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Immunogenicity and Tolerability to Human Papillomavirus-like Particle Vaccine in Girls and Young Women with Inflammatory Bowel Disease

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Abstract

Background—Female patients receiving immunosuppressive therapy may be at increased risk for human papillomavirus (HPV) infection and cervical neoplasia.

Methods—We administered the 3-dose HPV vaccine Gardasil® to 37 females aged 9–26 years with inflammatory bowel disease (IBD) prescribed immunosuppressive therapy (prospective cohort). Geometric mean titers (GMT) in milli-Merck (mMu/mL) units were determined before dose 1 and one month after dose 3 by competitive Luminex immunoassay (cLIA) and qualitatively compared to healthy females of similar age from Merck's database. Side effects and adverse events were evaluated. Concurrently, in 15 similar IBD patients previously vaccinated by their primary care provider we assessed antibody titers by cLIA and total IgG LIA after dose 3 of vaccine (range 0.5 to 27 months).

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Conflict of interest: The current study was an investigator-initiated protocol written by Drs. Jacobson, Lu and Bousvaros, and research support was provided by Merck and Co., Inc. During the same time the study was conducted, Dr. Bousvaros was a site investigator for studies sponsored by UCB pharmaceuticals, Abbott pharmaceuticals, and a consultant to Millenium pharmaceuticals.

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Results—The mean age of prospective patients was 15 years with 51% on anti-TNF therapy and 49% on immunomodulators: 33 of 37 completed all three doses. Seropositivity after dose 3 was 100% for types 6, 11 and 16 and 96% for type 18. GMT for HPV 6, 11, 16 and 18 was 1080, 1682, 3975 and 858, respectively, and did not qualitatively differ from healthy females. No serious adverse events were attributable to the vaccine. In the previously vaccinated cohort, seropositivity was 100% for types 6, 11, and 16, and 40% for type 18 by cLIA (93% for HPV18 by IgG LIA). Titers decreased with time since dose 3.

Conclusions—In this small study of IBD patients prescribed immunosuppressive therapy, Gardasil® was immunogenic and there were no clinically significant vaccine-associated adverse events.

Keywords

inflammatory bowel disease; immunosuppressive therapy; human papillomavirus vaccine; immunogenicity; girls and young women

INTRODUCTION

Cervical cancer is the second leading cause of cancer mortality among women worldwide. Cervical cancer occurs in approximately 500,000 women annually, and results in approximately 200,000 deaths a year, primarily in developing countries.(1) In countries where routine cervical cytology screening programs are in place, the incidence of invasive cervical cancer has declined through early detection and treatment of cervical lesions.(2) Human papillomavirus (HPV) types 16 and 18 cause about 70% of all cervical cancer and high-grade cervical intraepithelial neoplasia (CIN).(3) The first infection with at least one HPV type is most likely to occur within one year after the onset of sexual intercourse, and most infections resolve and do not cause cervical neoplasia in immunocompetent women.(4) Crohn's disease (CD) and ulcerative colitis (UC) often present in late adolescence or young adulthood, and patients receive immunosuppressive therapies which may increase their risks of infection or cancer. Whether or not IBD patients are at increased risk of cervical neoplasia remains controversial. Two studies have suggested an increased prevalence of abnormal Pap smears in patients with IBD,(5, 6) but a third study failed to show a higher prevalence of abnormal Pap smears in IBD patients.(7) While population estimates of CIN in IBD patients are not available, Bhatia et al. selected IBD patients with a Pap smear and matched them to non-IBD patients with a Pap smear.(5) They observed that 18% of IBD patients and 5% of non-IBD patients had an abnormal Pap smear with 3.4% and 0.9% having high grade squamous intraepithelial lesions.(5) Women prescribed immunosuppressive therapies or with immunosuppressive diseases, such as HIV(8), remain at elevated risk of cervical neoplasia. Therefore, vaccine prevention of initial infection with high risk HPV types seems an especially attractive strategy to reduce the incidence of cervical cancer in this immunosuppressed population.

Two vaccines have been licensed by the FDA for HPV prevention. Both vaccines (Cervarix®, Glaxo Smith Kline and Gardasil®, Merck and Co., Inc.) are composed of non-infectious virus-like particles (VLP) from the HPV 16 and 18 serotypes.(9) Additionally, the recombinant quadrivalent HPV vaccine (Gardasil®) also immunizes against HPV 6 and 11. (3, 10, 11) Given the young age at IBD diagnosis and use of immunosuppressive therapies, early vaccination against high risk HPV types could be an important strategy to reduce the risk of cervical intraepithelial neoplasia and cervical cancer in IBD patients.

While inactivated vaccines are generally recommended in immunosuppressed patients, there are limited data surrounding the immunogenicity of these vaccines. We and others have

shown that patients with IBD on immunomodulators and biologics mount responses to influenza and pneumococcal vaccines, though the response may be reduced in patients prescribed biologics.(11-15). In an open label prospective study of girls and young women with IBD, we investigated the immunogenicity and tolerability of the quadrivalent HPV vaccine (Gardasil®). We measured antibody titers at one month post-vaccination and recorded side effects and adverse events after each dose. In addition, we assessed antibody titers in a smaller group of patients who had been previously vaccinated by their primary care provider. We hypothesized that IBD patients on immunosuppressive therapies would have a similar response to the vaccine as healthy controls. To our knowledge, this is the first report evaluating HPV vaccine immunogenicity in children and young adults with IBD who are receiving immunosuppressive therapy.

MATERIALS AND METHODS

Population

This was an open label study of the immune response to the Gardasil® vaccine (Merck Research Laboratories, West Point, PA) among **immunosuppressed female IBD patients** aged 9-26 years old with CD, UC, or indeterminate colitis (IC) who were diagnosed by standard clinical, radiographic, endoscopic, or histologic criteria. Patients were receiving care at either Children's Hospital Boston or Maine Medical Center. Eligible subjects were prescribed maintenance immunosuppressive therapy for at least 30 days prior to enrollment, with any immunomodulator (azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus) and/or a TNF-alpha inhibitor (infliximab, adalimumab). Eligible patients could also be prescribed aminosalicylates, antibiotics and/or corticosteroids (e.g., prednisone). Patients were recruited for participation in the study during a scheduled clinical visit, a hospital admission, by contact through mail followed by a phone call, or through referral from local hospitals. After written informed consent, two cohorts of immunosuppressed IBD patients were enrolled in this study.

The **prospective cohort** had not previously received HPV immunization. This group had a pre-immunization titer, just prior to being administered the Gardasil® vaccine series, and a post-vaccine titer. We excluded patients from enrollment if they had a history of a bleeding disorder or were on anti-coagulation therapy because the vaccine was administered intramuscularly. We also excluded patients who had hypersensitivity to ingredients in the Gardasil® vaccine, were pregnant, or had previously received an HPV vaccine.

The **previously immunized cohort** consisted of patients who had already received the 3-dose Gardasil® HPV vaccine series from their primary care provider. They were on immunosuppressive therapy for at least 30 days prior to the first vaccine dose. HPV immunization history was obtained and a single post-vaccination titer was drawn.

Patients were enrolled into the prospective cohort between August 2008 and August 2009. The previously immunized cohort was enrolled between February 2009 and April 2010. Informed consent was obtained from the patients 18 years or older, and assent from patients younger than 18 along with parent/guardian consent. This study was approved by the Institutional Review Boards at Children's Hospital Boston (08-19-08), Maine Medical Center (9/30/2008), and The Harvard School of Public Health (08-21-08). It is listed on clinicaltrials.gov NCT00727636.

General Questionnaire and Disease Severity Scales

For the prospective cohort, chart review and patient and guardian questionnaires were completed at each of four study visits (Day 1, Month 2, Month 6, and Month 7). The

baseline questionnaire included sociodemographics (age, race), type of IBD diagnosis (CD, UC, or IC), disease location, IBD-related surgical history, and medications used within the last six months (type, dose, and duration on current medications). The follow-up questionnaires inquired about significant events (such as IBD flares, IBD-related hospitalizations and surgeries), new findings regarding disease (from endoscopy, histology or radiology), and changes in medications since the last visit. The height and weight were measured at each of the four visits. Disease location was documented using the Montreal classification.(16) Disease severity for participants with CD was assessed by the abbreviated Pediatric Crohn's Disease Activity Index (PCDAI)(17) and for UC and IC by the Pediatric Ulcerative Colitis Activity Index (PUCAI).(18)

Vaccination

The Gardasil® vaccine is a non-infectious, non-replicating, quadrivalent vaccine prepared from highly purified virus-like particles (VLPs) of the major capsid proteins of HPV types 6, 11, 16, and 18. Patients received three doses of the HPV vaccine over 6 months, administered as one 0.5 mL intramuscular injection per dose. The doses were given on Day 1 (1st dose), Month 2 (2nd dose), and Month 6 (3rd dose), according to the manufacturer's instructions. Specifically, Month 2 is 8 weeks after the 1st dose with a range of 5 to 11 weeks. Month 6 is 26 weeks after the 1st dose with a range of 22 to 30 weeks, as per the manufacturer's instructions.

Sample Collection

Ten ml of blood were collected on Day 1 (prior to immunization) and Month 7 (4 + 2 weeks after the 3rd dose) for HPV titers and laboratory measures of IBD activity (white blood cell count, hematocrit, sedimentation rate, and albumin). Specimens were processed locally and serum kept at -20°C until it was shipped on dry ice to Merck & Co., Inc. for determination of HPV titers.

Assessment of serum antibody against HPV antigens

In the prospective and previously immunized cohorts, a competitive Luminex immunoassay(19) (cLIA approved; reported in milli-Merck Units [mMu]/ml) measured serum antibodies to HPV 6, 11, 16 and 18 proteins. Half the numeric lower limit of detectability (LLD) for each subtype was assigned a given value. Thus, the LLD and assigned value were < 7 and 3.5 mMu/ml for HPV 6, < 8 and 4.0 mMu/ml for HPV 11, < 11 and 5.5 mMu/ml for HPV 16, and < 10 and 5.0 mMu/ml for HPV 18, respectively. Titers > 20, > 16, > 20 and > 24 mMu/ml for subtypes 6, 11, 16, and 18, respectively, were considered seroimmune. In the previously immunized cohort, an additional assay was performed which was a total IgG Luminex immunoassay (total IgG LIA)(20). For subtypes 6, 11, 16, and 18, titers > 15, > 15, > 7, and > 10 mMu/ml, respectively, were considered seroimmune. While the cLIA measures the antibody response to a unique conformation neutralizing epitope on each HPV type, the total IgG LIA measures a broader immune response.

Monitoring of Adverse Events

Participants were advised to contact the study physicians (AB, YL) immediately if they experienced any adverse events within 48 hours of vaccination and anytime during the study if they had concerns. In addition, every patient was contacted by the study staff within 48 hours after each vaccine dose to document any side effects or adverse events on the Adverse Event Reporting Form. The form included a list of common (e.g., soreness at injection site, headache) and uncommon (e.g., hives, respiratory distress) side effects or reactions, type and date of treatment for event (e.g., analgesics, PMD visit, emergency room visit), relationship

of study protocol to the event (likely or unlikely related to the event), and severity of the event.

Safety of the study was overseen by a Data Safety Monitoring Board (DSMB) consisting of three physicians (one infectious disease specialist, one gynecologist, and one pediatric gastroenterologist) unaffiliated with the study investigators. All events were logged, and serious adverse events (including hospitalizations) were reported to the DSMB, irrespective of vaccine causality.

Previously Immunized Cohort

Female IBD patients aged 9-26 years old were eligible for participation in the previously immunized cohort if they had already received three doses of the Gardasil® vaccine from their primary care provider and met the same criteria for immunosuppressive therapy as described for the prospective cohort at the time of their first dose of vaccine. After providing informed consent, information was obtained from the patient, with help from the parent/guardian, and from the medical records to complete the baseline questionnaire which included the same measures as described in the prospective cohort. For the previously immunized cohort, the information on disease and medication use refers to the time of the blood draw for titers. Ten ml of blood was drawn and processed in the same manner as for the prospective cohort, and then sent to Merck & Co., Inc. for testing of titers.

Comparison Group

In the product insert for prescribing information, Merck and Co, Inc. reported titers of healthy girls and young women aged 9-15 and 16-26 years who were vaccinated with Gardasil®.(21) The geometric mean titer (GMT) and 95% confidence interval (CI) are reported at Month 7 for each subtype. This information served as our comparison group.

Data analysis

Characteristics of the IBD patients in the prospective and previously immunized cohorts were described using the median for continuous variables and frequencies for categorical variables.

Titers to HPV subtypes

Each HPV subtype was analyzed separately. All titers were transformed using $\log_{10}$ before analyses and then the mean and 95% CI were determined for each subtype at Day 1 (pre-vaccination) and Month 7 (post-vaccination) for the prospective cohort, and at post-vaccination for the previously immunized cohort. Then the antilog of the mean and 95% CI were calculated to obtain the GMT and 95% CI for each subtype. The percent seropositive was calculated at each time point for each HPV type. Patients in the prospective study who were seropositive at Day 1 (pre-vaccination) were not included in the calculations of GMT and 95% CI at Month 7.

For each subtype, the GMT and 95% CI for IBD prospective participants was compared qualitatively to that of the Merck comparison group at Month 7 by age group (9-15 and 16-26 years old). Titers were also compared by medication group. For the previously immunized, time since the last dose (in months) was computed and a Spearman rank test was performed to evaluate the correlation with titer level for each subtype.

RESULTS

Thirty seven IBD subjects enrolled in the prospective cohort, of whom thirty-three completed the study with 3 doses of vaccine. Of the four who did not complete the study,

two were lost to follow-up and two discontinued. Among the two who discontinued, one was at the request of the primary gastroenterologist because they thought that it might be difficult to differentiate between vaccine and clinical events and the other because the mother did not want her daughter to have the full series. These subjects received only one dose of the vaccine. Of the 33 who completed all three doses, the median time between the third dose and the Month 7 blood draw was 0.9 months (range 0.2 to 2.9). There were 16 participants enrolled in the previously immunized cohort, of whom one withdrew before sample collection. The 15 samples from the previously immunized cohort were obtained at a median of 7.6 months after the third dose (range 0.5 to 27.2).

Characteristics of the Prospective and Previously Immunized Cohort

The majority of participants in the prospective and previously immunized cohorts were Caucasian (95% and 100%, respectively) and Non-Hispanic (92% and 100%, respectively), with median ages of 15 and 18 years at enrollment and 11 and 13 years at IBD diagnosis, respectively (Table 1). Most of the participants in both cohorts had CD (70%, 87%) followed by UC (24%, 13%) and IC (5%, 0%). Most CD participants (84%, 85%) were in remission based on the PCDAI and PUCAI scores. Montreal classifications of disease for CD and UC/IC participants are shown in Table 1. The majority of participants in both cohorts were on TNF-alpha inhibitor therapy (51%, 67%). No patients in either group were receiving tacrolimus or cyclosporine. Only two patients in the study were receiving prednisone (one patient 10 mg/day, one patient 20 mg/day) at time of enrollment.

Titers

For the prospective study participants, the percent seropositive and GMT (95% CI) at pre-vaccination (Day 1) and post-vaccination (Month 7) based on the cLIA assay are presented in Table 2. Prior to vaccination, 2 of 37 (5.4%) were seropositive for HPV 6 but none were seropositive for other HPV subtypes. After completion of the three-immunization series, all of the patients seroconverted to subtypes 6, 11 and 16. However, 2 of 33 (6%) did not seroconvert to HPV 18. By a qualitative assessment (Table 2, Figure 1), the post-vaccination GMT in the prospective cohort was as high or higher to all four HPV types compared to the Merck comparison group within each age group. The combined HPV titers, not stratified by age, for 6, 11, 16, and 18 were 1079.9 (95% CI 783, 1489.3), 1681.8 (95% CI 1189.9, 2377.2), 3975.1 (95% CI 2492.2, 6340.6) and 857.6 (95% CI 472.4, 1556.6), respectively.

We compared prospective patients on TNF-alpha inhibitor vs. immunomodulators for each HPV type. The mean (95% CI) titer and p value are as follows: HPV 6: 1511 (95% CI 955, 2393) vs. 788 (95% CI 526, 1180) $p = 0.038$; HPV 11: 1851 (95% CI 1087, 3154) vs. 1519 (95% CI 971, 2376) $p = 0.30$; HPV 16: 3751 (95% CI 1772, 7941) vs. 4227 (95% CI 2401, 7443) $p = 0.73$; HPV 18: 1039 (95% CI 407, 2656) vs. 699 (95% CI 334, 1465) $p = 0.09$.

For the study participants who had been previously immunized by their primary care physicians, the seropositivity rate and GMT (95% CI) by cLIA at post-vaccination are also shown in Table 2. Previously immunized participants were enrolled from 0.5 to 27.2 months after their third dose. All 15 participants were seropositive to types 6, 11, and 16. Six of the 15 participants (40%) had a titer below 24 (seronegative) for HPV18 by the cLIA assay. On the total IgG LIA assay, seropositivity on HPV types 6, 11, 16, and 18 was 93% (14/15), 87% (13/15), 100% (15/15) and 93% (14/15), respectively. There was a significant negative correlation between months since the third vaccine dose and cLIA titer levels for HPV 11 ($r = -0.64$, $p = 0.01$), HPV 16 ($r = -0.53$, $p = 0.043$) and HPV 18 ($r = -0.77$, $p = 0.0007$), suggesting that titers may decrease over the first year after the third dose. The correlation was not significant for HPV 6 ($r = -0.34$, $p = 0.21$).

Adverse events

Adverse event data were gathered only for the prospective cohort. After each dose of vaccine, almost half of the patients reported soreness at the injection site after all three doses. Other side effects were uncommon after any dose (Table 3). There were five serious adverse events (SAE) following vaccination with Gardasil® (Table 4). Two patients were hospitalized for IBD exacerbations, one for pneumonia, and one for an ovarian torsion secondary to endometriosis. The two patients hospitalized during the vaccine study had evidence of active ulcerative colitis in the weeks preceding enrollment in the study, and were being treated with immunosuppressants for active disease. While we cannot definitively exclude a role for the vaccine in their hospitalizations, both investigators and the DSMB felt that their hospitalizations were due to ongoing active disease, and that the vaccine was unlikely to have resulted in the hospitalizations. The fifth SAE was an emergency department visit for acute sinus pain. Review of the events by the investigators and DSMB determined that these SAE were either unrelated or unlikely to be related to the vaccine. There were 11 other minor adverse events that were deemed either unlikely or possibly related to the vaccine.

DISCUSSION

Our study of girls and young women with IBD prescribed immunomodulators and/or a TNF alpha inhibitor suggests they can be safely immunized with quadrivalent HPV vaccine. In addition, the immunogenicity of the vaccine appears comparable to healthy controls. Because of the small sample size, limited duration of follow-up, and lack of cervical sampling, we cannot definitely state that the good immunologic response confers protection to infection. However, other large studies in control populations have demonstrated that the quadrivalent vaccine effectively prevents HPV 16 and 18 infection, cervical intraepithelial neoplasia, and cervical cancer.(22) Thus, the vaccine has been licensed by the FDA for use in females aged 9-26 years since 2007, and for males in this age group since 2009.

In clinical trials of Gardasil® in healthy male and female children and young adults, the rate of seropositivity was over 99% to HPV 6, 11, 16 and 18 by one month after the third dose of vaccine (month 7).(21) The immune response in these healthy volunteers peaked at month 7, declined through month 24 and stabilized through month 36 where the response remained above baseline. This pattern of response was similar across sex and age groups. We achieved a similar rate of seropositivity in our cohort of immunosuppressed children and young women with IBD. All of our patients seroconverted to HPV 6, 11 and 16 at month 7 and 96% of patients seroconverted to HPV 18. Some studies suggest that response to type 18 is also lower in HIV-infected individuals, another immunocompromised population.(23) The strength of the antibody response in our patients at month 7 was within the range of that observed for the healthy volunteers described above. One limitation of our prospective study is that while we assessed titers one month after completion of the three vaccine series, we did not follow our patients long enough to evaluate the immune response over time. To address this limitation, we measured antibody titers in IBD patients who had been vaccinated outside of our study by their primary care providers. This second cohort underwent serum sampling and antibody titer assay up to 27 months after their third vaccine dose (median 7.6 months), and our analysis suggests that titers decrease over time. This is supported by Olsson et al, who showed that titers plateau at 24 months and remained stable for five years post-vaccine.(24) All of our previously immunized patients were seropositive to types 6, 11 and 16, but only 40% were seropositive to type 18 using the cLIA assay. The cLIA assay may underestimate seropositivity as it is restricted to the anti-VLP HPV type-specific conformational neutralizing antibody. Brown et al. found a 96.7% rate of seropositivity to type 18 at month 48 from the total IgG cLIA assay, which measures the total IgG antibody binding to the HPV VLPs.(25) Indeed, 93% of our previously immunized

patients were seropositive to HPV type 18 by the total IgG cLIA assay. Thus, our data suggest that IBD patients on immunosuppressive therapies develop an antibody response to the HPV vaccine types, but further follow-up is needed to understand the pattern of immune response over time.

Patients with IBD have a wide variety of genetic mutations that may result in subtle defects in innate and adaptive immunity.(26, 27) In addition, immunosuppressive agents of varying potency (including thiopurines, methotrexate, and anti-TNF agents) are used to treat these diseases.(28) In spite of the genetic and pharmacologically induced inhibitions of the immune system seen in IBD, the response to most inactivated vaccines appears either unaffected or only mildly affected. In a pediatric study of hepatitis A vaccine in children aged 2-18 years of age, the rate of seroconversion after the second dose was 97% in IBD patients and did not differ from the rate of 100% in healthy children.(29) The seropositivity rate in IBD patients was lower than controls after the first dose suggesting the second dose improved the immune response. Similarly, two studies of the influenza vaccine in children and young adults suggest that both seroprotection and seroconversion occur, though there might be a slight reduction of efficacy in patients prescribed biologics.(11, 12) In our study, the mean titers for types 6 and 18 tended to be higher among the prospective patients on TNF-alpha inhibitors. The high rate of seropositivity to the Gardasil® vaccine in our IBD patients is similar to rates of seropositivity to other vaccine studies among children and young adults with IBD, and should be reassuring to patients, parents, and providers that these vaccines are immunogenic and appear to be safe.

Our study has several limitations. Given the young age of our patient population (mean age 15 years old), we chose not to assess for cervical infection or inflammation during the study by performing Pap smears. To do this would have drastically curtailed recruitment. In addition, the HPV vaccine had already come into widespread use at the time we began the study, limiting the sample size of our prospective cohort. While we are able to demonstrate immunogenicity, the small sample size makes it difficult to compare the immune responses among different drug classes (e.g., immunomodulators vs. anti-TNF agents). In addition, rare adverse events would be difficult to detect in a small study, and are better studied by postmarketing surveillance and large registries. In addition, we cannot ascertain a protective effect against HPV infection, as our patients did not receive long term follow-up. While the young women in this study were prescribed immunomodulators, we do not have measures of adherence or drug levels to confirm that they actually took the medications as prescribed. Finally, we have no data on young men with IBD, as our study began before Gardasil® was licensed for administration in boys and men. In spite of these limitations, our study suggests that immunosuppressed patients with IBD receiving quadrivalent HPV vaccine can successfully mount an immune response to the vaccine, and are unlikely to develop any clinically significant vaccine associated adverse events. We conclude that as with other inactivated vaccines, quadrivalent HPV vaccine should be administered to IBD patients whether or not they are receiving immunosuppressive therapies.

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Figure 1. HPV Post-Vaccination Titers at Month 7 in Children and Young Adults with IBD (n=33) Compared to Healthy Historical Controls

The number (N) of participants in the IBD and control groups for each HPV subtype and age group is indicated below the mean (95% CI).

Table 1

Characteristics of IBD Patients in the Prospective and Previously Immunized Cohorts

Characteristic		Prospective ¹ (N=37)	Retrospective ^{1,2} (N=15)
Age (yrs)	Median (Min, Max)	15, (9, 26)	18 (14, 26)
Age at IBD diagnosis (yrs)	Median (Min, Max)	11 (3, 16)	13 (5, 17)
Caucasian - N (%)	No	2 (5%)	0 (0%)
	Yes	35 (95%)	15 (100%)
Hispanic or Latino ethnicity - N (%)	No	34 (92%)	15 (100%)
	Yes	3 (8%)	0 (0%)
IBD Diagnosis - N (%)	Crohn's	26 (70%)	13 (87%)
	Ulcerative colitis	9 (24%)	13 (13%)
	Indeterminate colitis	2 (5%)	0 (0%)
PCDAI score - N (%)	In remission	21 (84%)	11 (85%)
PUCAI activity level	In remission	11 (100%)	2 (100%)
Montreal Classification			
<i>Crohn's Disease</i>			
Disease location - N (%)	L2 (colon)	1 (4%)	0 (0%)
	L2+L4 (colon and upper GI)	2 (8%)	1 (8%)
	L3 (ileocolon)	9 (35%)	4 (33%)
	L3+L4 (ileocolon and upper GI)	14 (54%)	7 (58%)
Disease Behavior - N (%)	B1 (non-stricturing, nonpenetrating)	13 (50%)	5 (38%)
	B1p (non-stricturing, nonpenetrating and perianal)	4 (15%)	2 (15%)
	B2 (stricturing)	0 (0%)	2 (15%)
	B2p (stricturing and perianal)	0 (0%)	2 (15%)
	B3 (penetrating)	1 (4%)	0 (0%)
	B3p (penetrating and perianal)	8 (31%)	2 (15%)
<i>Ulcerative Colitis or Indeterminate Colitis</i>			
Extent of disease - N (%)	E2 (proctitis)	1 (9%)	1 (50%)
	E3 (pancolitis)	10 (91%)	1 (50%)
Medication group - N (%)	TNF-alpha inhibitor	19 (51%)	10 (67%)
	Immunomodulator	18 (49%)	5 (33%)

¹Frequencies may not add up to 100 due to rounding.²Information on disease status and medication use on retrospective patients was at the time of the first vaccine dose.

Table 2

Vaccine Titers by cLIA assay in IBD Patients in the Prospective and Previously Immunized Cohorts, Compared to Healthy Historical Controls

Subtype	Assessment	Age (yrs)	IBD Patients			Merck Historical Controls ⁵	
			N	Seropositive ⁴ N (%)	Geometric Mean Titer- mMU/mL ⁵ (95% CI)	N	Geometric Mean – mMU/mL ⁶ (95% CI)
HPV 6	Prospective						
	<i>Pre-vaccination</i> ¹	9-26	37	2 (5.4)	4.3 (3.6, 5.1)		
	<i>Post-vaccination</i> ²	9-15	19	19 (100)	1188.9 (820.3, 1723.3)	917	929.2 (874.6, 987.3)
		16-26	12	12 (100)	927.3 (509.8, 1686.8)	3333	545.2 (530.3, 560.6)
HPV 11	Previously Immunized						
	<i>Post-vaccination</i> ³	14-26	15	15 (100) ⁷	173.4 (91.3, 329.5)		
	Prospective						
	<i>Pre-vaccination</i> ¹	9-26	37	0 (0)	4.3 (4.0, 4.7)		
	<i>Post-vaccination</i> ²	9-15	20	20 (100)	1730.7 (1114.8, 2686.8)	917	1304.6 (1224.7, 1389.7)
		16-26	13	13 (100)	1609.4 (899.7, 2878.9)	3357	749.0 (726.1, 772.7)
	Previously Immunized						
	<i>Post-vaccination</i> ³	14-26	15	15 (100) ⁷	267.3 (122.4, 583.8)		
HPV 16	Prospective						
	<i>Pre-vaccination</i> ¹	9-26	37	0 (0)	5.7 (5.3, 6.1)		
	<i>Post-vaccination</i> ²	9-15	20	20 (100)	4155.1 (2339.5, 7379.7)	915	4918.5 (4556.6, 5309.1)
		16-26	13	13 (100)	3713.1 (1638.3, 8416.7)	3253	2411.3 (2311.1, 2515.9)
Previously Immunized	<i>Post-vaccination</i> ³	14-26	15	15 (100) ⁷	802.7	(391.1, 1676.6)	
HPV 18	Prospective						
	<i>Pre-vaccination</i> ¹	9-26	37	0 (0)	5.0 (5.0, 5.0)		
	<i>Post-vaccination</i> ²	9-15	20	19 (95)	1193.8 (571.1, 2495.5)	922	1042.6 (967.6, 1123.3)
		16-26	13	12 (92)	515.5 (195.0, 1362.8)	3571	475.6 (459.2, 492.6)
	Previously Immunized						
	<i>Post-vaccination</i> ³	14-26	15	6 (40) ⁷	79.5 (26.3, 240.5)		

¹Pre-vaccination (Day 1). Includes all patients with a pre-vaccination titer.

²Post-vaccination (Month 7). Excludes patients who were seropositive at the pre-vaccination titer.

³Post-vaccination titer at time of enrollment into the previously immunized cohort at a median of 7.6 months (range 0.5 to 27.2) after dose 3.

⁴Serostatus cutoffs for each HPV type by cLIA assay are: HPV 6 > 20; HPV 11 > 16; HPV 16 > 20; HPV 18 > 24.

⁵From Table 17 of the Product Insert for the Gardasil® vaccine.

⁶mMu/mL – milli Merck units by the cLIA assay.

⁷On the total IgG LIA assay, the rate of seropositivity was 93% (14/15), 87% (13/15), 100% (15/15) and 93% (14/15) for subtypes 6, 11, 16, and 18, respectively.

Table 3

Side Effects after Each Dose of the Vaccine in IBD Patients in the Prospective Cohort

Side Effect - N (%)	Dose 1 (N=35)	Dose 2 (N=32)	Dose 3 (N=33)
	N (%)	N (%)	N (%)
Soreness at injection site	17 (49)	15 (47)	17 (52)
Erythema of injection site	0 (0)	3 (9)	6 (18)
Swelling at injection site	0 (0)	3 (9)	2 (6)
Low grade fever	0 (0)	0 (0)	0 (0)
Itchiness	0 (0)	0 (0)	2 (6)
Nausea	1 (3)	0 (0)	3 (9)
Dizziness	0 (0)	0 (0)	0 (0)
Headache	3 (9)	3 (9)	2 (6)
Fatigue	1 (3)	3 (9)	1 (3)
Hives	0 (0)	0 (0)	0 (0)
Respiratory distress	0 (0)	0 (0)	0 (0)
Allergic asthma wheezing	0 (0)	0 (0)	0 (0)

Table 4

Adverse Events for IBD Patients in the Prospective Cohort

Adverse Event	Number of Events	Timing Relative to Dose	Relationship to vaccine
Serious Adverse Event			
Admitted to hospital/Went to emergency dept.			
Exacerbation of inflammatory bowel disease	2	3 days, 8 days	Unlikely related for both patients
Pneumonia	1	3 weeks	Unlikely related
Endometriosis with an ovarian torsion	1	2 days	Unrelated
Sinus pain and ED visit	1	3 weeks	Unrelated
Minor Adverse Event			
Leg pain	1	1 day	Possibly related
Asthma related symptoms	1	5 days	Unlikely related
Rectal bleeding and diarrhea	2	23 days, 2 days	Unlikely related, Possibly related
Axillary abscess	1	9 days	Unlikely related
Rash on their chin	1	2 days	Possibly related
Abdominal pain	3	2 weeks, Same day, Same day	Unlikely related, Possibly related, Possibly related
Migraine	1	3 months	Unlikely related
Swelling and severe pain in arm	1	3 days	Possibly related

Table 5

Number of IBD Patients with Flares, Hospitalizations and Surgeries after Each Vaccine Dose in the Prospective Study

Event	Dose 1 (N=33) ¹		Dose 2 (N=33) ¹		Dose 3 (N=33) ¹	
	N (%)	Days since dose ⁴	N (%)	Days since dose ⁴	N (%)	Days since dose ⁴
Flare ²	2 (6)	3, 21	3 (9)	47, 126, 127	1 (3)	11
Hospitalization ³	3 (9)	5, 15, 50	1 (3)	83 2	(6) 2,	20
Surgery	0 (0)		1 (3)	84	0 (0)	

¹ This is the total number of patients with data in the time period between that dose and the next dose.

² Five patients had flares, one of whom had a flare after doses 2 and 3.

³ Four patients were hospitalized. One patient was hospitalized after doses 1 and 2, another patient had two hospital admissions after dose 3, and a third patient had two hospitalizations after dose 1 and two hospitalizations after dose 3.

⁴ For those with multiple hospitalizations, the number of days between vaccine dose and the first admission.