from the AIN Substudy
- A summary of the overall Protocol 020 end of study data

The proposed new prescribing information is:

- **GARDASIL® is indicated in boys and men, and girls and women 9 through 26 years of age for the prevention of AIN grades 1, 2, and 3 caused by HPV types 6, 11, 16 and 18.**
- **GARDASIL® is indicated in boys and men, and girls and women 9 through 26 years of age for the prevention of anal cancer caused by HPV types 16 and 18.**

2. Introduction

GARDASIL® is a vaccine intended to prevent the full spectrum of anogenital cancer and its precursor lesions, genital warts, and infection caused by HPV types 6, 11, 16, and 18. These four HPV types cause the majority of anogenital HPV disease in the United States.

To date, the clinical development program for GARDASIL® has shown that administration of the vaccine is generally well tolerated in: (1) 9- to 15-year-old boys and girls, (2) 16- to 26-year-old women, (3) 27- to 45-year-old women, and (4) 16- to 26-year-old men. Efficacy against cervical, vulvar, and vaginal cancer caused by HPV types 16 and 18 has been proven in 16- to 26-year-old women, and efficacy against genital warts caused by HPV types 6 and 11 has been proven in both men and women. In

addition to providing extensive safety data, the studies in 9- to 15-year-old boys and girls have been the basis for immunobridging of efficacy from the adult studies to the target vaccination population of sexually naïve adolescents.

The scientific background information and clinical study data presented in this briefing document support the extension of the proposed indication for the use of GARDASIL® in the prevention of vaccine type-related AIN and anal cancer to all boys and men and all girls and women 9 through 26 years of age.

3. Background

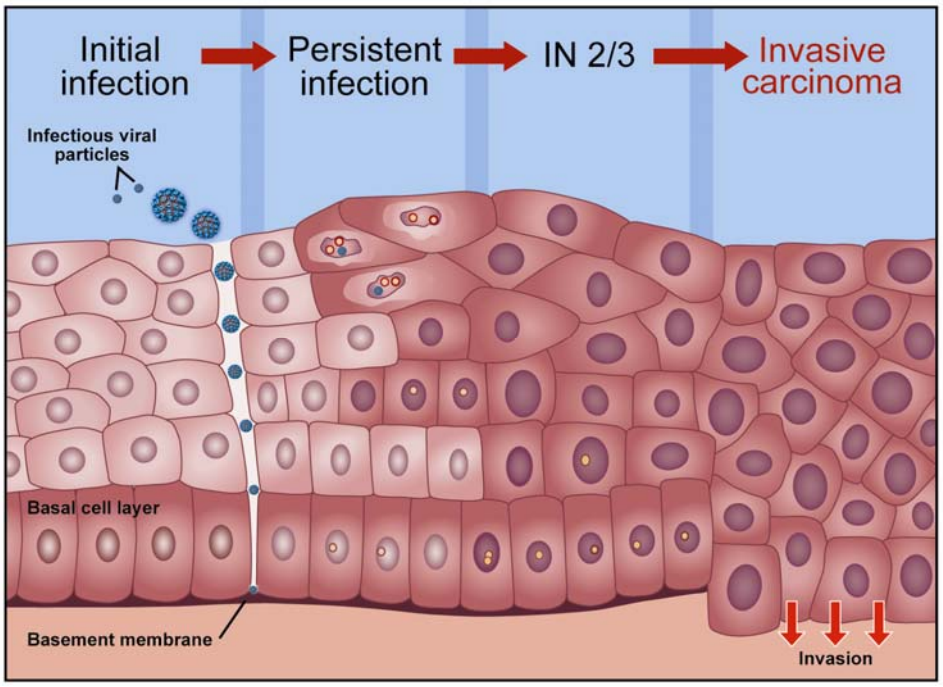
3.1 HPV-Related Pathogenesis in the Anogenital Tract

The HPV family consists of over 100 related epitheliotropic DNA viruses, which have the potential to infect virtually all epithelial surfaces of the anogenital tract, leading to a number of related benign and malignant diseases in men and women [1]. The pathogenetic process of disease development, from initial HPV infection of the basal cells of the epithelium, to ultimate development of precancerous lesions and invasive cancer, is similar in all affected epithelial tissues of the anogenital tract [1] [Figure 1]. Similar processes are also thought to occur in the oral cavity, where HPV infection has been shown to be strongly associated with malignancy [2]. Although all human papillomaviruses appear to disrupt cell cycle regulator mechanisms, HPV types are classified as high- and low-risk [3], based on their predominant associations with benign or malignant disease, respectively.

HPV infects its host by penetrating through mucosal tears to reach the basal keratinocytes on the basement membrane [4] [Figure 1]. From a pathophysiologic perspective, the E6 and E7 viral proteins are the most important viral genes, as they disrupt normal cell cycle control of infected cells, leading to uncontrolled cellular proliferation. Disruption of the E2 viral gene triggers uncontrolled expression of E6 and E7. The overexpressed E6 and E7 proteins associate with the tumor suppressor proteins p53 and pRB (retinoblastoma protein), leading to loss of regulated cell growth and malignant transformation [4].

Figure 1

HPV-Related Pathogenesis in the Anogenital Tract



IN=Intraepithelial Neoplasia

This process of HPV-related malignant transformation in the anogenital tract is associated with persistence of acquired infection [5], and the particularly malignant phenotypes of HPV 16 and 18 are thought to be related in part to the typically longer duration of infection with these types [6]. As a result, HPV 16 and 18 together cause the majority of HPV-related malignancies in both genders.

3.2 GARDASIL®- The Quadrivalent HPV (Types 6, 11, 16, 18) Vaccine

GARDASIL® is prepared from highly purified virus-like particles (VLPs) of the recombinant major capsid (L1) protein of HPV Types 6, 11, 16, and 18. The GARDASIL® VLPs are adsorbed on Merck's amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant. Each 0.5-mL dose of GARDASIL® is formulated to contain 20, 40, 40, and 20 µg of HPV 6, 11, 16, and 18 L1 proteins respectively. The final container product is a sterile suspension for injection in a single-dose vial or a prefilled syringe. For each image, the fill volume permits intramuscular injection of 0.5 mL of vaccine. GARDASIL® is not a live virus vaccine; it contains no viral DNA, and is therefore incapable of causing infection.

3.3 Summary of GARDASIL® Clinical Trial Findings

The efficacy program for GARDASIL® began in 16- to 26-year-old women, with the demonstration of a high degree of type-specific prevention of persistent genital HPV infection, which translated into prevention of HPV-related premalignant disease. GARDASIL® was shown to be highly efficacious in preventing HPV 16/18-related cervical, vulvar, and vaginal precancerous lesions (cervical intraepithelial neoplasia [CIN] 2/3 and Adenocarcinoma in Situ [AIS] vulvar intraepithelial neoplasia [VIN] 2/3, and vaginal intraepithelial neoplasia [VaIN] 2/3, respectively); HPV 6-, 11-, 16-, and 18-related cervical, vulvar, and vaginal dysplasia (CIN [any grade] or AIS; VIN, VaIN). GARDASIL® was also shown to prevent HPV 6/11-related genital warts. Demonstration of GARDASIL® efficacy was then extended to 16- to 26-year-old men, in whom type-specific prevention of genital infection was similarly shown to prevent the development of HPV 6/11-related genital warts, with limited favorable data on prevention of high-grade penile intraepithelial lesion (PIN 2/3), the precursor of HPV-related penile cancer. The efficacy results for HPV 6/11/16/18-related diseases against which GARDASIL® is currently licensed are summarized in Table 1.

Table 1

Summary of GARDASIL® Clinical Trial Efficacy Against HPV 6/11/16/18-Related Diseases in Men and Women

Population	Endpoint	Per Protocol Efficacy (%)	95% CI
16-26 year-old females	CIN 2/3, AIS	98	93, 100
	VIN 2/3	100	67, 100
	VaIN 2/3	100	55, 100
	Genital warts	99	96, 100
16-26 year-old males	Genital warts	90	65, 98

AIS=adenocarcinoma in situ; CIN=cervical intraepithelial neoplasia, VIN=vulvar intraepithelial neoplasia, VaIN=vaginal intraepithelial neoplasia.
Licensed against HPV types 6/11-related genital warts.

[7; 8]

GARDASIL® induces robust anti-HPV immune response in males and females 9-26 years of age. Based on the demonstrated non-inferiority of anti-HPV responses among boys and girls 9-15 years of age compared to that observed in men and women 16-26 years of age, efficacy was bridged to the HPV-naïve pre-adolescent and adolescent population in which efficacy studies were not feasible.

Administration of GARDASIL® has been generally-well tolerated in all populations in which it has been evaluated. The proportions of subjects who reported serious adverse experiences, or who discontinued due to an adverse experience were low and comparable to placebo recipients. There were slightly higher incidences of injection-site adverse experiences and low-grade fever following vaccination compared to placebo recipients.

Overall, the postlicensure experience with GARDASIL® collected through passive reporting of spontaneous adverse experiences to Merck & Co., Inc. has confirmed the favorable safety profile of the vaccine. With a low proportion of reported serious adverse experiences, the benefit-risk profile for the product remains favorable. Assessment based on the Vaccine Adverse Events Reporting System (VAERS) and Vaccine Safety Datalink (VSD) project indicates that the postlicensure safety data continue to support the favorable benefit-risk profile of GARDASIL®. The postlicensure epidemiologic safety study conducted by Merck has also provided data that support the favorable safety profile of GARDASIL®.

4. The Role of HPV in Anal Cancer Pathogenesis

Detection of HPV in tumors is accepted as the best estimate of the etiologic fraction of potentially HPV-related cancers [9]. On this basis, virtually 100% of cervical cancers are caused by HPV, with types 16 and 18 together responsible for approximately 70%. Up to 90% of all anal cancers are caused by HPV [10; 11], and compared with cervical cancer there is an even higher attribution of types 16 and 18 (approximately 80%, as detailed below). In fact, with regard to overall prevalence of HPV positivity, AIN and anal cancers resemble CIN and cervical cancers more than the other anogenital cancers and precancers [12]. The high proportion of anal tumors with detectable HPV and the pathogenetic similarities between anal and cervical cancers support the role of HPV as a necessary cause of anal cancer [11]. The literature reviewed in this section is summarized in [Appendix 1].

One of the largest and most comprehensive studies of HPV in the etiology of anal cancer is a population-based case-control study conducted by Daling et al. in western Washington State [13]. This study evaluated anal cancers diagnosed between 1986 and 1998 in 119 men (41.7% MSM) and 187 women, 102 and 146 of whom respectively had tumor tissue samples tested for HPV. Among all cancers with detectable DNA, 87.9% were HPV positive (86.3% in males; 89.0% in females). Among squamous cell anal carcinomas, HPV positivity was 92.2% (92.6% in males; 91.8% in females). Neither the proportion of tumors that were positive for HPV, the type of HPV detected, nor the proportion of invasive tumors that were positive for HPV were statistically significantly different between men and women. However, MSM had a higher proportion of HPV positivity than heterosexual males (HM) (97.7% vs. 78.0%, p=0.004).

The overall findings of the Daling study are consistent with another large, population-based case-control study, conducted in Denmark and Sweden [14]. This study included 93 men (15% MSM) and 324 women, and included cancers diagnosed between 1991 and

1994. In close agreement with Daling et al., a total of 88% of anal biopsy samples were positive for HPV, and 89% of females had HPV positive cancers. However, the proportion of males with HPV positivity was lower than the U.S. study and statistically significantly different than females (69% of males, $p<0.001$). Similarly, in a case-control study of 394 anal cancer cases, 100% of men (11/11) reporting sexual activity with male partners had cancers that were positive for high-risk HPV types compared with 58% (38/66) of men who did not report same sex partners. In the same study, 90% (228/253) of women had high-risk HPV positive cancers [11]. A recent French study of 147 men and 225 women who had anal cancer also found a high prevalence of any HPV type (96% for males; 97% females) and high-risk HPV types (83% for males, 96% for females) in these cancers [15].

Two recent meta analyses report overall HPV positivity in anal cancers ranging from 71% to 84% [16; 17]. Current estimates from the World Health Organization rely on the DeVuyst meta analysis and several of its underlying studies, including Daling et al. [18]. The variation in the natural history literature regarding the proportion of anal cancers caused by HPV is likely explained by the cancer sites and histologies included in the various studies (i.e., inclusion of *in situ* carcinoma in some studies and exclusion in others), small sample sizes in some studies, variation in laboratory methods used to assess HPV DNA in biopsy samples, and the specific populations and sampling methods (such as females, MSM, HM, or convenience samples). As described by Hoots, it is notable that more recently published studies report higher rates of HPV detection in cancer specimens, likely reflecting improvements in PCR HPV detection methodologies [16].

Overall, HPV types 16 and 18 together account for approximately 80% of all anal squamous cancers, a larger proportion than for cervical cancers [19; 13; 20; 12]. This factor strongly supports the necessary role of these HPV types in development of the vast majority of anal cancers. HPV 16 is consistently reported as the most common high-risk HPV type identified in anal cancers, followed by type 18. In the Washington State study, 73.0% and 6.9% of all anal cancers examined were positive for HPV 16 and 18 respectively, for a total prevalence of HPV 16/18 of 79.9%, with little variation between men and women [13]. Notably, these estimates of HPV 16/18 prevalence were higher (85.6%) when restricted to squamous cell anal carcinoma, which accounts for the majority of anal cancers in the U.S. and Europe [13; 16]. Low-risk HPV types 6 and 11 have lower oncogenic potential than types 16 and 18, and are identified in fewer than 5% of anal cancers in the U.S. However, although frequently accompanied by high-risk types, low-risk types are also identified alone in anal precursor and invasive cancer lesions [16; 21].

Many of the risk factors for anal cancer in both men and women are notably similar to the well-known risk factors for cervical cancer, and support the role of HPV in anal carcinogenesis [14; 22]. Case control studies suggest that risk factors for anal cancer in both men and women are: evidence of previous infection with HPV 16 or 18, history of

HPV-related dysplasia or cancer, history of genital warts, history of receptive anal intercourse, multiple anal intercourse partners, higher number of lifetime number of sexual partners, having other sexually transmitted infections or history of sexual transmitted infection in a partner, smoking, immunocompromised status (due to HIV and other causes, including iatrogenic immunosuppression for organ transplant), and older age [23; 24; 14; 25; 26; 13; 27]. Although history of anal intercourse is a risk factor for anal cancer, anal cancers commonly arise in individuals who report no prior anal intercourse [14; 13]. As an example, Daling et al. showed that of individuals with invasive anal cancer, 100% of 54 heterosexual men and 66-74% of women reported no history of anal intercourse [13].

In addition to the detection of HPV 16 and 18 in a substantial proportion of anal cancers and the epidemiologic evidence that prior HPV 16 or 18 infection is a risk factor for anal cancer, the pathogenetic role of HPV in anal cancer is further supported by the observation that the vast majority of anal cancers are squamous cell carcinomas that are characterized by the same histologic and molecular patterns that are typically identified with other HPV-related cancers. In a large case-control study of anal cancer, for example, tumor lesions were associated with characteristics commonly associated with cervical cancers, including basaloid features, adjacent high-grade AIN, poor or absent keratinization, and predominance of small or medium neoplastic cells [11]. In this study, these findings were all positively associated with high-risk HPV status. The histologic patterns of anal cancers are also identical between the genders, supporting the same pathogenetic processes of anal cancer development in males and females [11]. Many of the molecular changes identified in anal cancers, such as overexpression of P16 and Ki67, are similar to the HPV-related genital cancers, and are also the same in men and women [28; 29; 30].

5. The Burden of Anal Cancer in Men and Women-An Unmet Medical Need

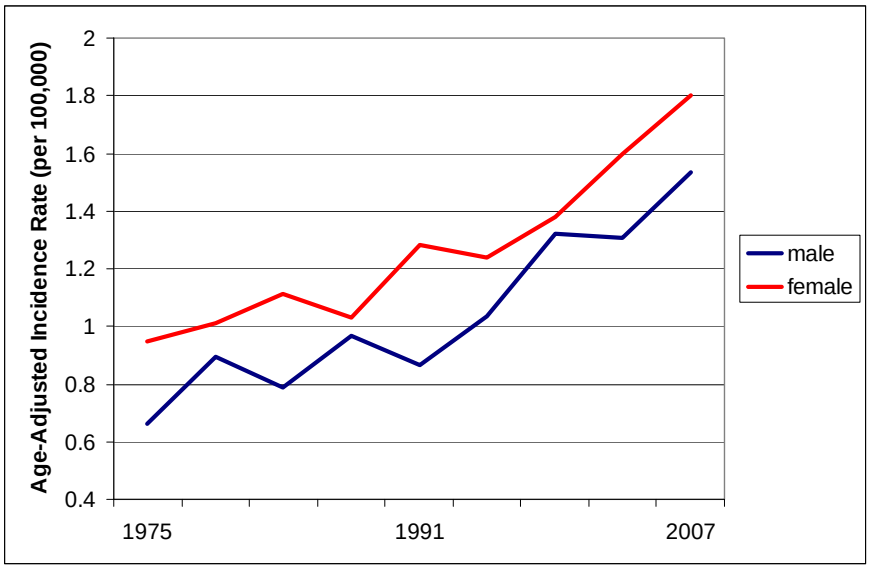
It is anticipated that in 2010, 5260 new cases of anal cancer will be diagnosed in the United States, and 720 individuals will die from the disease [31]. The median age at diagnosis of anal cancer is approximately 60 years [32], and median age at death is 65 years [32; 31]. Screening for anal cancer has not yet been shown to be effective, and is not routine for any general patient population. As with most cancers, early anal cancer is asymptomatic. Thus, approximately 40% of invasive anal cancers are diagnosed with either regional or distant spread, for which survival is lower [10]. The burden of anal cancer in women is increasingly being recognized; in the United States, roughly 60% of anal cancer cases and deaths occur in women [33].

In contrast to the incidence trends for many other cancers, anal cancer incidence has been increasing in men and women at approximately 2.2% per year over the past several decades [Figure 2]. The National Cancer Institute has determined that this increase is statistically significant [33]. It is particularly notable that the largest increase in anal cancer incidence rates is in women ages 50-64 years, for whom the rate in 2007 was 1.5

times the rate in 2000 [33]. U.S. mortality rates from anal cancer in both men and women have followed a similar trend as the rising incidence rates, showing statistically significant increases between 1992 and 2007 [33].

Figure 2

Anal Cancer Incidence in Men and Women
Surveillance, Epidemiology and End Results (SEER) Data 1975 to 2007



As is the case with cervical cancer, certain population groups are at particularly high risk of developing anal cancer, due to the preponderance of risk factors in these populations (described earlier). Specific to anal cancer, these groups include MSM, HIV-infected men and women, and other immunocompromised individuals (e.g. organ transplant recipients). Before the HIV epidemic, the incidence of anal cancer in men with a history of receptive anal intercourse was estimated at 35 per 100,000, similar to cervical cancer rates before the introduction of cervical screening [34].

In average-risk individuals, treatment of premalignant lesions detected through cytologic screening of the anal canal may not be as successful as cervical screening in lowering anal cancer incidence rates. Reports on the sensitivity and specificity of anal cytology in healthy individuals have been variable. While some studies suggest that the sensitivities of anal and cervical cytology are similar, others suggest that the concordance between histologic and cytologic grades of abnormalities is poor, and that particularly in healthy individuals, anal cytology may underestimate the severity of anal neoplasia [26; 22; 35]. Also, although treatment of AIN lesions is feasible, treatment options are limited to surgical approaches, as there are no effective pharmacologic therapies. As a result, side

effects including post-operative pain, bleeding, and anal stenosis are not uncommon [36; 37]. In addition, unlike surgical treatment for CIN, which entails removal of the transformation zone and thus removal of the most susceptible epithelial tissue, the transformation zone of the anal canal cannot be removed. Associated with these therapeutic limitations, high recurrence rates have been reported [38].

For those patients who develop invasive anal cancer, treatment consists primarily of chemotherapy and radiation. While often successful, these therapies are associated with significant acute and chronic morbidity, including radiation proctitis, chronic diarrhea, post-radiation anal stenosis and stricture which may be accompanied by anal incontinence [39] [Appendix 2]. These acute and chronic side effects of treatment may negatively impact various aspects of quality of life [39].

Given these factors, coupled with the burden of disease, the optimal approach to address anal cancer is primary prevention of anal HPV infection. The Protocol 020 AIN Substudy was designed to show the potential of vaccination with GARDASIL® to address this important unmet medical need.

6. Epidemiology and Natural History of Anal HPV Infection

Acquisition of anal HPV infection is the initial event that can lead to persistent infection, high-grade anal intraepithelial neoplasia (AIN), and ultimately, malignant transformation to invasive anal cancer. Anal HPV infection, high-grade AIN, and anal cancer all share a similar set of risk factors that are largely behavioral and not gender-specific. Type-specific anal HPV infections are acquired in both the general population and specific subpopulations, such as MSM. However, there is no mechanism to predict which of these infections will regress, which will persist, and which will progress. Therefore, type-specific prevention of persistent anal HPV infection through HPV vaccination is the optimal approach to preventing anal precancers and ultimately anal cancers, the majority of which are due to HPV 16 and 18.

The risk factors for HPV anal infection provide clear and consistent evidence that the factors associated with infection are ultimately those that also lead to high-grade AIN and anal cancer [40; 22]. In brief, these factors include: higher lifetime number of sex partners, history of anal intercourse, history of tobacco use, history of HPV-associated anogenital infections or diseases (such as HPV-associated cervical disease, HPV infection on the penis and/or scrotum, and anogenital warts), history of sexually transmitted diseases (STDs), and short duration of relationship with primary sex partner [41; 42; 43; 44; 45; 46; 47; 48]. Studies suggest that individuals with immunosuppression related to HIV seropositivity [49] and organ transplantation [50; 51] are also at an increased risk for acquiring anal HPV infection.

Anal HPV infections, especially type 16 infections, are common in women and men, with prevalence varying by the extent that risk factors are prevalent within a given population. For example, among women, anal HPV prevalence was 42.5% in a study of 431 healthy

Hawaiian women in a cervical screening population [52]. This population may have had higher risk behaviors than the general U.S. population (e.g., mean lifetime number of sex partners was 17). Palefsky et al. reported a similar prevalence of HPV anal infection in a San Francisco study of 57 HIV-negative women at high risk for anal and HIV infections (43%) and a much higher prevalence of HPV anal infection in 223 HIV-positive women (79%) [43]. In that study, as well as the Hawaiian cohort, the prevalence of anal HPV infection was similar to or higher than the prevalence of cervical infection [43; 44]. Among anal HPV-positive women in the Hawaiian cohort, the prevalence of type 16 was 4.4% [52]. Among anal HPV-positive but HIV-negative women in the San Francisco cohort, type 16 was not detected, but type 18 had a 9.5% prevalence. In contrast, among the women who were both anal HPV-positive and HIV-positive, the prevalence by PCR of types 16 and 18 was 14.5% and 6.6%, respectively [43].

All males are susceptible to anal HPV infection. For example, a cross-sectional study of 222 healthy, low-risk 18- to 40-year-old men who have sex with women and who provided anal canal specimens was conducted in Arizona and Florida. These men were sampled from the general community and reported having sexual intercourse with a female within the previous year; no history of genital warts, penile cancer or anal cancer; no current diagnosis of a sexually transmitted disease; and no history of anal or oral sex with a man. Among these men, the prevalence of HPV infection in the perianal area or anal canal was 24.8%, with 16.6% prevalence specifically in the anal canal. Of men infected with any HPV type in the anal canal, 13.9% had HPV 16 or 18 anal canal infections [40].

Among HIV-negative MSM, anal HPV prevalence is higher than in heterosexual males. In a Dutch study of 241 MSM visiting an STD clinic, anal HPV was detected in 32.8% of men, and types 16 and 18 were detected in 9.1% and 3.7%, respectively, of men with anal infection [46]. Among HIV-positive MSM, the prevalence of HPV anal infection is notably high, consistent with the high rates of anal cancer in this population. In the Dutch cohort, among 17 HIV-positive MSM, anal HPV was detected in 64.7%, with types 16 and 18 found in 29.4% and 23.5%, respectively, of men with anal HPV detected [46]. In a study of 357 HIV-positive MSM in California, 95% had anal HPV infection [53]. Similarly, in a Montreal cohort of 247 HIV-positive MSM (median age 43 years), HPV anal infection was detected in 97.9% of the cohort at baseline, with type 16 (38.2%) and type 18 (24.5%) being the two most commonly detected high-risk HPV types [54]. The incidence rate of new anal infections in this cohort was very high; by month 36 of the study, 33.2% of the cohort who were initially negative for type 16 developed an incident infection, and similarly, 13.1% developed a type 18 infection [54].

The differences observed in the prevalence of anal HPV infection among low-risk (community-based) heterosexual males (16.6%), low-risk (community-based) HIV-negative females (42.5%), high-risk HIV-positive females (79%) and MSM (95-97.9%) can likely be explained by the overall behavioral differences among each group. For example, MSM and females are more likely than heterosexual males to engage in

receptive anal intercourse, a risk factor for anal infection. This is supported by data from Daling et al.; in a western Washington state cohort, 87.5% of MSM and 26-34% of females reported a history of anal intercourse, whereas no males who self-identified as heterosexual reported a history of anal intercourse [13]. In addition, the female and MSM studies described above included subpopulations at higher risk for anal HPV infection, such as women with a mean of 17 lifetime number of sex partners and HIV-positive MSM [43; 52; 53; 54]. In contrast, the heterosexual male study from Arizona and Florida was limited to only include healthy men with no history of sexually transmitted diseases, including HIV and genital warts [40]. These lower risk men are expected to have a lower prevalence of anal HPV infection.

Anal HPV infection is important because it is the first step in the biologic pathway that leads to high-grade AIN, similar to the association between cervical HPV infection and CIN. In fact, anal HPV infection is the most significant risk factor for AIN development in both males and females [26; 22]. For example, in a prospective study, HIV-negative men who were HPV-positive for high- and low-risk HPV types had a 9-fold increased risk of developing anal high-grade squamous intraepithelial lesions [55]. Detection of only high-risk HPV types was associated with a 4-fold increased risk in high-grade lesions. In another prospective study among HIV-negative men, HPV types 16 or 18 were associated with a 16.6-fold higher risk of developing high-grade AIN [42]. Natural history data from the placebo group in the Protocol 020 AIN Substudy are also consistent with these findings. For example, the incidence of AIN was higher in subjects who had anal HPV infection at baseline. Notably, the mean and median durations of anal HPV 16 and 18 infection were found to be comparable to genital HPV infection duration in women, further supporting the similar pathogenic potential of HPV infection in these anatomic sites [48].

In summary, both women and men are at risk for acquiring HPV anal infections, and in fact, acquire these infections at a substantial rate. The risk factors for HPV anal infection are largely behavioral, are not gender-specific, and are consistently reported across a variety of studies. Moreover, these risk factors for anal infection are similar to those for AIN and anal cancer, which provides the important epidemiologic link between anal infection leading to high-grade AIN and subsequent progression to anal cancer. Similar epidemiologic links have been shown for HPV cervical infection, CIN, and cervical cancer. It is not possible to predict which anal infections will progress and which will regress. Therefore, it must be assumed that any anal infection with high-risk HPV types has the potential to progress to high-grade AIN and subsequent anal cancer. Type-specific prevention of anal HPV infection will prevent anal cancers associated with those types, the overwhelming majority of which are due to HPV types 16 and 18.

7. High-Grade Anal Intraepithelial Neoplasia (AIN 2/3): The Precursor of Anal Cancer in Men and Women

In demonstrating the efficacy of GARDASIL® against anal cancer, a relevant endpoint for study was required. Analogous to the endpoints evaluated in the cervical, vulvar, and vaginal cancer prevention studies of GARDASIL®, the relationship of this endpoint as an obligate precursor to cancer needed to be well-established, such that its prevention through vaccination would provide sufficient evidence for cancer prevention. There is a substantial body of evidence demonstrating that high-grade AIN (AIN 2/3) is the obligate precursor of HPV-related anal cancer in men and women, and that the process of anal HPV infection and progression to anal cancer is analogous to the process of HPV-related carcinogenesis that occurs in the cervix. It is notable that the National Cancer Institute and other expert groups such as the American Cancer Society and the American Society for Colon and Rectal Surgeons consider this evidence sufficient to state that high-grade AIN is a premalignant lesion.

Collectively, the evidence is sufficient to support the use of AIN 2/3 as the relevant endpoint for demonstration of anal cancer prevention in the context of the GARDASIL® clinical trials, and includes the items mentioned below, which are discussed in further detail in this section:

- The rate of anal cancer among individuals with high-grade AIN is substantially higher than the rate of anal cancer in the general population;
- There are notable anatomic similarities between the anus and the cervix relevant to HPV-related neoplasia;
- There are similarities in biologic characteristics between AIN and CIN;
- There are similarities in the molecular characteristics of high-grade AIN and high-grade CIN; and
- The proportion of anal lesions with high-risk HPV types 16 and 18 increases with increasing lesion severity.

Evidence supporting the premalignant potential of intraepithelial lesions is found in prospective natural history data showing evidence of progression from high-grade to invasive disease. The natural history of cervical neoplasia is well-established, and it is on the basis of the preponderance of evidence of high-grade CIN as a precursor of cervical cancer that prevention of the lesion by vaccination was accepted as a basis for licensure of GARDASIL® for cervical cancer prevention [56]. The data summarized in the following paragraphs demonstrate that analogous to CIN 2/3, AIN 2/3 is the obligate precursor of HPV-related anal cancer, and has significant malignant potential [57].

An analysis of the literature suggests that individuals with high-grade AIN have a substantially increased risk of developing anal cancer compared to the general U.S. population. As shown in [Figure 3] and detailed in[Appendix 3], annual rates of anal cancer were developed based on available prospective studies of this association

identified in the literature. The studies of this association are relatively few, have small sample sizes, and include one study for which progression was not observed [58]. Nonetheless, 4 of the 5 studies of this association show a clearly elevated rate of anal cancer among patients with a history of high-grade AIN [59; 60; 61; 62; 63]. For example, annualized rates of anal cancer among patients with a history of high-grade AIN were estimated to range up to 17.8% (17,800 per 100,000 persons based on Scholefield et al., 1994 [60], which is dramatically increased compared to the 1.65 per 100,000 persons age-adjusted annual incidence of anal cancer in the general U.S. population [33]. However, it should be noted that the sample sizes of these studies were often limited, some study populations had an over-representation of HIV-positive patients, and the age distribution and demographic characteristics of study subjects may not reflect those in the general population. Thus, the reported data may not be directly comparable to the age-adjusted general population data. Nonetheless, the incidence rates in the general population are consistent with the malignant potential of high-grade AIN regardless of sexual orientation, gender and immune status, and it is unlikely that these differences alone could account for the substantially increased risk of anal cancer observed among individuals with a history of high-grade AIN, in comparison to the general population. Thus, these data are very supportive of the association between high-grade AIN and anal cancer.

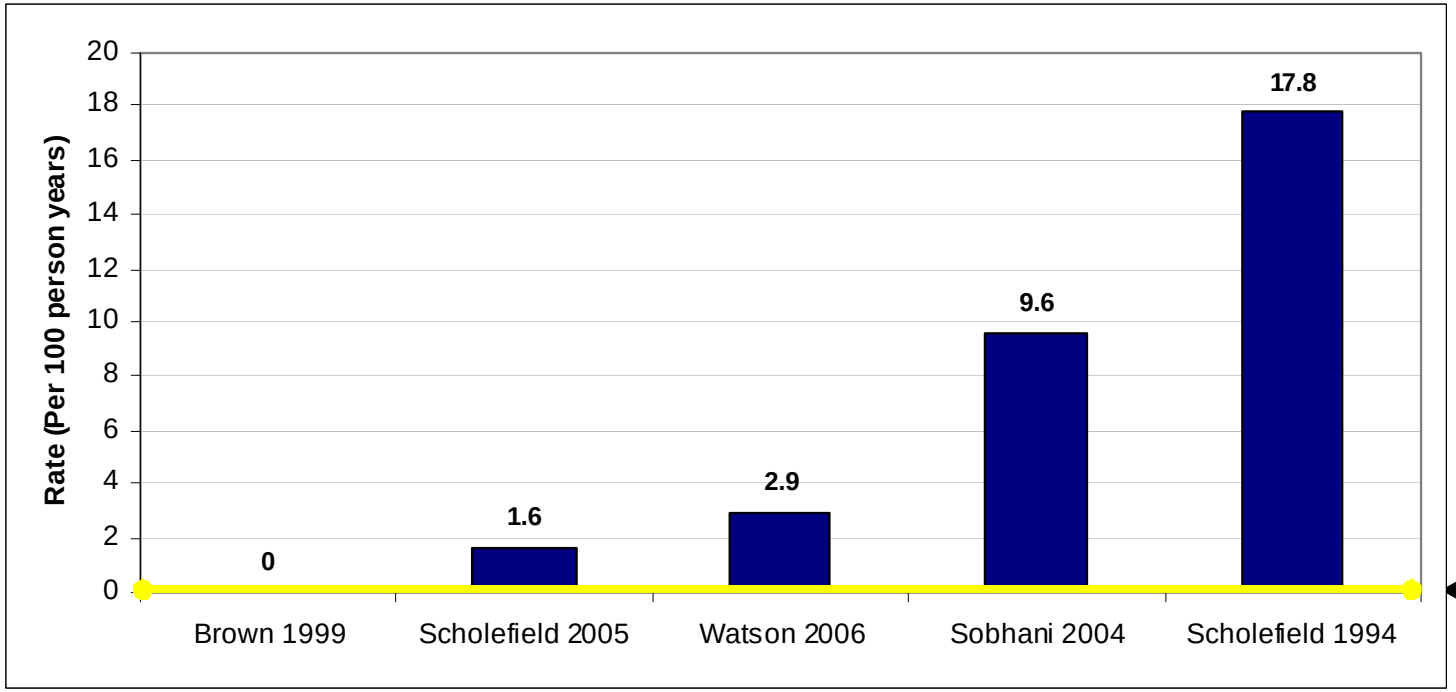
In the largest prospective study to date, 199 consecutive patients with recently treated anal condyloma (84% male; 72% HIV-positive) were recruited. Seven of these patients developed invasive anal carcinoma (6 HIV-positive men aged 26-53 years and one 62 year-old HIV-negative woman) over a median of 26 months (range 13-112 months after study entry). Prior to diagnosis of anal cancer, 6 of these 7 patients had high-grade AIN present at baseline and during follow-up. The one remaining patient had high-grade AIN diagnosed during follow-up. Having a high-grade lesion at baseline was significantly associated with developing invasive cancer (23% vs. 0.5%, $p<0.01$) [59].

In another prospective study of 72 subjects with AIN 1, 2, or 3 (52 females/20 males), 8 subjects (2/10 subjects with AIN 2 and 6/45 subjects with AIN 3) developed invasive cancer over a median follow-up time of 60 months [62]. The authors of this study concluded that "there is no reason to believe…that the conversion of intraepithelial neoplasia to invasive cancer at any site should be different from any other", and "high-grade intraepithelial neoplasia...in all anogenital sites can be a precursor to invasive malignancy…" Similarly, in a recent prospective study of AIN progression in 446 HIV-positive men in Germany, all 5 men who refused treatment for high-grade AIN progressed to anal cancer in a median time of 8.6 months, and the authors concluded that "high-grade (anal) dysplasia…can progress to invasive cancer…" [63]. This study demonstrates one of the challenges in assessing the natural history of AIN; most patients diagnosed with high-grade AIN receive treatment, which likely alters the progression to anal cancer.

In another study conducted in San Diego between 1995 and 2003, 40 HIV-positive men with an abnormal anal examination, anal squamous dysplasia of the anal margin or anal canal, and at least one year of follow-up with physical examination and biopsy (if needed) were evaluated [64]. The mean age of study subjects was 39 years (range 24-57) and the mean follow-up time was 32 months (range 13-130 after diagnosis of dysplasia). Of the 40 patients, 28 had severe dysplasia, and 3 of these 28 patients developed invasive anal carcinoma during the follow-up period.

Figure 3

Summary of the Literature on Annualized Progression Rates of AIN to Anal Cancer

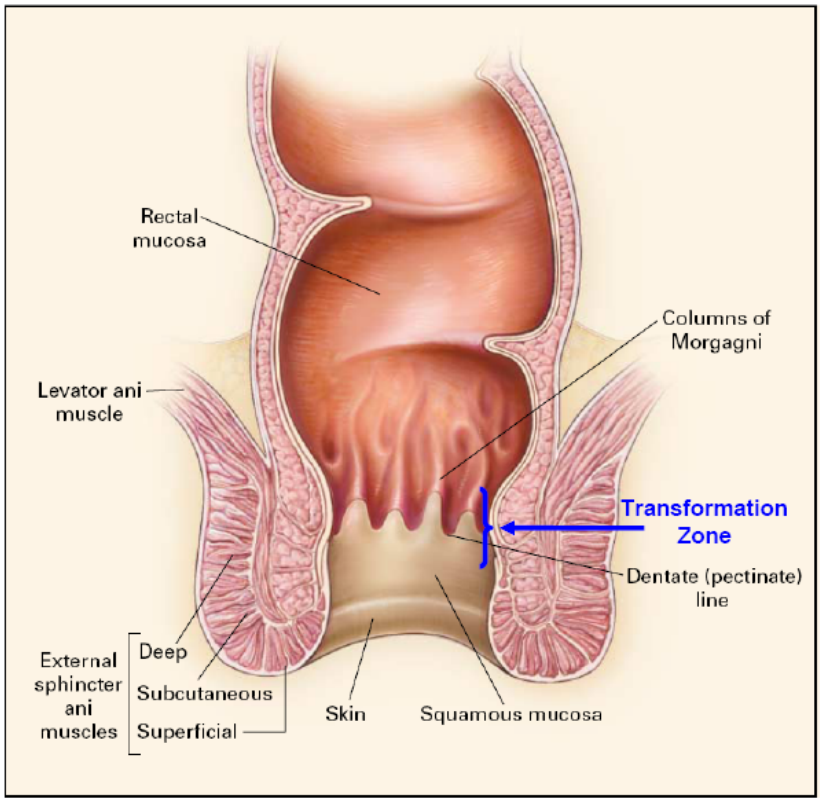


Studies of cervical and anal disease also report increasing HPV prevalence with increasing lesion severity. In a review of 111 anal surgical specimens, high-risk HPV types were detected in a progressively greater number of AIN lesions from low- to high-grade (from 56% in low-grade to 88% in high-grade) [20]. A recent meta-analysis of HPV distribution in lesions included 35 peer-reviewed studies of anal cancer, LSIL (low-grade squamous intraepithelial lesion) (defined as cytologically confirmed LSIL or histologically diagnosed AIN 1) and HSIL (high-grade squamous intraepithelial lesion) (defined as cytologically confirmed HSIL or histologically diagnosed AIN 2-3) publications up to July 2007 [16]. While HPV was found in all lesions, the prevalence of HPV 16 and/or HPV 18 was markedly higher in HSIL and invasive cancers (68.7% and 72.2%, respectively), in comparison to LSIL (27.4%) [16]. Of note, the prevalence of these two high-risk types was similar between HSIL and invasive anal cancer.

The evidence supporting the relationship of high-grade AIN to anal cancer is further substantiated by convincing similarities between anal and cervical neoplastic disease. The anatomy of the anus and cervix canals is similar, both structures possessing a transformation zone, where the squamous and columnar epithelial linings of the anal or cervical canal meet [Figure 4]. The epithelium of the transformation zone in both anatomic sites is characterized by immature metaplastic squamous epithelium that is highly susceptible to HPV infection and neoplastic change, and is where anal and cervical cancers arise [65].

Figure 4

Anatomy of the Anal Canal



[65] Reprinted with Permission

AIN 2/3 and CIN 2/3 share similar biological characteristics of progressively increasing proliferation and replacement of the normal epithelium by malignant cells with a basal morphology and large nuclear-cytoplasmic ratios, and mitoses in the more superficial cell layers [66]. The two lesions are, in fact, essentially indistinguishable from a histologic standpoint [Appendix 4]. Molecular changes such as p16 and Ki67 overexpression, are also shared between high-grade AIN and CIN, suggesting a common molecular pathway [67; 68; 69].

As commonly seen in cervical cancer, co-existent high-grade anal intraepithelial neoplastic changes are often identified adjacent to invasive anal cancers, showing histologic evidence of progression from the non-invasive AIN 3 to the invasive component of lesions [Appendix 5], supporting that anal cancer arises in sites of neoplasia [70]. Moreover, as observed with co-existent CIN and cervical cancers, studies

have reported similar prevalence of high-risk HPV types in AIN and invasive anal carcinomas in surgical specimens containing both lesions [20].

The similarity in HPV prevalence between high-grade AIN and cancer, and the progressive increase in high-risk HPV positivity from low- to high-grade lesions are consistent with the observation that high-grade AIN is the true precursor of squamous anal carcinoma. When identified together, high-grade and invasive anal lesions also commonly share anatomical, biological, and molecular characteristics [11; 70], providing further support for the premalignant nature of high-grade lesions.

There is no evidence that the process of anal cancer pathogenesis is different in men and women; anal cancer is the same disease in men and women. Thus, high-grade AIN is thus the obligate precursor of HPV-related anal cancer in both genders. First, as described earlier, the causal relationship of HPV to anal cancer exists in both men and women. Anal anatomy in men and women is the same, characterized by the same epithelial lining and transformation zone. The histologic progression of AIN to anal cancer is observed in anal cancers from both men and women, and as mentioned above, anal cancers in men and women show the same molecular changes and are grossly and histologically identical [Appendix 6] and [Appendix 7].

Taken together, the totality of the natural history, anatomic and pathogenetic data provide convincing evidence that high-grade AIN is the obligate precursor of invasive HPV-related anal cancer in both men and women. This evidence supports the use of high-grade AIN as a clinically relevant endpoint for anal cancer prevention in clinical studies and as the basis for an indication for GARDASIL® for the prevention of HPV-related anal cancer in men and women.

8. Approach to the Evaluation of GARDASIL® in the Prevention of Anal Cancer

8.1 Introduction to the Protocol 020 AIN Substudy

Protocol 020 was a randomized, placebo-controlled, international, multicenter, double-blind safety, immunogenicity, and efficacy study operating under in-house blinding procedures. The primary objective of Protocol 020 was to evaluate the efficacy of GARDASIL® in the prevention of external genital lesions. The October 2009 licensure of GARDASIL® for use in boys and men 9 to 26 years of age to prevent HPV 6/11-related genital warts was based on efficacy and safety data from Protocol 020, supported by safety and immunogenicity data from the adolescent (9- to 15-year-old) GARDASIL® trials.

The AIN Substudy for evaluation of the efficacy of GARDASIL® against AIN and anal cancer was undertaken in a population of 16- to 26-year-old MSM, who also participated in the main study of external genital lesions. In order to ensure optimal evaluation of anal disease endpoints, substudy subjects were enrolled at selected sites with expertise in High Resolution Anoscopy (HRA).

8.2 Rationale for the Study Population

As described above, although anal intercourse is not required for anal HPV acquisition, this sexual practice is known to be associated with infection. Existing epidemiologic data on rates of anal HPV infection in 16 to 26 year-old MSM strongly supported performing the study in this population in order to maximize the likelihood of achieving a successful demonstration of vaccine efficacy. Other than their higher risk of exposure to anal HPV infection, there is no evidence to suggest that MSM are at particular biologic risk of anal HPV infection or disease, or that the pathogenetic process of AIN and anal cancer development is different in MSM than in heterosexual males or females.

8.3 Study Design

The AIN Substudy enrolled 602 MSM subjects, who were randomized in a 1:1 ratio to GARDASIL® or placebo at 0, 2, and 6 months, and followed every six months for up to 36 months. Key inclusion criteria included no more than 5 lifetime sexual partners, in order to minimize the proportion of enrolled subjects who were HPV sero- and/or PCR positive at baseline. MSM subjects must have engaged in either insertive or receptive anal intercourse with another male sexual partner within the year prior to enrollment; however, MSM who denied having had anal sex were also eligible if they had engaged in oral sex with a male partner within the past year. Key exclusion criteria included a history of HPV-related disease or infection, prior HPV vaccination, or HIV positive status. Potential subjects were examined prior to enrollment for visible signs of HPV infection. Those with lesions felt to be definitely, probably or possibly related to HPV or of unknown etiology were not randomized. Subjects were not screened for PCR- or seropositivity prior to enrollment; subjects were enrolled regardless of their HPV status. All MSM subjects were monitored for HIV status during the study, and any subjects diagnosed with HIV were excluded from the per protocol efficacy analysis.

The AIN efficacy objective was as follows:

> **To investigate the impact of administration of a 3-dose regimen of quadrivalent HPV (Types 6, 11, 16, 18) L1 VP vaccine on the combined incidence of HPV 6-, 11-, 16-, or 18-related anal intraepithelial neoplasia (AIN) or anal cancer in MSM subjects who are naïve to the relevant HPV type.**
>
> The AIN analysis was to be performed when 17 cases of HPV 6/11/16/18-related AIN or anal cancer were observed. The protocol stated that if the case target for the AIN analysis had not been met at the time of the primary EGL efficacy analysis, the AIN efficacy analysis would be the subject of a separate report.

The corresponding hypothesis was as follows:

> **Administration of a 3-dose regimen of quadrivalent HPV (Types 6, 11, 16, 18) L1 VLP vaccine reduces the combined incidence of HPV 6-, 11-, 16-, and 18–related AIN or anal cancer in 16- to 26-year-old MSM who are seronegative at Day 1 and PCR negative from Day 1 through Month 7 to the relevant HPV type, compared to placebo recipients.**

The overall study secondary endpoints of persistent infection and DNA detection were also evaluated in the context of the AIN study; however, there were no separate prespecified hypotheses limited to the substudy.

The EGL endpoint was assessed when at least 32 cases of HPV 6/11/16/18-related EGL were accrued. This occurred prior to the end of study, which was planned to continue for approximately 36 months of follow-up for each subject. At the time the EGL endpoint was assessed, fewer than 17 HPV 6/11/16/18-related AIN or anal cancer cases had accrued in MSM subjects and the hypothesis for this endpoint was therefore not tested. No further case counts were obtained until the end of the study, at which time a total of 29 6/11/16/18-related AIN cases were observed.

8.4 AIN Efficacy Endpoint Ascertainment and Adjudication

8.4.1 Disease Ascertainment

The comprehensive approach to anal infection and disease assessment, with anal Pap testing and anal sampling for HPV PCR detection at 6 month intervals, in addition to the use of HRA for evaluation of abnormal cytology, allowed for complete ascertainment of HPV-related anal disease in the study population. Routine evaluation of study subjects consisted of the procedures that were performed for the overall study, in addition to anal swabs for HPV PCR testing, anal ThinPrep™ cytology, anorectal examination, and anoscopy if indicated for evaluation of any anorectal abnormality identified on examination. All anal lesions identified on physical examination or anoscopy that in the opinion of the investigator, were possibly, probably, or definitely HPV-related, or whose relationship to HPV infection could not be determined, were biopsied. HRA was performed for evaluation of either abnormal cytology (defined as Pap results of atypical squamous cells of undetermined significance [ASC-US] or worse) or any potentially HPV-related intra- or perianal lesion observed on routine examination. Anal biopsies were performed for any identified abnormalities. Mandatory HRA examination was also performed on all substudy patients at the final visit, regardless of cytology results or anal examination findings.

The pathology infrastructure used in the substudy was identical to that used in all prior Phase II/III GARDASIL® efficacy studies. All biopsies were adjudicated to a consensus diagnosis by the same Pathology Panel as for previous GARDASIL® studies. All pathologists were blinded to individual treatment allocation, HPV PCR- and serostatus,

study visit number, the clinical impression, and the Program Central Laboratory pathology diagnosis (which was utilized for patient clinical management). All panel members utilized the same histologic grading system. For the purposes of the current analysis, any cases with a Pathology Panel consensus diagnosis of condyloma acuminatum in an anal biopsy specimen were grouped under AIN 1, using a diagnostic approach similar to that used for cervical and vulvar disease [71]. Low-grade anal intraepithelial neoplasia lesions with histologic characteristics of condyloma were classified as acuminate AIN 1, and non-warty, flat, low-grade lesions were classified as non-acuminate AIN 1.

Because uniform standards for the management of anal disease do not exist, the management of confirmed HPV-related anal lesions was based on local standards as determined by the individual study investigators. Clinical management decisions were based on pathology diagnoses made by the Program Central Laboratory, independent of the Pathology Panel.

8.4.2 Persistent Infection

The persistent infection endpoint was defined as detection of the same vaccine HPV type DNA in 2 consecutive anal samples collected 6 months (±1 month) apart. The 6-month visit interval was designed to ensure maximal ascertainment of persistent HPV 6, 11, 16, or 18 infection. Since the data on the duration of incident HPV 6, 11, 16, or 18 infection in men were sparse, the duration of HPV infection in women of similar age and risk profile was referenced. Since the median duration of HPV 16 infection in women is approximately 12 months, a 6-month visit interval allowed for complete ascertainment of such infections.

8.4.3 Detection of HPV Types in Biopsy and Swab Specimens

All biopsy and swab specimens were sent to Merck Research Labs (MRL) for HPV testing. Each biopsy specimen was tested by PCR for HPV, regardless of whether an HPV-related histologic diagnosis was made, for the purpose of determining the causal HPV type in the lesion. Testing was performed on Thinsection microtomy specimens prepared by the Program Central Laboratory. In addition, all swab samples were tested by PCR. All laboratory staff were blinded to vaccination allocation, as well as to subject identity, visit interval, or histologic diagnosis rendered by the Program Central Laboratory or the Pathology Panel. All biopsy and swab samples were tested for HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

8.4.4 Disease Endpoint Adjudication

Individual biopsies, collected using separate biopsy instruments and placed into individual containers, underwent embedding and sectioning under ultraclean conditions (to minimize PCR contamination). A total of 13 sections were generated for each biopsy. The first and last two sections were used for histologic analysis. Each of the remaining 9

sections was placed into an individual tube for PCR testing. As the section in which HPV testing occurred was adjacent to the section in which histopathology was read, this procedure allowed for precise co-localization of the histopathologic finding with the associated HPV type.

The disease endpoint adjudication process for anal endpoints was identical to the process for EGL endpoints.

An AIN endpoint occurred if on a single biopsy or excised tissue block, the following conditions were met:

- The Pathology Panel consensus diagnosis was anal intraepithelial lesion of any grade or anal cancer;

  and

- At least one of HPV types 6, 11, 16, or 18 was detected by Thinsection PCR in an adjacent section from the same tissue block.

8.5 Statistical Methods

Statistical analyses were prespecified in the Statistical Analysis Plan (SAP). All analyses were performed according to standardized and validated methods.

The evaluation of anal disease followed a fixed-case study design whereby statistical analyses were to be performed when a prespecified number of cases had been observed. The case target for the AIN Substudy was at least 17 cases of HPV 6/11/16/18-related AIN or anal cancer in the substudy per-protocol population.

8.5.1 Definition of Analysis Populations

The key elements for the efficacy analysis populations are summarized here. Data in this background document are presented for the PPE, FAS, and GHN populations.

- The per-protocol efficacy (PPE) population was used for the primary efficacy analysis. Briefly, PPE subjects were seronegative at Day 1and HPV DNA negative through Month 7 to the relevant HPV type, were HIV negative, and had no major protocol violations; case counting started after 4 weeks following completion of the vaccination series (i.e., after Month 7).
- The Generally HPV Naïve (GHN) population included subjects who were HPV DNA negative at Day 1 to all 14 HPV types tested in the multiplex PCR assay (HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), seronegative to HPV 6, 11, 16, 18, and had a negative anal Pap test at Day 1; case counting started after Day 1.
- The Full Analysis Set (FAS) included all enrolled subjects who received at least one dose of vaccine and had follow-up after Day 1; subjects with prevalent

infection (i.e., HPV infection present at Day 1) and disease with any HPV type were therefore included; case counting started after Day 1.

8.5.1.1 Prophylactic Efficacy Analysis Populations

The PPE and FAS analysis populations are summarized in Table 2. The primary efficacy analysis for the prophylactic efficacy of GARDASIL® against AIN utilized the PPE population, since this allowed measurement of the full benefit of GARDASIL® in individuals who were negative to the relevant vaccine HPV type through the completion of the 3-dose vaccination regimen. As full vaccine benefit is not expected until after the 3-dose vaccination series is completed (i.e., starting after 4 weeks following dose 3, or Month 7), cases in the PPE population were counted starting after Month 7.

Supportive analyses in the FAS population were performed. The FAS analysis population includes all HPV-negative subjects, as well as subjects with evidence of prior exposure or active infection with a vaccine or non-vaccine HPV type at enrollment. Because this population includes virtually all study subjects, the FAS approximates the general population of sexually active 16- to 26-year-old men. Evaluation of prophylactic efficacy impact in the FAS population was measured on vaccine-type disease only.

Table 2

Definition of Populations Used in the Prophylactic Efficacy Analyses

Parameter	PPE	FAS
Definition	Included subjects who: (1) were sero- and PCR-negative at Day 1 and PCR-negative through Month 7 to the appropriate vaccine HPV types; (2) received all 3 vaccinations within a one year period, (3) generally did not deviate from the protocol, and (4) were HIV negative.	Included all subjects who received at least 1 vaccination and had follow-up after Day 1.
Case Counting	Cases were counted starting after Month 7.	Cases were counted starting after Day 1.
Relevant Endpoints	HPV 6-, 11-, 16-, and 18-Related AIN HPV 6, 11, 16, and 18 Persistent Infection and DNA detection	HPV 6-, 11-, 16-, and 18-Related AIN HPV 6, 11, 16, and 18 Persistent Infection and DNA detection
Role in the Analysis Plan	Primary efficacy analysis population.	Supportive to primary efficacy analysis.
Value of Population in Evaluating Vaccine Efficacy	Measurement of the full benefit of GARDASIL® in persons who were negative to the relevant HPV type through the completion of 3-dose vaccination regimen.	Measurement of vaccine impact on vaccine type AIN in the general population of 16 to 26 year-old men, starting immediately after the first dose.
HPV = Human papillomavirus; Pap = Papanicolaou; PCR = Polymerase chain reaction.		

8.5.1.2 Population Benefit Analysis Populations

The analysis of efficacy with respect to the population benefit endpoints (i.e., evaluation of the impact of GARDASIL® on the incidence of any type anal disease) was performed using the GHN and the FAS populations. These populations are summarized in Table 3.

The GHN population was designed to approximate a population of adolescent and young adult men who were either sexually naïve or sexually experienced but not yet exposed to any HPV type. Given that it is impossible to evaluate GARDASIL® disease efficacy in the target population of boys prior to sexual debut, the GHN population is the most appropriate subpopulation of Protocol 020 to provide insight regarding the potential impact of vaccination of males when vaccinated in young adolescence, prior to HPV exposure. With cases being counted after the first dose of vaccine, efficacy for this population is anticipated to be lower than for the per-protocol population.

The FAS population represents the general population of sexually-active 16- to 26-year-old men, and therefore includes subjects with prevalent HPV infection (vaccine and non-vaccine HPV types). Since only incident vaccine-type disease is anticipated to be prevented by GARDASIL®, efficacy for this population is also anticipated to be lower than for the PPE population. The population impact analysis of the FAS population was for efficacy against any HPV type.

Table 3

Definition of Populations Used in the Population Benefit Analyses

Parameter	GHN	FAS
Definition	Generally HPV-naïve (GHN): Included all subjects who: (1) were seronegative and PCR negative to all 4 vaccine HPV types at Day 1; (2) were PCR negative to HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, *and* 59 at Day 1; (3), had a Pap test result at enrollment that was negative for SIL; and (4) received at least 1 vaccination.	Full analysis set (FAS): Included all subjects who received at least 1 vaccination and had follow-up after Day 1.
Case Counting	Cases were counted starting after Day 1.	Cases were counted starting after Day 1.
Relevant Endpoints	AIN (caused by vaccine or non vaccine HPV types) Anal Procedures and Therapies	AIN (caused by vaccine or non vaccine HPV types) Anal Procedures and Therapies
Role in the Analysis Plan	Key analysis population for the evaluation of the population benefit of GARDASIL®.	For the evaluation of the population benefit of GARDASIL®, supportive population.
Value of Population in Evaluating Vaccine Efficacy	GHN population approximates a population of adolescent and young adult men who were either sexually-naïve or sexually-experienced and had not yet been exposed to *any* HPV type. This population provides insight on the potential impact of vaccination on males when vaccinated in young adolescence, prior to HPV exposure.	FAS population provides information on overall vaccine impact when used in a general population of sexually active 16 to 26 year-old men.
HPV = Human papillomavirus; Pap = Papanicolaou; PCR = Polymerase chain reaction.		

8.5.2 Efficacy Statistical Methodology

The AIN Substudy objective was addressed by testing the hypothesis that administration of a 3-dose regimen of GARDASIL® reduces the incidence of HPV 6/11/16/18-related AIN and anal cancer in subjects who are naïve to the relevant HPV type compared with placebo recipients. The statistical criterion for success on this hypothesis required that the lower bound of the multiplicity-adjusted two-sided 95% confidence interval for vaccine efficacy against HPV 6/11/16/18-related AIN in the PPE population exclude 0%. In this case, vaccine efficacy is the relative risk reduction of developing an HPV 6/11/16/18-related AIN or anal cancer in the vaccine group compared to the placebo group. Note that the multiplicity adjustment was made to account for an interim summary of AIN cases done at the time of the primary EGL efficacy analysis (details are provided later in this section).

The estimate for vaccine efficacy was computed under the assumption that the number of primary efficacy cases among vaccine recipients followed a Binomial distribution given the total number of cases, which followed from an assumption that incidence rates among vaccine and placebo recipients were means of independent Poisson processes. The 95% confidence interval for vaccine efficacy was determined using exact methods. All estimates obtained were adjusted for differences in the person-years of follow-up time accrued in the vaccine and placebo groups. Estimates of vaccine efficacy for the secondary endpoints were obtained using the same methods used for the primary endpoint analysis. The statistical criteria used in the study were agreed to by the Food and Drug Administration (FDA).

At the time of the primary EGL efficacy analysis, an interim summary of the substudy endpoint was performed, since the case target of 17 in substudy had not yet been achieved. Although no inference was made regarding the substudy endpoint based on this interim summary, a Haybittle-Peto group sequential interim analysis plan was used for the interim summary and substudy efficacy hypothesis. Under this plan, a nominal α=0.001 (2-sided) was used to account for the interim summary. Since a stringent nominal alpha level was used for the interim summary, there was negligible impact on the type 1 error of the final analysis, so a nominal α=0.05 (2-sided) could have still been applied for the final analysis. In this particular case, with the target of 17 cases, due to the discreteness of the binomial case split, the exact alpha level used at the final analysis was 0.049 (2-sided). No monitoring of efficacy data was conducted for the purpose of monitoring the emerging efficacy results prior to the final frozen file, which was executed to update the safety, primary and secondary efficacy, and immunogenicity results and conduct the substudy efficacy analysis.

9. AIN Substudy Results

This section describes baseline characteristics and subject accounting in the vaccine and placebo groups of Protocol 020 and provides data on efficacy findings. Overall, the substudy groups were similar in terms of all baseline characteristics evaluated, and eligibility for the efficacy analyses was comparable between the two groups. The AIN Substudy data indicate that GARDASIL® is efficacious in the prevention of AIN and anal cancer and persistent infection related to HPV types 6, 11, 16, and 18.

9.1 Subject Accounting and Baseline Characteristics

The final cut-off date for study visits was July 31, 2009. The median duration of efficacy follow-up at the end of the study was 32.2 months post-enrollment for the substudy population.

A summary of the number of AIN Substudy subjects who were randomized, vaccinated, who completed or discontinued during the vaccination period of the study, who entered and who discontinued during the efficacy follow-up phase, by vaccination group, is provided in Table 4. Study subjects were to have been followed for 36 months from the date of enrollment, however after the primary analysis was completed and efficacy was demonstrated, the Data and Safety Monitoring Board (DSMB) was consulted and agreed with the decision to compress study visits to end the study early. Sites were notified of the early study termination and an amendment was written to describe the compression of study visits through Month 36. This visit compression largely impacted the interval between the Month 30 and Month 36 visits for substudy subjects.

Table 4

Subject Disposition-AIN Substudy Subjects

	GARDASIL®		Placebo		Total	
	n	(%)	n	(%)	n	(%)
SCREENING FAILURES					99	
RANDOMIZED	301		301		602	
VACCINATED AT:						
Dose 1	299	(99.3)	299	(99.3)	598	(99.3)
Dose 2	286	(95.0)	286	(95.0)	572	(95.0)
Dose 3	278	(92.4)	277	(92.0)	555	(92.2)
Vaccination Period (Day 1 Through Month 7)						
ENTERED	299		299		598	
COMPLETED[†]	272	(91.0)	274	(91.6)	546	(91.3)
DISCONTINUED	27	(9.0)	25	(8.4)	52	(8.7)
WITH LONG-TERM FOLLOW-UP[‡]	4	(1.3)	3	(1.0)	7	(1.2)
Clinical AE	2	(0.7)	0	(0.0)	2	(0.3)
Other reasons	2	(0.7)	2	(0.7)	4	(0.7)
Uncooperative	0	(0.0)	1	(0.3)	1	(0.2)
WITHOUT LONG-TERM FOLLOW-UP [§]	23	(7.7)	22	(7.4)	45	(7.5)

Subject Disposition-AIN Substudy Subjects (Cont.)

	GARDASIL®		Placebo		Total	
	n	(%)	n	(%)	n	(%)
Lost to follow-up	18	(6.0)	14	(4.7)	32	(5.4)
Moved	0	(0.0)	1	(0.3)	1	(0.2)
Other reasons	3	(1.0)	2	(0.7)	5	(0.8)
Protocol deviations	1	(0.3)	0	(0.0)	1	(0.2)
Withdrew consent	1	(0.3)	5	(1.7)	6	(1.0)
Followup Period (After Month 7)						
ENTERED	274		276		550	
COMPLETED	20	(7.3)	22	(8.0)	42	(7.6)
CONTINUING	234	(85.4)	239	(86.6)	473	(86.0)
DISCONTINUED	20	(7.3)	15	(5.4)	35	(6.4)
Lost to follow-up	12	(4.4)	6	(2.2)	18	(3.3)
Moved	3	(1.1)	2	(0.7)	5	(0.9)
Other reasons	2	(0.7)	0	(0.0)	2	(0.4)
Withdrew consent	3	(1.1)	7	(2.5)	10	(1.8)

† Subjects completed 3 doses of vaccinations and entered the long-term follow-up period.

‡ Subjects received fewer than 3 doses of vaccinations and entered the long-term follow-up period.

§ Subjects discontinued on or before Month 7 and did not enter the long-term follow-up period.

Status percentages are calculated based on the number of subjects who entered the respective time period.

A summary of AIN Substudy subject baseline characteristics is presented in Table 5. MSM subjects represented 14.8% of the overall study population and had a median age of 22 years, with a mean age of 22.1. Baseline characteristics, including sexual history, were comparable between vaccine and placebo groups. Of the substudy subjects, 30.5% of subjects were PCR positive at Day 1 to a vaccine HPV type, 22.8% were seropositive to a vaccine HPV type, and 39.1% were positive to a vaccine HPV type by PCR or serology. Only 0.2% of subjects were HPV PCR positive to all 4 vaccine HPV types at Day 1. With regard to baseline prevalence of non-HPV anogenital infection, 9.4% of MSM subjects were positive for rectal chlamydia. Approximately 10% of baseline anal Pap tests were reported to have squamous intraepithelial lesions present.

Table 5

Summary of Subject Characteristics by Vaccination Group
AIN Substudy Subjects

	GARDASIL® (N = 301)	Placebo (N = 301)	Total (N = 602)
	n (%)	n (%)	n (%)
Gender			
Male	301 (100)	301 (100)	602 (100)
Age (years)			
Mean	22.2	22.1	22.1
Standard Deviation	2.5	2.5	2.5
Median	22	22	22
Range	16 to 26	17 to 27	16 to 27
Race/Ethnicity			
Asian	15 (5.0)	18 (6.0)	33 (5.5)
Black	22 (7.3)	20 (6.6)	42 (7.0)
Hispanic American	72 (23.9)	77 (25.6)	149 (24.8)
White	185 (61.5)	178 (59.1)	363 (60.3)
Other	7 (2.3)	8 (2.7)	15 (2.5)
Region			
Asia-Pacific	45 (15.0)	44 (14.6)	89 (14.8)
Europe	61 (20.3)	61 (20.3)	122 (20.3)
Latin America	65 (21.6)	67 (22.3)	132 (21.9)
North America	130 (43.2)	129 (42.9)	259 (43.0)
Smoking Status			
Current smoker	121 (40.2)	120 (39.9)	241 (40.0)
Ex-smoker	26 (8.6)	30 (10.0)	56 (9.3)
Never smoked	153 (50.8)	150 (49.8)	303 (50.3)
Missing or Unknown	1 (0.3)	1 (0.3)	2 (0.3)
Circumcision			
Yes	134 (44.5)	133 (44.2)	267 (44.4)
No	167 (55.5)	167 (55.5)	334 (55.5)
Missing or Unknown		1 (0.3)	1 (0.2)

Percent calculated as 100*(n/N); N=Number of subjects randomized

Table 6 provides the number of subjects in the AIN PPE population eligible for each efficacy endpoint analysis. Among the 598 MSM subjects enrolled who received at least one injection in the study, 50%, 58%, and 63% were eligible for the MSM PPE analyses related to HPV types 6/11, 16, and 18, respectively. The most common reasons for exclusion from each of the HPV 6/11, HPV 16, and HPV 18 PPE populations were Day 1 through Month 7 positivity to the relevant HPV type (i.e., prevalent disease [Day 1] or incident disease before the full vaccination series take effect [through Month 7]), missing the 2nd and 3rd vaccinations, missing Day 1 or Month 7 swab PCR results, Day 1 or Month 7 swab samples not collected within the acceptable day range, inadequate PCR or serology samples at Day 1 or Month 7, and general protocol violations. The numbers of subjects excluded within each vaccination group for each reason were generally comparable.

Table 6

Subject Accounting for the Efficacy Analysis Populations by Vaccination Group
AIN Substudy Subjects

	GARDASIL® (N=301)	Placebo (N=301)	Total (N=602)	Population to Which Exclusion Category Applies: PPE
Number of Subjects who received at least 1 injection[†]	299	299	598	
Eligible for the PPE Analysis Related to:				
HPV 6/11	149	148	297	
HPV 16	173	175	348	
HPV 18	179	200	379	
Ineligible for the PPE Analysis Related to:				
HPV 6/11	150	151	301	
HPV 16	126	124	250	
HPV 18	120	99	219	
Reason for Ineligibility[‡]				
General protocol violation	36	26	62	X
Received incorrect clinical material or dose amount	1	0	1	X
Received non-study vaccination[§]	7	6	13	X
Received immunosuppressives, IgG, or blood products	5	2	7	X
With a history of immune disorder	3	4	7	X
Diagnosed with HIV or is otherwise immunocompromised	19	14	33	X
Received vaccine stored outside acceptable temperature range	2	0	2	X
Subject prematurely unblinded	2	0	2	X
Vaccination series not completed within 12 months[‖]	2	0	2	X
Missed 2nd and 3rd vaccination	13	13	26	X
Missed 3rd vaccination	8	9	17	X
Without Day 1 serology results within acceptable day range[¶]	2	5	7	X

Subject Accounting for the Efficacy Analysis Populations by Vaccination Group
AIN Substudy Subjects (Cont.)

	GARDASIL® (N=301)	Placebo (N=301)	Total (N=602)	Population to Which Exclusion Category Applies
				PPE
Without Day 1 swab PCR results within acceptable day range[#]	7	4	11	X
Without Month 7 swab PCR results within acceptable day range[‖#]	32	31	63	X
HPV 6 or 11 Positive by Serology or PCR[††]				
At Day 1	76	89	165	X
At or before Month 7	85	110	195	X
HPV 16 Positive by Serology or PCR[††]				
At Day 1	43	56	99	X
At or before Month 7	57	66	123	X
HPV 18 Positive by Serology or PCR[††]				
At Day 1	37	31	68	X
At or before Month 7	46	39	85	X

[†] Subjects who did not receive at least 1 injection were excluded from all analysis populations.

[‡] Subjects are counted once in each applicable exclusion category. A subject may appear in more than one category.

[§] Includes live virus vaccines received within 21 days before or 14 days after study vaccination or inactivated or recombinant vaccines received within 14 days of study vaccination.

[‖] Among subjects who received all 3 vaccinations.

[¶] Includes subjects with a missing serum sample or missing cLIA results for ≥ 1 HPV type.

[#] Includes HM subjects who are missing at least two required swab samples or PCR results for ≥ 1 HPV type and MSM subjects who are missing at least three required swab samples or PCR results for ≥ 1 HPV type.

[††] Day 1 includes seropositivity or PCR positivity. Post-Day 1 includes PCR positivity only. Applies only to the analysis populations for the respective HPV type(s).

N = Number of subjects randomized to the respective vaccination group.

cLIA = Competitive Luminex immunoassay; HIV = Human immunodeficiency virus; HM = Heterosexual men; HPV = Human papillomavirus; PCR = Polymerase chain reaction; PPE = Per-Protocol efficacy.

9.2 Efficacy Results

9.2.1 Prophylactic Disease Efficacy

Table 7 and the associated time to event curve Figure 5 show the results of the analysis of efficacy performed in the PPE population to address the AIN efficacy hypothesis. Success was achieved in the test of the AIN efficacy hypothesis, showing significant vaccine efficacy against HPV 6/11/16/18-related AIN and anal cancer (p-value < 0.001). With 5 HPV 6/11/16/18-related AIN cases in the GARDASIL® group and 24 cases in the placebo group, vaccine efficacy against this endpoint was 77.5% (multiplicity-adjusted 95.1% CI : 39.6, 93.3). All of the cases in the GARDASIL® group and the majority of the cases in the placebo group had positive PCR results for HPV types 6 and/or 16.

Table 7

Analysis of Efficacy Against HPV 6/11/16/18-Related AIN and Anal Cancer[†] by HPV Type and Lesion Type
(AIN Per-Protocol Efficacy Population)

	GARDASIL® (N=299)				Placebo (N=299)						
Endpoint	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	CI[‡]	P-value[§]
HPV 6/11/16/18-Related AIN and Anal Cancer	194	5	381.1	1.3	208	24	411.6	5.8	77.5	(39.6, 93 3)	< 0.001
By HPV Type											
HPV 6-Related AIN and Anal Cancer	141	3	275.2	1.1	144	10	298 5	3.4	67.5	(-26.4, 94.2)	
HPV 11-Related AIN and Anal Cancer	141	0	279.2	0.0	144	6	298.2	2.0	100	(9.3, 100)	
HPV 16-Related AIN and Anal Cancer	167	2	330.6	0.6	170	6	341.9	1.8	65.5	(-92.8, 96.6)	
HPV 18-Related AIN and Anal Cancer	173	0	345.3	0.0	193	4	387.4	1.0	100	(-70.0, 100)	

Analysis of Efficacy Against HPV 6/11/16/18-Related AIN and Anal Cancer[†] by HPV Type and Lesion Type
AIN Per-Protocol Efficacy Population (Cont.)

	GARDASIL® (N=299)				Placebo (N=299)						
Endpoint	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	CI[‡]	P-value[§]
By Lesion Type											
AIN 1	194	4	383.1	1.0	208	16	413.8	3.9	73.0	(16.3, 93.4)	
Condyloma Acuminatum	194	0	386.8	0.0	208	6	418.2	1.4	100	(8.2, 100)	
Non-acuminate	194	4	383.1	1.0	208	11	416.7	2.6	60.4	(-33.5, 90.8)	
AIN 2 or worse	194	3	383.9	0.8	208	13	417.2	3.1	74.9	(8.8, 95.4)	
AIN 2	194	2	384.5	0.5	208	9	418.6	2.2	75.8	(-16.9, 97.5)	
AIN 3	194	2	385.4	0.5	208	6	419.7	1.4	63.7	(-103.0, 96.4)	
Anal Cancer	194	0	386.8	0.0	208	0	421.1	0.0	NA	NA	

[†]Cases found from performing an HRA due to the presence of perianal external lesions are not included in this analysis to eliminate potential ascertainment bias.

[‡] A 95.1% CI is reported for the HPV 6/11/16/18-related AIN and anal cancer endpoint. For all analyses by HPV type and lesion type, a 95% CI is reported. The CI reported for the HPV 6/11/16/18-related AIN and anal cancer endpoint differs from the other analyses due to the alpha adjustment applied.

[§]A p-value<0.0245 (one-sided) corresponds to a lower bound of the confidence interval for vaccine efficacy greater than 0% and supports the conclusion that the vaccine is efficacious against the given endpoint

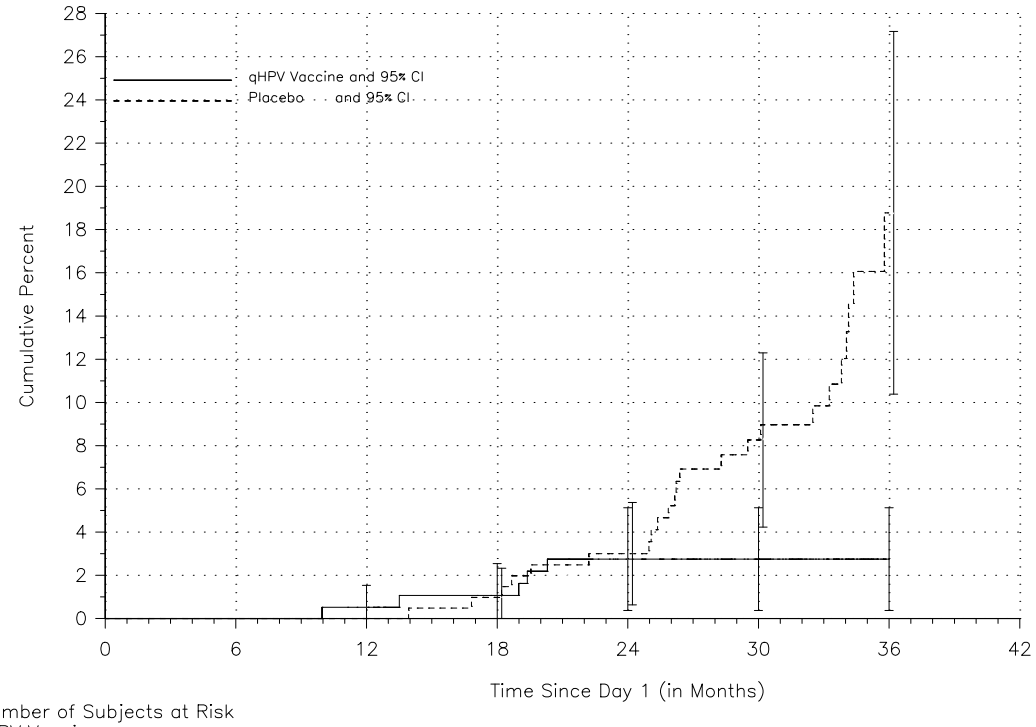
N = Number of subjects in the AIN substudy randomized to the respective vaccination group who received at least 1 injection.

n = Number of subjects in the AIN substudy who have at least one follow-up visit after Month 7.

AIN = Anal intraepithelial neoplasia; CI = Confidence interval; HPV = Human papillomavirus; HRA = High resolution anoscopy.

Figure 5

Analysis of Time to HPV 6/11/16/18-Related AIN and Anal Cancer
(AIN Per-Protocol Efficacy Population)



Number of Subjects at Risk

qHPV Vaccine	194	194	189	178	165	128	34
Placebo	208	208	206	199	178	130	29

qHPV vaccine = quadrivalent HPV vaccine (GARDASIL®)

Having achieved success on the prespecified endpoint of HPV 6/11/16/18-related AIN, efficacy against high-grade AIN (AIN 2 or worse) was evaluated. Of the 24 cases in the placebo group, 13 were identified with diagnoses of AIN 2 or worse. In the vaccine group, there were 3 subjects identified with diagnoses of AIN 2 or worse out of the total of 5 cases. Thus, the vaccine efficacy estimate for HPV 6/11/16/18-related high-grade AIN was 74.9% (95% CI: 8.8, 95.4), which indicates that the vaccine efficacy for this endpoint is above 0%. Although this was not a prespecified endpoint, the consistency of this observed vaccine effect with other demonstrations of GARDASIL® efficacy against high-grade HPV-related precancers provides support for the efficacy of GARDASIL® against high-grade AIN, the obligate precursor of HPV-related anal cancer.

In order to further evaluate consistency of the effect, vaccine efficacy against high-grade AIN related to HPV types 16 and 18 was assessed. One (1) case was observed in the vaccine group and 8 cases were observed in the placebo group, for an efficacy estimate of 86.6% (95% CI: 0.03, 99.7). There was no *a priori* hypothesis test of efficacy against high-grade AIN related to types 16 and 18; however the observed efficacy against 6/11/16/18-related AIN and this extension of the effect seen in prior demonstrations of GARDASIL® efficacy against disease related to these types provide further support for vaccine efficacy against HPV 16/18-related high-grade anal disease and therefore against anal cancer caused by these important types.

Analysis of the Per-Protocol Vaccine Group AIN Cases

Throughout the GARDASIL® program, a consistent approach has been taken to the attribution of incident lesions to a causal HPV type. As described above, an endpoint of AIN related to HPV 6, 11, 16, or 18 is defined to occur if that HPV type is identified in an adjacent section from the same tissue block in which the lesion is diagnosed. Thus, a given lesion is attributed to the vaccine type, regardless of the presence of other high-risk types, even if infection with those types was present at baseline or preceded development of the lesion in the absence of any evidence of preceding infection with the vaccine type. Other less conservative approaches could have been taken, but this choice was made so as to avoid potential over-estimation of vaccine efficacy.

With this in mind, and in an effort to better understand the potential involvement of non-vaccine types in the development of the five AIN cases that occurred in the per-protocol vaccine group subjects, these cases were reviewed with regard to the vaccine and non-vaccine HPV types identified in anogenital swabs and biopsy specimens before and subsequent to the diagnoses for which the subjects became cases. For each of the five subjects who became cases, the patterns of HPV detection suggest possible alternate explanations of the causal HPV type. In each case, there was either more than one high-risk HPV type detected in the AIN lesion, or the subject was infected at baseline with an HPV type different from the type detected in the lesion. Although co-infection was also observed in the placebo group cases, non-vaccine HPV types were rarely detected in the lesional tissue, and preceding persistent infection with the vaccine HPV type was more consistently seen, supporting the causal role of the vaccine HPV type in those cases.

To summarize the vaccine group cases briefly (cases are also summarized in [Appendix 8]), one subject who became a case of type 6-related AIN 1, AIN 2, and AIN 3 was infected at baseline and for most of the study with HPV 16, but the three AIN lesions were positive only for HPV 6, and thus the subject was counted as having type 6-related disease (Case 1 in Appendix). Another vaccine group case of AIN 3 was counted as a type 16-related case; however, this subject was infected at baseline with types 6, 18, 45, 39, and 59, and had developed HPV 39-positive AIN 2 prior to becoming a case of AIN 3. Type 39 was also detected in the type 16-related biopsy for which the subject became a case (Case 2). Another subject had HPV 6-related AIN 2, and was also

positive for HPV 45, which was detected in the lesion as well as in subsequent anal swabs for the duration of the study (Case 3). A subject with type 6-related AIN 1 was infected at baseline with high-risk HPV types 45 and 51, as well as type 56, which was also detected in the AIN 1 lesion for which the subject became a case (Case 4). Another case of type 16-related AIN 1 was also positive for type 45 in the lesion, as well in an adjacent biopsy for which pathology was read as negative (Case 5). It is notable that in some of these cases, attribution to a vaccine HPV type was based on a single timepoint of detection of the given type, without evidence of persistent infection (as was seen with the non-vaccine types identified in the case lesions). Despite these findings, these subjects were counted as cases in the group that received GARDASIL®, according to the prespecified case definition.

In addition to the patterns of HPV infection just described, the timing of disease occurrence in the vaccine as compared to the placebo group also suggests possible undetected prevalent disease in the vaccine cases. In contrast to the vaccine group, in which some cases occurred relatively early during post-vaccination follow-up, all of the cases in the placebo group occurred after Month 12, strongly suggesting that the placebo cases were related to new HPV infections that occurred following completion of the vaccination regimen. This finding is also supported by the observation that the median time to the development of AIN in the five per-protocol population vaccine recipients was 1.00 years, compared to 1.82 years in the 24 placebo group subjects.

9.2.2 Efficacy Against Persistent Anal HPV Infection

It is accepted that HPV-related premalignant and malignant disease do not develop in the absence of preceding infection. Demonstration of type-specific vaccine efficacy against persistent infection in the relevant disease site therefore provides important supportive evidence that complements and strengthens disease efficacy findings. To support the efficacy analysis of AIN and anal cancer in the PPE population, an analysis was performed to evaluate vaccine efficacy against HPV 6/11/16/18-related persistent anal infection using only the anal specimens from subjects in the PPE population. Table 8 shows the results of the analysis of efficacy against HPV 6/11/16/18-related persistent anal infection for the PPE population. Vaccine efficacy for this population was 94.9% (95% CI: 80.4, 99.4). Notably, type-specific efficacy estimates against high-risk HPV type 16 and 18 infection were each over 90% and reached statistical significance. When considered in the context of the greater than 90% observed type-specific efficacy against persistent anal infection related to HPV 16 and 18, the strong trend in the disease endpoint analyses provides compelling support for the efficacy of GARDASIL® against HPV 16/18-related high-grade AIN.

Table 8

Analysis of Efficacy Against HPV 6/11/16/18-Related Anal Persistent Infection by HPV Type
(AIN Per-Protocol Efficacy Population)

	GARDASIL® (N=299)				Placebo (N=299)					
Endpoint	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number Of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	95% CI
HPV 6/11/16/18-Related Intra-Anal Persistent Infection	193	2	385.6	0.5	208	39	381.2	10.2	94.9	(80.4, 99.4)
By HPV Type										
HPV 6-Related Intra-Anal Persistent Infection	140	1	277.9	0.4	144	13	286.8	4.5	92.1	(47.2, 99.8)
HPV 11-Related Intra-Anal Persistent Infection	140	0	279.4	0.0	144	5	295.6	1.7	100	(-15.5, 100)
HPV 16-Related Intra-Anal Persistent Infection	166	1	331.5	0.3	170	16	329.9	4.9	93.8	(60.0, 99.9)
HPV 18-Related Intra-Anal Persistent Infection	172	0	346.3	0.0	193	10	376.2	2.7	100	(51.5, 100)

N = Number of subjects randomized to the respective vaccination group who received at least 1 injection.
n = Number of subjects who have at least one follow-up visit after Month 7.
CI = Confidence interval; HPV = Human papillomavirus.

9.2.3 Supportive Efficacy Analyses

Analysis of the FAS population showed reductions in AIN due to HPV 6/11/16/18, supporting the efficacy findings in the PPE population [Table 9]. As expected, given the inclusion of subjects irrespective of baseline HPV status, vaccine efficacy was lower in the FAS population than in the PPE population. It is notable, however, that even in this population, which included subjects who were baseline HPV positive for vaccine HPV types, the lower bound of the 95% confidence interval for vaccine efficacy against HPV 6/11/6/18-related AIN 2 or worse was above 0%. The associated time to event curve [Figure 6] in this population shows similar rates of disease in the vaccine and placebo groups early in the study, reflecting the dominance of cases caused by prevalent infection. After 12 months, the curves separate, showing prevention of incident cases within the vaccine group. This provides further support for the effect of HPV vaccination in this non HPV-naïve population.

Table 9

Analysis of Efficacy Against HPV 6/11/16/18-Related AIN and Anal Cancer by HPV Type and Lesion Type
(AIN Full Analysis Set Population)

	GARDASIL® (N=299)				Placebo (N=299)					
Endpoint	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	95% CI
HPV 6/11/16/18-Related AIN and Anal Cancer	275	38	607.1	6.3	276	77	611.9	12.6	50.3	(25.7, 67.2)
By HPV Type										
HPV 6-Related AIN and Anal Cancer	275	18	644.8	2.8	276	47	645.3	7.3	61.7	(32.8, 79.1)
HPV 11-Related AIN and Anal Cancer	275	13	651.2	2.0	276	25	660.5	3.8	47.3	(-7.1, 75.2)
HPV 16-Related AIN and Anal Cancer	275	8	668.7	1.2	276	18	678.6	2.7	54.9	(-9.0, 83.0)
HPV 18-Related AIN and Anal Cancer	275	5	671.9	0.7	276	11	684.5	1.6	53.7	(-44.6, 87.4)

Analysis of Efficacy Against HPV 6/11/16/18-Related AIN and Anal Cancer by HPV Type and Lesion Type (AIN Full Analysis Set Population) (Cont.)

	GARDASIL® (N=299)				Placebo (N=299)					
Endpoint	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	95% CI
By Lesion Type										
AIN 1	275	31	619.3	5.0	276	62	624.1	9.9	49.6	(21.2, 68.4)
Condyloma Acuminatum	275	13	651 3	2.0	276	31	664.2	4.7	57.2	(15.9, 79.5)
Non-acuminate	275	27	636.0	4.2	276	48	641.3	7.5	43.3	(7.3, 66.0)
AIN 2 or worse	275	18	660.1	2.7	276	39	655.2	6.0	54.2	(18.0, 75.3)
AIN 2	275	11	668.0	1.6	276	29	671.5	4.3	61.9	(21.4, 82.8)
AIN 3	275	10	665.9	1.5	276	19	672.8	2.8	46.8	(-20.2, 77.9)
Anal Cancer	275	0	678.4	0.0	276	0	694.8	0.0	NA	NA

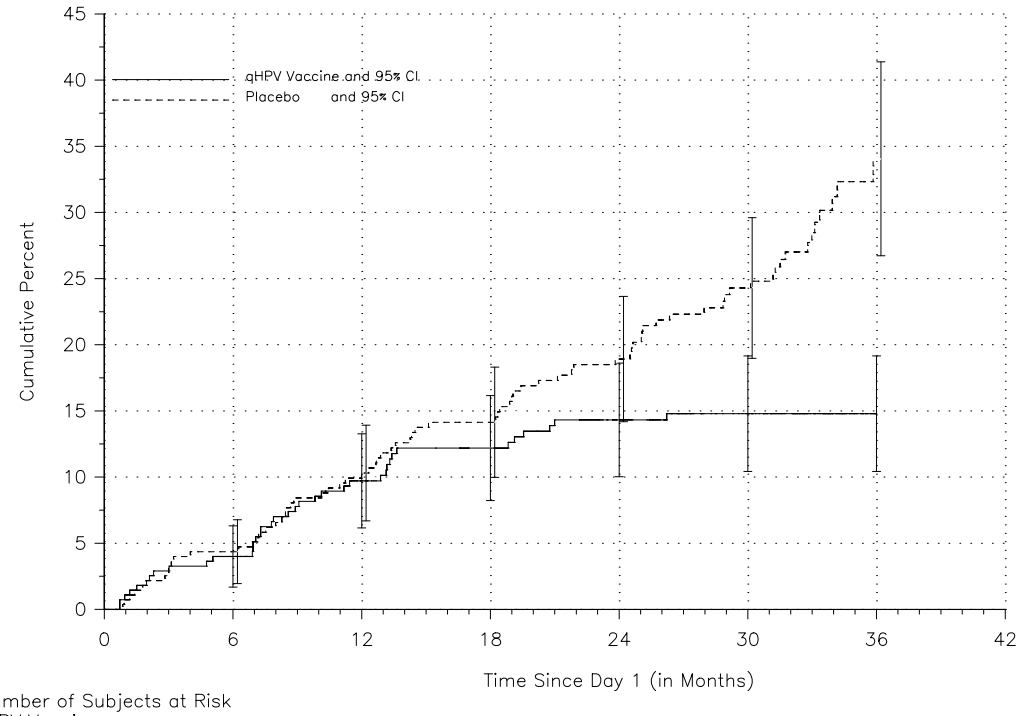
N = Number of subjects in the AIN substudy randomized to the respective vaccination group who received at least 1 injection.

n = Number of subjects in the AIN substudy who have at least one follow-up visit after Day 1.

AIN = Anal intraepithelial neoplasia; CI = Confidence interval; HPV = Human papillomavirus; HRA = High resolution anoscopy.

Figure 6

Analysis of Time to HPV 6/11/16/18-Related AIN and Anal Cancer
(AIN Full Analysis Set Population)



qHPV vaccine = quadrivalent HPV vaccine (GARDASIL®)

9.2.4 Population Benefit Analyses

The estimate of GARDASIL® efficacy against AIN and anal cancer due to any HPV type in the GHN population was 54.9% (95% CI: 8.4, 79.1), supporting the potential of HPV vaccination to lower the overall burden of HPV-related anal disease in the population. As stated earlier, with cases being counted after the first dose of vaccine and the contribution of AIN caused by types not contained in the vaccine, efficacy for this population is anticipated to be lower than for the per-protocol population. Not unexpectedly, efficacy against this endpoint in the FAS analysis did not reach statistical significance (data not shown), given the contribution of disease from prevalent vaccine type and non-vaccine type infections in this population.

9.2.5 AIN Efficacy Conclusions

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16 to 26 year old men is efficacious in preventing the development of HPV 6/11/16/18-related high-grade AIN (AIN 2 or worse).**

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16 to 26 year old men is efficacious in preventing development of HPV 6/11/16/18-related AIN of any grade.**

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16 to 26 year old men is efficacious in preventing development of 6/11/16/18 persistent anal infection.**

10. Protocol 020 End of Study Results-Overall Study

This section provides a brief update of the overall Protocol 020 study findings. These end of study efficacy, safety, and immunogenicity results were all consistent with those obtained and presented at the time of the primary analysis of Protocol 020.

10.1 Efficacy

10.1.1 Overall End of Study Efficacy Results

Table 10 shows the end of study results of analyses of efficacy performed in the PPE population to address the primary (EGL) Protocol 020 efficacy hypothesis. Vaccine efficacy against HPV 6/11/16/18-related EGL was 90.6% (95% CI: 70.1, 98.2). Subsequent to the previous analysis, there was a single additional case in the placebo group; this was an MSM placebo recipient with a diagnosis of HPV 16-related PIN 2/3. With 4 cases of 6/11/16/18-related PIN in the placebo group and no cases in the vaccine group, the final study data are also favorable, although limited, regarding efficacy of GARDASIL® against HPV-related penile malignancy. Updated efficacy against HPV 6/11/16/18-related persistent infection was 85.5% (95% CI: 77.0, 91.3). The supportive analysis in the FAS population (data not shown) was consistent with the primary analysis in the PPE population and also supports the previous conclusion that GARDASIL® is efficacious against HPV 6/11/16/18-related EGL in males.

Table 10

Analysis of Efficacy Against HPV 6/11/16/18-Related EGL by Sexual Orientation, HPV Type, and Lesion Type
(Per-Protocol Efficacy Population)

	GARDASIL® (N=2,025)				Placebo (N=2,030)					
Endpoint	n	Number Of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	95% CI
HPV 6/11/16/18-Related EGL	1,394	3	3,109.2	0.1	1,404	32	3,106.0	1.0	90.6	(70.1, 98.2)
By Sexual Orientation										
HM Subjects	1,200	2	2,722.4	0.1	1,196	26	2,689.7	1.0	92.4	(69.6, 99.1)
MSM Subjects	194	1	386.9	0.3	208	6	416.3	1.4	82.1	(-47.8, 99.6)
By HPV Type										
HPV 6-Related EGL	1,242	3	2,779.8	0.1	1,243	19	2,790.3	0.7	84.2	(46.2, 97.0)
HPV 11-Related EGL	1,242	1	2,781 2	0.0	1,243	11	2,790.7	0.4	90.9	(37.2, 99.8)
HPV 16-Related EGL	1,292	0	2,883 5	0.0	1,270	3	2,841.1	0.1	100	(-138.4, 100)
HPV 18-Related EGL	1,331	0	2,978.0	0.0	1,352	1	3,013.4	0.0	100	(-3846.4, 100)

Analysis of Efficacy Against HPV 6/11/16/18-Related EGL by Sexual Orientation, HPV Type, and Lesion Type (Per-Protocol Efficacy Population) (Cont.)

	GARDASIL® (N=2,025)				Placebo (N=2,030)					
Endpoint	n	Number Of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	n	Number of Cases	Person-Years at Risk	Incidence Rate per 100 Person-Years at Risk	Observed Efficacy (%)	95% CI
By Lesion Type										
Condyloma	1,394	3	3,109.2	0.1	1,404	28	3,108.0	0.9	89.3	(65.3, 97.9)
PIN 1 or worse	1,394	0	3,112.2	0.0	1,404	4	3,124.9	0.1	100	(-52.1, 100)
PIN 1	1,394	0	3,112.2	0.0	1,404	2	3,126.6	0.1	100	(-434.9, 100)
PIN 2/3 or Cancer	1,394	0	3,112.2	0.0	1,404	2	3,125.1	0.1	100	(-434.7, 100)
PIN 2/3	1,394	0	3,112.2	0.0	1,404	2	3,125.1	0.1	100	(-434.7, 100)
Penile/Perianal/Perineal Cancer	1,394	0	3,112.2	0.0	1,404	0	3,126.8	0.0	NA	NA

N = Number of subjects randomized to the respective vaccination group who received at least 1 injection.

n = Number of subjects who have at least one follow-up visit after Month 7.

CI = Confidence interval; EGL = External genital lesions with a diagnosis of Condyloma, PIN, or Penile/Perianal/Perineal Cancer; HM = Heterosexual men; HPV = Human papillomavirus; MSM = Men having sex with men; PIN = Penile/Perianal/Perineal intraepithelial neoplasia.

10.1.2 Overall Study Efficacy Conclusions

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16 to 26 year old men is efficacious in preventing development of HPV 6/11/16/18-related external genital lesions.**

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16 to 26 year old men is efficacious in preventing development of HPV 6/11/16/18 persistent infection.**

10.2 Immunogenicity

10.2.1 Immunogenicity Results

Immunogenicity results at Months 7 and 24 were presented in the detail in the previous submission. End of study data show that at Month 36, Geometric Mean Titers (GMTs) in vaccinated subjects were lower than at Month 7 for all vaccine HPV types [Table 11]. Similar to observations in the female studies, among all four vaccine HPV types, anti-HPV 18 antibodies show the highest rate of decline. Month 36 anti-HPV 18 GMTs were comparable to the estimated antibody level induced by natural infection; however, seroconversion percentages at Month 36 were comparable to the Month 24 rates for each of the vaccine types [Table 12]. No HPV 18-related AIN or EGL were observed among vaccinees in the PPE population of Protocol 020, supporting durability of efficacy despite nominal seronegativity. Overall, the final immunogenicity findings from Protocol 020 are similar to the experience in the female GARDASIL® efficacy studies.

Table 11

Summary of Anti-HPV Geometric Mean Titers by Vaccination Group
(Per-Protocol Immunogenicity Population)

Assay (cLIA) Study time	GARDASIL® (N=2,025) n	GMT (mMU/mL)	95% CI	Placebo (N=2,030) n	GMT (mMU/mL)	95% CI
Anti-HPV 6						
Day 1	1,092	< 7	(<7, <7)	1,108	< 7	(<7, <7)
Month 7	1,092	447.6	(422.6, 474.1)	1,108	< 7	(<7, <7)
Month 24	941	79.8	(75.8, 84.1)	949	< 7	(<7, <7)
Month 36	847	71.5	(67.5, 75.8)	834	< 7	(<7, <7)
Anti-HPV 11						
Day 1	1,092	< 8	(<8, <8)	1,107	< 8	(<8, <8)
Month 7	1,092	624.0	(594.1, 655.4)	1,107	< 8	(<8, <8)
Month 24	941	94.6	(90.0, 99.5)	948	< 8	(<8, <8)
Month 36	847	82.6	(78.3, 87.1)	833	< 8	(<8, <8)
Anti-HPV 16						
Day 1	1,135	< 11	(<11, <11)	1,127	< 11	(<11, <11)
Month 7	1,135	2,404.3	(2,272.2, 2,544.0)	1,127	< 11	(<11, <11)
Month 24	979	342.7	(324.7, 361.7)	951	< 11	(<11, <11)
Month 36	877	293.3	(276.5, 311.2)	839	< 11	(<11, <11)
Anti-HPV 18						
Day 1	1,174	< 10	(<10, <10)	1,202	< 10	(<10, <10)
Month 7	1,174	402.3	(380.2, 425.7)	1,202	< 10	(<10, <10)
Month 24	1,011	38.4	(36.0, 41.0)	1,010	< 10	(<10, <10)
Month 36	905	33.1	(30.9, 35.4)	882	< 10	(<10, <10)

The estimated GMTs and associated CIs are calculated using an ANOVA model with a term for vaccination group.

N = Number of subjects randomized to the respective vaccination group who received at least 1 injection.

n = Number of subjects contributing to the analysis.

ANOVA = Analysis of variance; CI = Confidence interval; cLIA = Competitive Luminex immunoassay; GMT = Geometric mean titer; HPV = Human papillomavirus; mMU = Milli Merck units; PCR = Polymerase chain reaction.

Table 12

Summary of Anti-HPV Percent Seroconversion by Vaccination Group
(Per-Protocol Immunogenicity Population)

Anti-HPV Response Study Time	GARDASIL® (N=2,025)				Placebo (N=2,030)			
		Seroconversion				Seroconversion		
	n	m	Percent	95% CI	n	m	Percent	95% CI
HPV 6 cLIA ≥ 20 mMU/mL								
Day 1	1,092	0	0.0	(0.0%, 0.3%)	1,108	0	0.0	(0.0%, 0.3%)
Month 7	1,092	1,080	98.9	(98.1%, 99.4%)	1,108	18	1.6	(1.0%, 2.6%)
Month 24	941	855	90.9	(88.8%, 92.6%)	949	20	2.1	(1 3%, 3.2%)
Month 36	847	753	88.9	(86.6%, 90 9%)	834	26	3.1	(2.0%, 4.5%)
HPV 11 cLIA ≥ 16 mMU/mL								
Day 1	1,092	0	0.0	(0.0%, 0.3%)	1,107	0	0.0	(0.0%, 0.3%)
Month 7	1,092	1,083	99.2	(98.4%, 99.6%)	1,107	23	2.1	(1 3%, 3.1%)
Month 24	941	900	95.6	(94.1%, 96 9%)	948	13	1.4	(0.7%, 2.3%)
Month 36	847	796	94.0	(92.2%, 95 5%)	833	19	2.3	(1.4%, 3.5%)
HPV 16 cLIA ≥ 20 mMU/mL								
Day 1	1,135	0	0.0	(0.0%, 0.3%)	1,127	0	0.0	(0.0%, 0.3%)
Month 7	1,135	1,121	98.8	(97.9%, 99 3%)	1,127	20	1.8	(1 1%, 2.7%)
Month 24	979	970	99.1	(98.3%, 99.6%)	951	7	0.7	(0 3%, 1.5%)
Month 36	877	859	97.9	(96.8%, 98.8%)	839	18	2.1	(1 3%, 3.4%)

Summary of Anti-HPV Percent Seroconversion by Vaccination Group (Per-Protocol Immunogenicity Population) (Cont.)

Anti-HPV Response / Study Time	GARDASIL® (N=2,025)				Placebo (N=2,030)			
		Seroconversion				Seroconversion		
	n	m	Percent	95% CI	n	m	Percent	95% CI
HPV 18 cLIA ≥ 24 mMU/mL								
Day 1	1,174	0	0.0	(0.0%, 0.3%)	1,202	0	0.0	(0.0%, 0.3%)
Month 7	1,174	1,143	97.4	(96.3%, 98 2%)	1,202	21	1.7	(1 1%, 2.7%)
Month 24	1,011	630	62.3	(59.2%, 65.3%)	1,010	12	1.2	(0.6%, 2.1%)
Month 36	905	516	57.0	(53.7%, 60 3%)	882	9	1.0	(0 5%, 1.9%)

Percent is calculated as 100*(m/n).

The CIs are computed based on exact methods.

N = Number of subjects randomized to the respective vaccination group who received at least 1 injection.

n = Number of subjects contributing to the analysis.

m = Number of subjects with the indicated response.

CI = Confidence interval; cLIA = Competitive Luminex immunoassay; HPV = Human papillomavirus; mMU = Milli Merck units.

10.2.2 Immunogenicity Conclusion

- **Prophylactic administration of a 3-dose regimen of GARDASIL® to 16- to 26- year-old men generates robust anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 responses that result in a high level of protective efficacy through approximately 36 months of study follow-up.**

10.3 Safety

At the time of the primary EGL analysis for Protocol 020, all study subjects were well beyond the vaccination phase of the study. The final safety data are nearly identical to those in the EGL sBLA, with the exception of new medical history data, which contains data that extend beyond the vaccination phase. This section therefore provides a brief update on the end of study safety data.

The approach to evaluation of vaccine safety was presented previously, and will not be reviewed in detail here. Briefly, subjects were observed for 30 minutes after each vaccination. All subjects were followed up for adverse experiences (AEs) using a vaccination report card (VRC)-aided surveillance for 14 days after each injection of study vaccine. Study subjects recorded temperature values and injection-site adverse experiences for 5 days (Days 1 through 5 post-vaccination), and systemic adverse experiences and any other medications administered for 15 days (Days 1 through 15 post-vaccination). The investigator determined seriousness, action taken, and relationship to study vaccine for any VRC-recorded adverse experience. New medical history data were collected throughout the study.

10.3.1 Summary of End of Study Safety Data

The following observations can be made from clinical adverse experiences reported by subjects at any time during the study. The proportions of subjects who reported at least one clinical adverse experience and who reported at least one injection-site adverse experience were slightly higher in the GARDASIL® group than in the placebo group. The proportion of subjects who reported at least one systemic adverse experience was generally comparable between the vaccine and placebo groups. Few subjects discontinued the study due to an adverse experience, and the proportion of subjects who discontinued due to an AE was slightly higher in the placebo group than in the GARDASIL® group.

A total of 19 subjects (8 in the GARDASIL® group and 11 in the placebo group) reported serious adverse experiences over the entire duration of the study; none were vaccine-related. A total of 13 subjects died during the study (3 in the GARDASIL® group and 10 in the placebo group); none of the deaths were considered to be vaccine related. Overall, the proportions of subjects who reported new medical conditions, including conditions potentially indicative of an autoimmune phenomenon, were comparable between vaccination groups. The overall clinical adverse experience study is summarized in Table 13.

Table 13

Clinical Adverse Experience Summary
(Days 1 to 9999 Following Any Vaccination Visit) (All Vaccinated Subjects)

	GARDASIL® (N=2020)		Placebo (N=2029)	
	n	(%)	n	(%)
Subjects in analysis population	2020		2029	
Subjects without follow-up	75		79	
Subjects with follow-up	1945		1950	
Number (%) of subjects:				
with no adverse experience	599	(30.8)	698	(35.8)
with one or more adverse experiences	1346	(69.2)	1252	(64.2)
injection-site adverse experiences	1169	(60.1)	1047	(53.7)
systemic adverse experiences	616	(31.7)	622	(31.9)
with vaccine-related[†] adverse experiences	1242	(63.9)	1134	(58.2)
injection-site adverse experiences	1169	(60.1)	1046	(53.6)
systemic adverse experiences	274	(14.1)	284	(14.6)
with serious adverse experiences	8	(0.4)	11	(0.6)
with serious vaccine-related adverse experiences	0	(0.0)	0	(0.0)
who died	3	(0.2)	10	(0.5)

Clinical Adverse Experience Summary
(Days 1 to 9999 Following Any Vaccination Visit) (All Vaccinated Subjects) (Cont.)

	GARDASIL® (N=2020)		Placebo (N=2029)	
	n	(%)	n	(%)
discontinued [‡] due to an adverse experience	5	(0.3)	14	(0.7)
discontinued due to a vaccine-related adverse experience	2	(0.1)	3	(0.2)
discontinued due to a serious adverse experience	3	(0.2)	10	(0.5)
discontinued due to a serious vaccine-related adverse experience	0	(0.0)	0	(0.0)

[†] Determined by the investigator to be possibly, probably, or definitely related to the vaccine.
[‡] Discontinued = Subject discontinued from therapy.
Percentages are calculated based on the number of subjects with follow-up.

10.3.2 Safety Conclusion

- **Prophylactic administration of a 3-dose regimen of GARDASIL® is generally well tolerated in 16- to 26-year-old men.**

11. Summary of Postlicensure Surveillance and Studies

Merck has continued to monitor the safety profile of GARDASIL® since its original licensure, including conducting postmarketing safety surveillance and a postlicensure safety study. In addition, the United States Centers for Disease Control and Prevention (CDC) conducts surveillance for safety of vaccines, including GARDASIL®.

11.1 Updated Postlicensure Safety Surveillance

From the International Birthdate (i.e., the date of first regulatory approval worldwide, 1-June-2006) through 31-May-2010, over 65 million doses of GARDASIL® were distributed worldwide; there were no countries where marketing applications have been rejected, withdrawn, suspended, or revoked for safety reasons. The eighth 6 month Periodic Safety Update Report (PSUR) covering the time period of 01-Dec-2008 thru 31-May-2010 contains a list of 131 countries where GARDASIL® has received marketing approval up to 31-May-2010.

To permit safety surveillance for its products, Merck & Co., Inc. maintains the New Worldwide Adverse Experience System (NWAES) database. Postmarketing safety surveillance is a worldwide, passive, spontaneous, and voluntary reporting system. At Merck & Co. Inc., the NWAES database contains all spontaneous adverse experience reports from the marketed environment, serious reports from clinical trials, and reports from the medical literature. This is a dynamic database, and adverse experience information is updated continuously. The retrieval of data is provided as a snapshot in time. The data are compiled and reviewed on a periodic basis and reported in a PSUR.

All of the reports are entered into NWAES and are coded using the terminology of the reporter. The Medical Dictionary for Regulatory Activities (MedDRA) is the dictionary used to code adverse experience terms in the NWAES database. Inclusion of the report in the database implies only a temporal association and not necessarily a causal association. Each report represents one individual who may experience one or more adverse experiences. Since each adverse experience is coded to a body system, one report may contain multiple adverse experiences in the same or different body systems.

Routine Pharmacovigilance practices include continuous monitoring of the safety profile of approved products. Data from the NWAES database are routinely reviewed as individual reports and in aggregate. The purpose of the review is to evaluate adverse experience reports for possible safety signals, to determine if further investigation is warranted to clarify the safety profile of the product, and to ensure completeness of safety information in worldwide package circulars. This approach is in line with

generally accepted pharmacovigilance approaches including the European Union requirements.

A query of the NWAES database performed on 14-Jun-2010 revealed that 37,884 spontaneous reports have been received from 1-June-2006 through 31-May-2010; 4,598 reports (12%) were considered serious. The 5 most frequently reported adverse experiences included no adverse event (8096), inappropriate schedule of drug administration (6975), drug exposure during pregnancy (3289), dizziness (3098), and syncope (3089). The events of syncope and dizziness are listed in the United States product label. The term "no adverse event" is coded when another event, such as a medication error term such as "overdose" is coded. It is important to note whether or not an adverse event was associated with these medication errors.

There have been reports of males being vaccinated with this product but there is no way to estimate the number of males who have been vaccinated. As of 14-Jun-2010, 260 spontaneous reports involving male patients have been received in the NWAES database from 1-June-2006 through 31-May-2010 (these were included in the total of 37,884); 12 reports (5%) were considered serious. The 5 most frequently reported adverse experiences which involved male patients included no adverse event (108), off label use (103), wrong drug administered (40), inappropriate schedule of drug administration (31), and accidental exposure (22). The 12 serious reports include 28 serious adverse experiences as follows: 4 events of convulsion, 2 events of Guillain Barre Syndrome, and 1 event each of 22 individual adverse experience preferred terms.

Overall, the post licensure experience with GARDASIL® collected through passive reporting of spontaneous adverse experiences to Merck & Co., Inc. has confirmed the favorable safety profile of the vaccine, with a low proportion of reported serious adverse experiences; the benefit-risk profile for the product remains favorable. Because the product has not been widely used in male patients, the data from the post marketing environment is insufficient to draw conclusions regarding the safety profile of the vaccine relative to males. To date, however, the type of adverse experiences spontaneously reported in male vaccine recipients, does not suggest a unique safety concern for that gender.

Merck & Co., Inc will continue to monitor the safety of GARDASIL® in the post-licensure period.

11.2 Post-Licensure Safety Studies

The GARDASIL® Postlicensure Surveillance Program (i.e., the "GARDASIL® Safety Study") in females was conducted at two large managed care organizations, Kaiser Permanente Northern California and Kaiser Permanente Southern California. An independent, external Safety Review Committee (SRC) reviewed the study's surveillance data for evidence of any safety signals associated with GARDASIL®. No safety signals associated with GARDASIL® vaccination were detected for pre-specified, new onset

autoimmune conditions, for death, or adverse pregnancy outcomes in a base population of 189,629 females who received ≥1 dose of GARDASIL® between August 1, 2006 and March 31, 2008. Additionally, with the exception of syncope and possibly cellulitis, no safety signals were detected for emergency room visits and hospitalizations. The SRC noted that some cellulitis cases may have been injection site reactions rather than acute infection. However, limited information in available medical charts impeded further assessment of these cases. In summary, the results from the GARDASIL® Safety Study in females indicate a favorable safety profile consistent with what has been reported from other sources. Interim and final reports have been issued for this study.

The GARDASIL® Postlicensure Safety Study in males is similar to the safety study in females, described above. All males vaccinated with GARDASIL® at this managed care organization as of October 16, 2009 (the date of FDA licensure for the prevention of genital warts in males) will be included in this study, up to 135,000 males with at least one dose, 44,000 males with 3 doses, or all males vaccinated over a 6-year period (whichever occurs first). An independent, external SRC will review the study's surveillance data for evidence of any safety signals associated with GARDASIL® in males. Interim reports and a final report of safety in males will be issued.

11.3 Postlicensure Study of Anal Disease

Merck is conducting an extension of Protocol 020 to evaluate the longterm safety and effectiveness of GARDASIL® in men. All eligible subjects from Protocol 020 will be invited to participate in the longterm extension to assess safety and immunogenicity, and to monitor for development of EGLs. As part of this extension, all continuing MSM subjects will also undergo yearly anal cytology and biennial high resolution anoscopy to monitor for anal disease development. The first subject was enrolled into the extension on August 10, 2010.

11.4 Overall Safety Conclusion

- **Clinical trial safety data, passive postlicensure safety surveillance and active, controlled evaluations of selected conditions of clinical interest continue to support the overall positive safety profile of GARDASIL®.**

12. Overall Summary and Conclusions

There is a substantial and increasing burden of anal cancer in men and women, almost all of which originates from anal HPV infection, which is common in both genders. HPV is one of the most common sexually transmitted infections, and it is well-documented that anal intercourse is not required for anal HPV acquisition. Thus, all sexually active individuals, regardless of gender, are potentially at risk for anal HPV infection and subsequent disease. The HPV types contained in GARDASIL® are together responsible for the vast majority of anal cancers. A vaccine such as GARDASIL® that has been

demonstrated to prevent the infections responsible for anal cancer thus has the potential for a significant impact on anal cancer incidence in both genders.

There is no proven method for prevention of anal HPV infection. High-grade AIN is a precursor for anal cancer, and although public health authorities recognize anal cancer as an important entity for which screening should be considered, there is currently no standardized or prospectively studied screening method for anal disease. Results from the AIN Substudy, in which a substantial number of the observed AIN cases were diagnosed at the mandatory end-of-study anoscopy, underscore these potential limitations of cytologic screening for anal premalignant and malignant disease in a generally healthy population. Additionally, treatment of high-grade AIN, while currently performed in high-risk populations, has not been prospectively proven to eliminate anal cancer development. For those who progress from high-grade AIN to develop anal cancer, treatment is associated with significant morbidity and longterm sequelae. Risk factors for anal cancer include a history of HPV-related genital tract disease, including genital warts. Given the limitations of anal screening and the high incidence of genital HPV-related disease in both males and females, a broad strategy of vaccinating sexually naïve males and females to prevent anal HPV infection prior to exposure is more likely to be an effective approach to anal cancer prevention.

The new efficacy data on anal cancer prevention support and further extend the potential benefit of GARDASIL® to address the important unmet medical need for the prevention of HPV-related disease in both men and women. Protocol 020 has demonstrated that, in addition to high efficacy in males against external genital lesions, GARDASIL® is efficacious against HPV 6/11/16/18-related AIN, and has statistically significant efficacy against high-grade AIN, the obligate precursor of HPV-related anal cancer. Primary prevention through vaccination therefore provides the greatest potential to have a significant impact on this unmet medical need.

The Protocol 020 AIN study was performed in an MSM population due to the significantly elevated risk of anal infection and disease in this population. As presented in this document, HPV infection and disease of the anal canal are not, however, limited to MSM. Anal infection with HPV, in particular HPV type 16, is common and can lead to cancer in heterosexual men and women, as well as MSM. The predominant HPV types that cause anal cancer are the same in men and women. The pathogenesis, histopathologic, and clinical presentation of HPV-related anal disease are identical across genders and populations (women, HM, MSM). The anatomy and histology of the anal canal is identical in males and females, and there are no gender-specific characteristics of these lesions that differentiate AIN and anal cancers in men and women. AIN and anal cancer are indistinguishable between the genders. Furthermore, based on the clinical trials experience to date, there is no evidence that efficacy of GARDASIL® is gender-dependent. Demonstration of vaccine efficacy against AIN in MSM should therefore reflect efficacy in the general population.

The proposed new indication should thus be broadly applicable to all groups, and in light of the Protocol 020 AIN efficacy data, Merck proposes an expansion of the GARDASIL® label to:

- **GARDASIL® is indicated in boys and men, and girls and women 9 through 26 years of age for the prevention of AIN grades 1, 2, and 3 caused by HPV types 6, 11, 16 and 18.**

- **GARDASIL® is indicated in boys and men, and girls and women 9 through 26 years of age for the prevention of anal cancer caused by HPV types 16 and 18.**

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14. Appendices

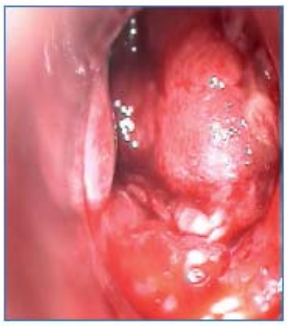
Appendix 1
Summary of Type-Specific Prevalence of HPV6, 11, 16, and 18 in Anal Cancer

Study	Geographic Location	Tumor Characteristic	Tumor Behavior	Population	HPV6 (%)	HPV11 (%)	HPV6/11 (%)	HPV16 (%)	HPV18 (%)	HPV16/18 (%)
Daling et al. (2004)	U.S. (western Washington State)	All sites and histology	Invasive	All Male	--	--	--	66.7	7.6	74.3
				Female	--	--	--	75.9	6.3	82.2
				Both	--	--	--	72.5	6.7	79.2
		All sites and histology	In Situ	All Male	--	--	--	80.6	5.6	86.2
				Female	--	--	--	67.7	8.8	76.3
				Both	--	--	--	74.3	7.1	81.4
		All sites and histology	Invasive and In Situ	All Male	--	--	--	71.6	6.9	78.5
				Female	--	--	--	74.0	6.8	80.8
				Both	--	--	--	73.0	6.9	79.9
		Seer-defined anal cancer[b]	Invasive and In Situ	All Male	--	--	--	74.2	4.8	79.0
				Female	--	--	--	76.7	6.8	83.5
				Both	--	--	--	75.8	6.1	81.9
		All sites, squamous cell carcinoma only	Invasive and In Situ	All Male	--	--	--	77.8	7.4	85.2
				Female	--	--	--	74.5	10.2	84.7
				Both	--	--	--	76.0	8.9	85.9
Frisch et al. (1997)	Denmark and Sweden	All sites and histology[c]	Invasive and In Situ	All Male	2.0	0.0	2.0	57.0	5.0	62.0
				Female	1.0	0.0	1.0	77.0	6.0	83.0
				Both	1.3	0.0	1.3	72.9	5.7	78.6
Devuyst et al. (2009)	Predominantly U.S. & Europe	"Anal cancer" as defined within studies[d]	Invasive and In situ	All Male	--	--	--	--	--	--
				Female	--	--	--	--	--	--
				Both	5.1	1.0	6.1	65.6	5.1	70.1
Hoots et al. (2009)	Predominantly U.S. & Europe	"Anal cancer" as defined within studies[d]	Invasive	All Male	0.0	8.3	8.3	60.0	7.8	67.8
				Female	3.4	3.4	7.0	73.7	7.6	81.3
				Both	2.9	0.3	3.2	73.4	5.2	78.6
WHO (2010)	U.S.	"Anal cancer" as defined within studies[d]	Not specified	All Male	--	--	--	--	--	--
				Female	--	--	--	--	--	--
				Both	--	--	2.0	61.7	10.4	72.1

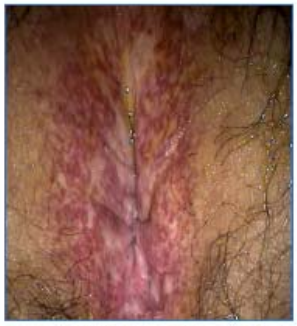
[a] All sites include: rectum, anal canal, anus, anorectum, and perianal skin
[b] Anal canal, anus, anorectum
[c] All sites include: rectum, anal canal, anus, anorectum, and perianal skin
[d] There is variability in the ways various studies define anal cancer; meta analyses and WHO reports incorporate that variability into their estimates.
WHO = World Health Organization.

Appendix 2

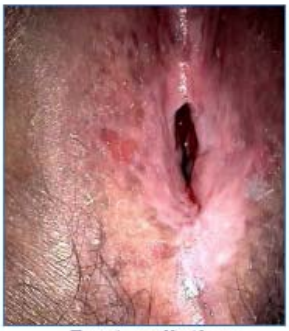
Selected Effects of Anal Radiation Therapy



Radiation proctitis



Radiation-induced skin changes



Post-radiation anal stenosis

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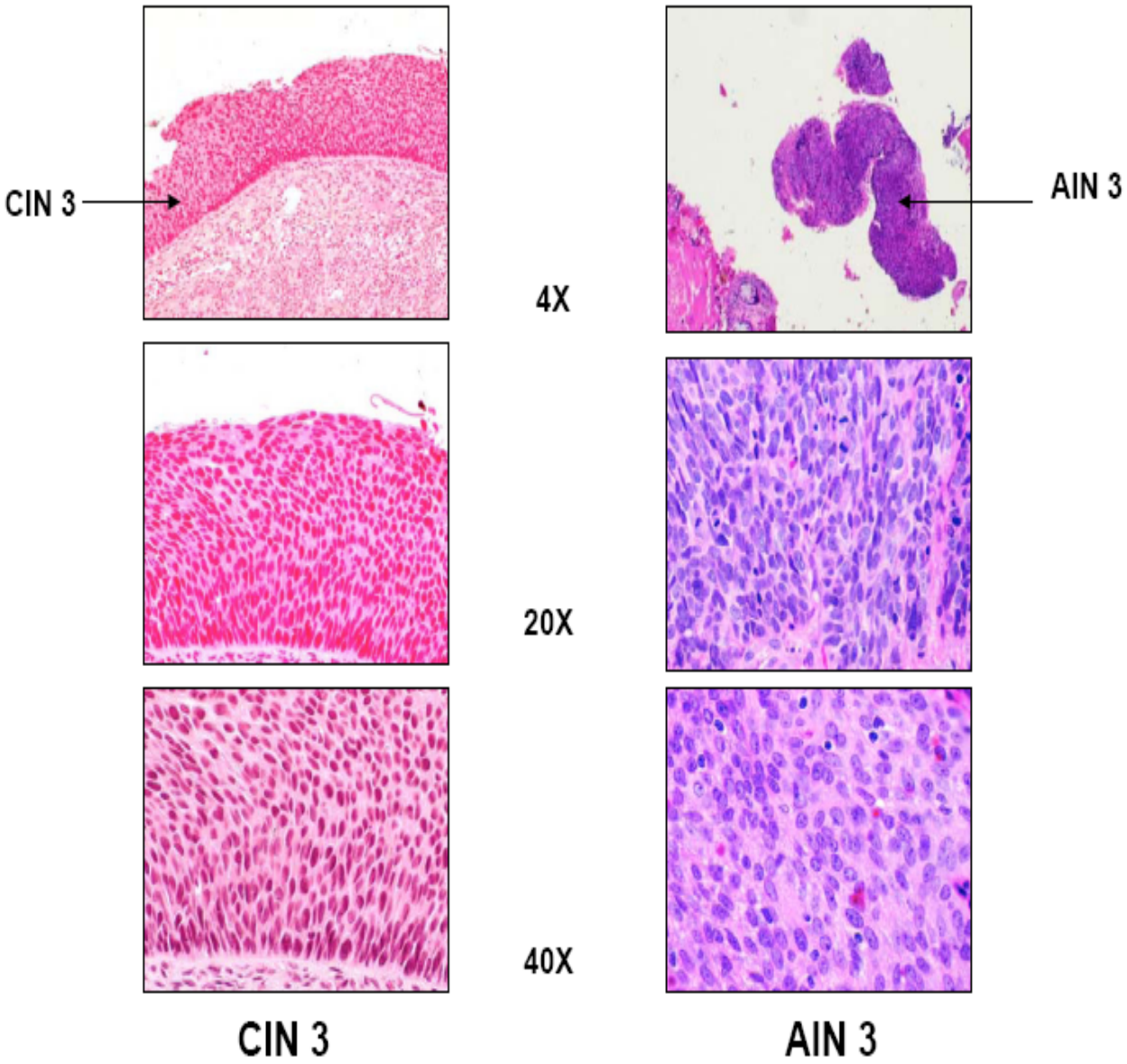
Appendix 3
Anal Cancer Risk in Patients with AIN

Study	Study Population	Baseline Diagnosis (# of subjects)	# Progressed to Invasive Anal Cancer	Annualized Incidence Rates of Invasive Anal Cancer Per 100 PY
Watson et al., 2006	• New Zealand • Patients identified from prospective database from 1996-2004 • Median follow-up time ~60 months • Some patients underwent perianal skin excision at baseline	AIN 2 (10)	2	4
		AIN 3 (45)	6	2.7
		AIN 2/3 (55)	8	2.9
Sobhani et al., 2004	• France • Consecutive patients referred to department of coloproctology from 1993-2002 • Median follow-up time ~23 months • All patients treated for condyloma and then had 3-6 month follow-up visits • 88% male; 50% HIV positive • All who progressed to cancer (6 male; 1 female) had high-grade AIN at baseline	Condyloma (with or without AIN diagnosis) HIV-negative (141)	1	0.4
		HIV-positive (58)	6	5.4
		Either HIV+ or HIV-	7	9.6
Scholefield et al., 1994	• United Kingdom • Patients with history of high-grade CIN , invasive vulvar cancer, and immunosuppressed renal allograft recipients • Median follow-up time ~20 months • All 27 patients with AIN were treated with surgical excision.	AIN 3 (27)	8	17.8
		Any AIN (32 AIN 1, 11 AIN 2, 27 AIN 3) (70)	8	6.8
Brown et al., 1999	• United Kingdom • All patients diagnosed in 1 hospital from 1989-1996 (many of whom were also included in Scholefield et al. 1994) • Mainly female, apparently high risk for AIN due to presence of other anogenital disease; unclear patient selection process • All high-grade AIN cases treated and then intensively followed up • Over 50% had incomplete excision; high rates of recurrence or residual disease	High-grade AIN (34)	0	0
Scholefield et al., 2005	• United Kingdom • Patients identified from prospective database between 1994-2003 • All patients had excision of localized lesions and then followed up every 6 months • Followed for median of ~63 months	AIN 3 Immunocompetent (29)	0	0
		Immunosuppressed (6)	3	9.5
		Immunocompetent & Immunosuppressed (35)	3	1.6
U.S. annual incidence rate, SEER 2010		General population of men and women in U.S. (vast majority do not have AIN & are HIV-negative)	---	0.00165

Appendix 4
AIN 2/3 and CIN 2/3 are Histologically Identical



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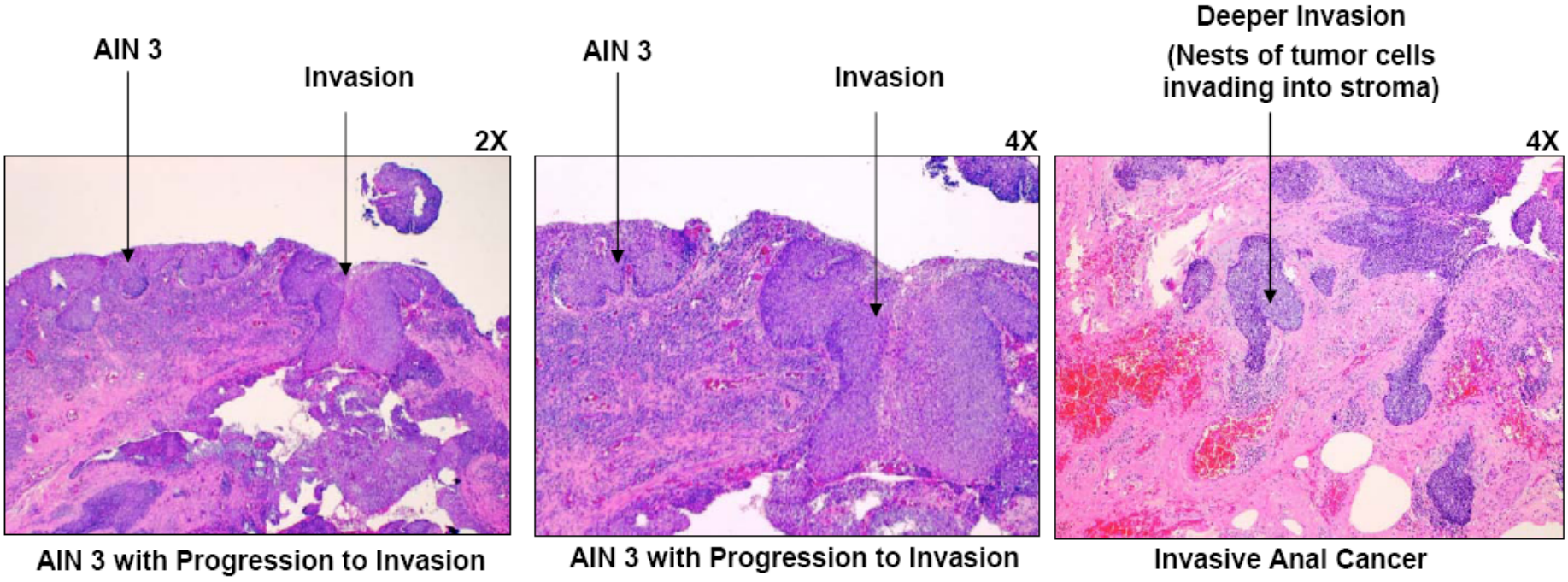
Appendix 5
High-Grade AIN Progresses to Invasive Anal Cancer



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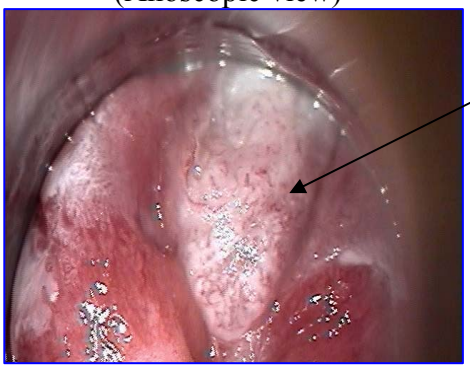
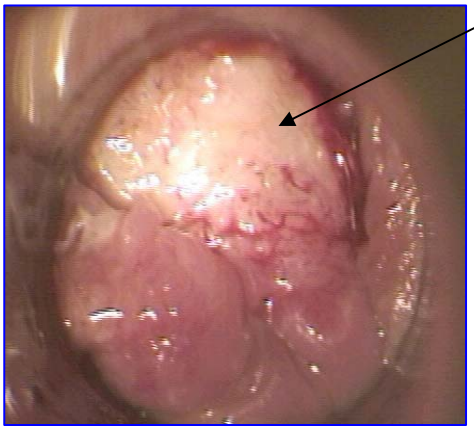
Appendix 6

Anal Cancer is the Same Disease in Men and Women

Anal Cancer in a Woman
(Anoscopic view)



Anal Cancer in a Man
(Anoscopic view)



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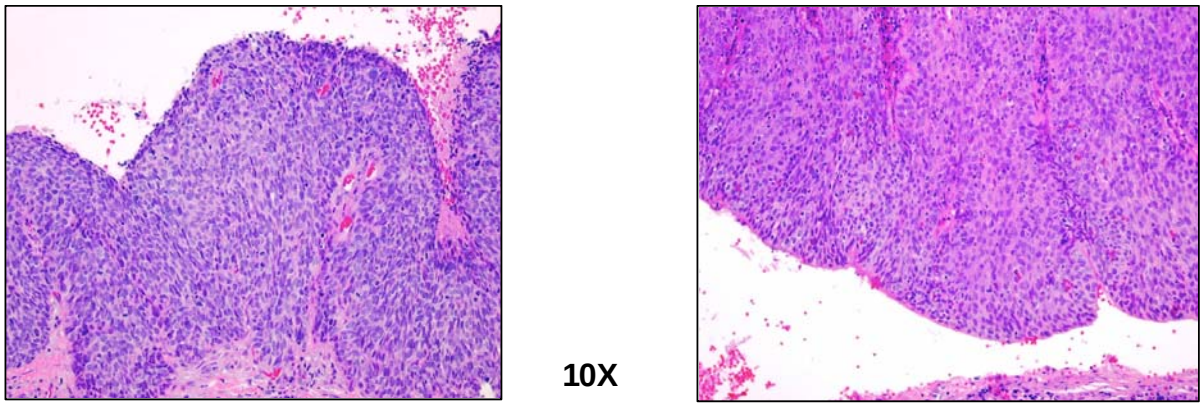
Appendix 7

Histology of AIN and Anal Cancer is the Same in Men and Women

AIN 3 in a Man **AIN 3 in a Woman**



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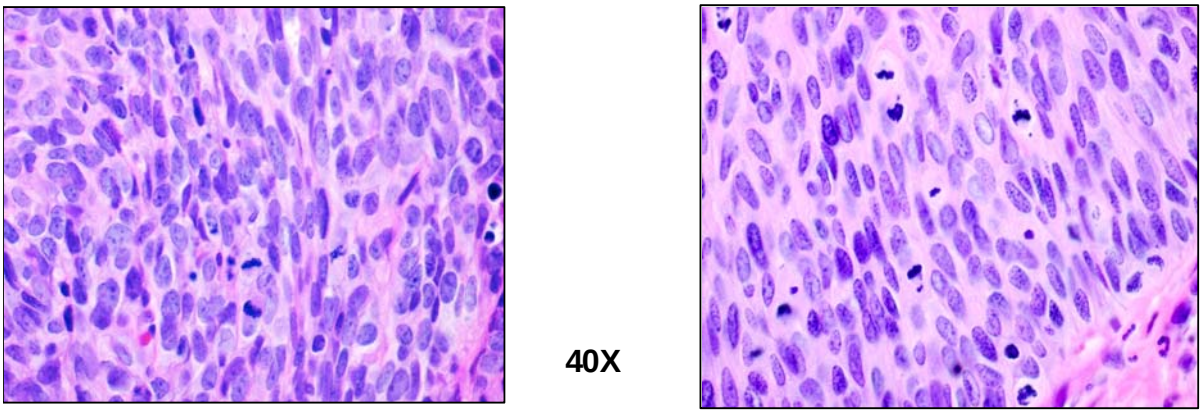

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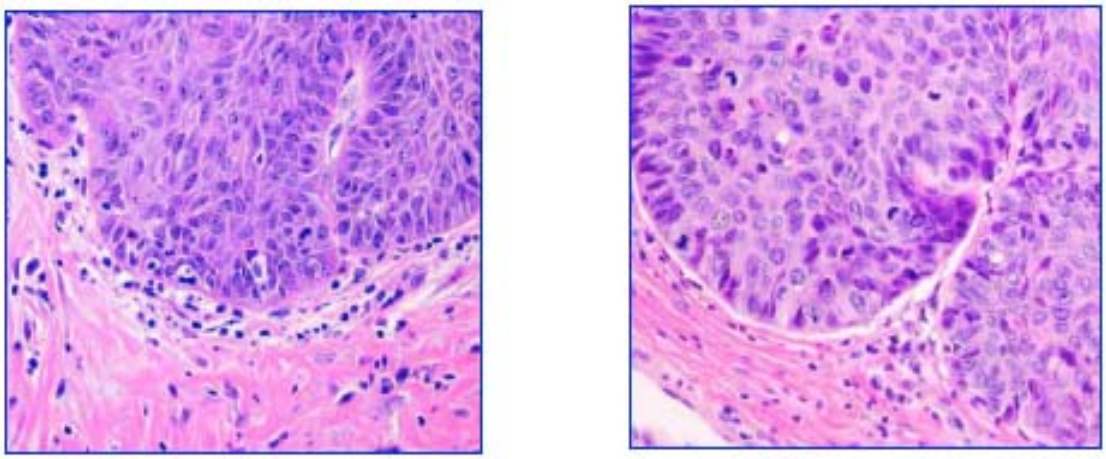

Anal Cancer in a Man **Anal Cancer in a Woman**

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Appendix 8
Summary of PPE Vaccine Group 6/11/6/18-Related AIN Cases

Month	Case 1	Case 2	Case 3	Case 4	Case 5
0	Swabs: HPV 16	LSIL; Swabs: HPV 6, 18, 39, 45 and 59	Negative swabs	Swabs: HPV 45, 51 and 56	Negative swabs
		Condyloma: HPV 18; AIN 2: PCR Negative	--	--	--
7	Swabs: HPV 16	ASC-US; Swabs: HPV 39, 45 and 59	ASC-US; Swabs: HPV 31, 45, and 56	ASC-US; Swabs: HPV 35, 45 and 56	Negative swabs
		Negative biopsy: PCR Negative; Negative biopsy: PCR Negative	**AIN 2:** HPV **6** and 45	AIN 1: HPV 35; Negative biopsy: PCR negative	--
12	Swabs: HPV 6 and 16	ASC-US; Swabs: HPV 59	Swabs: HPV 45 and 56	LSIL; Swabs: HPV 35, 45 and 56	Swabs: HPV 45
--		AIN 2: HPV 39; Negative biopsy: HPV 59; Negative biopsy: HPV 39 and 59	--	**AIN 1:** HPV **6** and 56; AIN 1: HPV 35; AIN 1: HPV 56	--
18	LSIL; Swabs: HPV 6 and 16	LSIL; Swabs: HPV 39	Swabs: HPV 45 and 56	LSIL; Swabs: HPV 11, 35 and 56	ASC-US; Swabs: HPV 45
--	**AIN 1:** HPV **6**; **AIN 2:** HPV **6**; **AIN 3:** HPV **6**	**AIN 3**: HPV **16** and 39; Negative biopsy: HPV 39; Negative biopsy: PCR negative	--	Negative biopsy: PCR negative; Negative biopsy: PCR negative	**AIN 1:** HPV **16** and 45; Negative biopsy: HPV 45
24	LSIL; Swabs: HPV 6 and 16	LSIL; Swabs: HPV 39	ASC-US; Swabs: HPV 45	ASC-US; Swabs: HPV 35, 45 and 56	Negative swabs
--	AIN 1: HPV 6; Negative biopsy: PCR negative	--	--	Negative biopsy: PCR negative; Negative biopsy: PCR negative; Negative biopsy: PCR negative	LSIL; Negative biopsy: PCR negative
30	--	--	--	Swabs: HPV 45	Negative swabs
36	Negative biopsy: PCR negative; Negative biopsy: PCR negative	LSIL; Swabs: HPV 16 and 39; **AIN 1**: HPV **16** and 39; AIN 1: HPV 39; Negative biopsy: PCR negative	Swabs: HPV 45; AIN 1: HPV 45	LSIL; Swabs: HPV 35 and 56; Negative biopsy: HPV 35; Negative biopsy: PCR negative Negative biopsy: PCR negative	Negative swabs
FINAL Case(s)	**HPV 6-related AIN 1, AIN 2 and AIN 3**	**HPV 16-related AIN 3 and AIN 1**	**HPV 6-related AIN 2**	**HPV 6-related AIN 1**	**HPV 16-related AIN 1**

Bolded font=Diagnosis (es) for which subject became a case of 6/11/16/18-related AIN; Final case(s) with HPV ascertainment is presented in last row of each column.
ASC-US=Atypical squamous cells of undetermined significance; LSIL=Low-grade squamous intraepithelial lesion; PCR = Polymerase chain reaction.