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PERSISTENCE OF MEMORY B AND T CELL RESPONSES TO THE QUADRIVALENT HPV VACCINE IN HIV-INFECTED CHILDREN

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Abstract

Objectives—To determine the magnitude and persistence of human papillomavirus (HPV)16 and HPV18 B and T cell memory after three or four doses of quadrivalent HPV vaccine (QHPV) in HIV-infected children.

Methods—74 HIV-infected children immunized with four doses and 23 with three doses of QHPV had HPV16 and HPV18 IgG B cell and IFN γ and IL2 T cell ELISPOT performed at 2, 3.5 and 4-5 years after the last dose.

Results—HPV16 and HPV18 T cell responses were similar in both treatment groups, with higher responses to HPV16 vs. HPV18. These HPV T cell responses correlated with HIV disease characteristics at the study visits. Global T cell function declined over time as measured by nonspecific mitogenic stimulation. B cell memory was similar across treatment groups and HPV genotypes. There was a decline in HPV-specific B cell memory over time that reached statistical significance for HPV16 in the four-dose group.

Conclusions—B and T cell memory did not significantly differ after either three or four doses of QHPV in HIV-infected children. The clinical consequences of decreasing global T cell function and HPV B cell memory over time in HIV-infected children requires further investigation.

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Conflicts of interest:

AW and MJL receive research grants from Merck & Co and from GlaxoSmithKlein (money goes to the University of Colorado). MJL has intellectual property.

MJL and ABM and are advisors for Merck & Co and receive honoraria.

AS is employed by Merck & Co.

Keywords

Human papillomavirus; vaccine; HIV infection; B cell memory; T cell memory

INTRODUCTION

High risk human papillomavirus (HPV) types 16 and 18 are associated with the development of anogenital precancers and cancers and oropharyngeal cancers [1, 2]. Low-risk HPV types 6 and 11 cause genital warts as well as a large proportion of abnormal cervical cytologies [3]. HPV-related complications are more common and persistent in patients with human immunodeficiency virus (HIV) infection [4, 5]. Reconstitution of immune function following treatment with combination antiretroviral therapy (ART) prevents many, but not all the complications of HPV in HPV/HIV dually-infected patients [6-10]. The persistently increased incidence of HPV complications in HIV-infected individuals has been ascribed to either defective or incomplete immune reconstitution, inadequate adherence to ART, or inability of the immune response to eliminate established HPV infection [11].

Ecologic data confirm the high efficacy of HPV vaccines demonstrated in the vaccine trials in HIV-uninfected women [12]. Protection against squamous intraepithelial lesions is also observed in populations with high vaccine coverage [13]. Rates of HPV and genital warts have decreased dramatically in women under 25 years of age in populations that have greater than 50% coverage of targeted age groups [14-16]. In addition, rates of genital warts have decreased in men less than 25 years of age in populations where greater than 50% of women have been vaccinated, suggesting a strong herd effect [15].

Protection conferred by HPV vaccines results from the high antibody titers induced against the vaccine genotypes [17]. There is a decline in antibody titer over time, such that at 9 years after quadrivalent HPV vaccine (QHPV) administrations >90% vaccinees retain antibody concentrations above the level that defines positive serostatus for HPV6 and 16 and >60% for HPV11 and 18 when using a competitive Luminex immunoassay (cLIA) [14, 18-20]. With an noncompetitive ELISA assay >90% vaccinees remain seropositive at 9 years against all 4 vaccine genotypes [20, 21].

QHPV is safe and immunogenic in HIV-infected adults and children, but the magnitude of type-specific HPV antibody responses is generally diminished compared to uninfected individuals. This difference is most apparent in patients with a low number of CD4+ T cells and high plasma HIV viral load [22-27]. This is consistent with observations that antibody responses to other vaccine immunogens are decreased in HIV-infected patients [28]. Information on the duration of HPV antibody persistence in HIV-infected vaccinees is limited to 5 years or less [29].

Since neutralizing antibodies against vaccine HPV genotypes are lower in HIV-infected individuals than in the general population, memory and anamnestic responses may play a more important role in the long-term protection against HPV infection. In a previous study we showed that 18 months after completion of a 3-dose immunization regimen HIV-infected children had anamnestic responses to a QHPV booster dose regardless of the antibody titers

before booster administration [30]. Here we address additional potential protective mechanisms by investigating the persistence of memory B and T cell responses to QHPV for 4 to 5 years after vaccination.

METHODS

Study design

In 2010 children 7 to < 12 years of age with HIV infection were enrolled into a parent protocol [International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) P1047] if their baseline CD4% was ≥ 15 and they had been receiving ART for ≥ 3 months [27]. Exclusion criteria prior to administration of any dose of QHPV or placebo included other diseases or medications causing immune suppression; prior sexually transmitted infections; or treatment with any blood-derived product within the six months prior to the first injection or planned during the study. The administration of QHPV or identical placebo was assigned randomly, in a double-blinded fashion, in a 3:1 ratio, to 40 subjects in each group at entry (90 to receive QHPV and 30 to receive placebo; Fig S1). Each subject received the same assigned study vaccine 8 and 24 weeks later. At week 96 subjects who previously received the QHPV 3-dose standard regimen received a fourth dose of QHPV, while the previous placebo recipients began the QHPV 3-dose standard regimen. All subjects in the current protocol (P1085; CLINICALTRIALS.GOV identifier: NCT01206556) were enrolled between 1 and 2 years of their last QHPV vaccination to study the persistence of HPV-induced immune responses. Subjects were excluded if they received any additional dose of QHPV or blood-containing products. Interval blood samples for HPV-specific immune responses and HIV status were obtained at 2 and 3.5 after completion of the QHPV schedule for each arm of the P1047 study as per protocol. The last blood draw was originally scheduled at 5 years, but the study was closed early by the network, such that the last visit in some participants occurred as early as 4 years (Fig S1). The study was approved by the Ethical Review Committee of each participating site and the parents or guardians of all subjects provided written informed consent; assent was obtained from subjects at sites where this was standard of care.

B Cell ELISPOT

Peripheral blood mononuclear cells (PBMC) were cryopreserved at the clinical site laboratories using a standardized protocol (<http://www.hanc.info/labs/Pages/SOPs.aspx>). For the detection of IgG secreting memory B cells, PBMC were thawed, counted and stimulated with 1 μ g/ml R848 and 10 ng/ml IL-2 in RPMI 1640 (Gibco) containing 10% fetal bovine serum (Gemini Bio-Products), 0.4% penicillin and streptomycin and 1% HEPES buffer (Corning Cellgrow), for 96 hours at 37° C and 5% humidified CO₂. PBMCs with viability $\geq 55\%$ after stimulation were used at 250,000 cells/well in duplicate wells of ELISPOT IgG kits (Mabtech Inc). Assays were performed as per manufacturer's instructions using HPV16 or HPV18 VLP at 0.2 μ g/well and mouse anti-human IgG monoclonal antibodies for coating. IgG spot-forming cells (SFC) were counted with an ImmunoSpot II instrument (Cell Technologies Ltd.). Results were expressed as IgG SFC/10⁵ stimulated PBMCs.

T Cell ELISPOT

T cell responses were measured with a dual color IFN γ and IL2 ELISPOT assay (ELISPOT MabTech FluoroSpot kit) as per manufacturer's instructions. Thawed PBMC with viability 70% before and after overnight resting were added at 250,000 cells/well in 100 μ L of RPMI 1640 with glutamine (Gibco), containing 10% human AB serum (Gemini Bio-Products) and 1% antibiotics (Gibco). Cells were stimulated in duplicate wells with HPV16 or HPV18 VLP at the pre-optimized concentration of 10 μ g/ml or phytohemagglutinin A (PHA) at 10 μ g/ml or medium control. After 48 hours at 37°C in 5% humidified CO₂, plates were developed and analyzed with an Immunospot II plate reader. T cell responses were calculated as the difference between HPV- or PHA-stimulated wells and unstimulated control wells. Results were reported as mean SFC/10⁵ PBMC.

Statistical Analysis

Baseline characteristics were summarized and compared among the two treatment arms using Wilcoxon rank sum tests for continuous variables and Fisher's exact test for categorical variables. To assess the persistence of the T cell and B cell responses after completion of QHPV vaccine, longitudinal scatter plots, with estimated mean and 95% CIs from mixed effects longitudinal data analyses were constructed. To further examine the time and treatment effects on T cell and B cell responses, mixed effects analyses were conducted, with PHA and total IgG as covariates for T-cell and B-cell responses, respectively, to adjust the potential confounding effects. To compare T-cell HPV-16 and HPV-18 specific responses, basic summary statistics and paired Wilcoxon signed rank test were performed at each study visit. Spearman's rank order correlation coefficients were calculated to examine the strength and statistical significance of associations of T-cell and B-cell responses with HPV antibody levels and other continuous variables; Wilcoxon rank sum or Kruskal-Wallis tests were used to assess the associations between T-cell or B-cell responses and categorical variables.

RESULTS

Characteristics of the study population

This persistence study enrolled 97 participants, including 74 in Arm A and 23 in Arm B (Fig S1). The two arms were well balanced with respect to demographic and HIV disease characteristics except for ART, which was used in 96% of participants in Arm A and 74% in Arm B (Table 1). Antibody concentrations were also significantly higher in Arm A compared with Arm B at all time points by virtue of Arm A having received four and Arm B three doses of QHPV.

Persistence of T cell responses after three and four doses of QHPV

HPV16- and HPV18-specific and PHA-nonspecific T cell responses measured by IFN γ and IL2 ELISPOT at 2, 3.5, and 4.5 to 5 years after the last dose of vaccine are presented in Figure 1. Participants had 0.1 IFN γ SFC/10⁵ PBMC before vaccination, which was in accordance with their HPV-naïve status [30]. There was a general trend in HPV and PHA IL2 T cell responses to decrease over time. The decrease in PHA responses reached significance

for IL2 in both Arms ($p = 0.05$) and for IFN γ in Arm B ($p=0.02$). The decrease in PHA responses indicated a global loss of T cell functionality. To identify potential losses specific to HPV we adjusted the HPV-specific ELISPOT analyses for the global loss of T cell functionality by using the response to PHA as a covariate in mixed effects analysis (Table S1). After this adjustment, we did not observe a significant decrease over time in IFN γ or IL2 responses that was specific to HPV16 or HPV18 in either treatment Arm. Moreover, in the adjusted analysis, there were no differences in the responses to HPV16 or HPV18 between the two Arms at any time point.

HPV 16-specific IL2 and IFN γ T cell responses were significantly higher than HPV18-specific responses at most time points (Table 2). The immunization regimen did not have a significant effect on the differences in responses to HPV16 compared with HPV18.

An analysis of select demographic and HIV disease characteristics at each visit showed positive correlations of T cell responses to HPV16 and HPV18 with CD4+ T cell counts and percentages, and negative correlations with plasma HIV RNA copies/mL and with CD8+ T cell counts and percentages (Table S2). The correlations were most readily detected in Arm A, which had a larger sample size than Arm B.

The correlation analysis of T cell responses with antibody concentrations at each visit showed robust positive associations of IFN γ and IL2 SFC with cLIA antibody concentrations for HPV16 and HPV18 in both Arms (Table 3).

Persistence of memory IgG B cell responses after 3 and 4 doses of QHPV

Total and HPV16- and HPV18-specific IgG memory B cell results are summarized in Figure 2. There were no significant changes in total IgG memory B cells over time in either Arm. HPV-specific memory B cell responses tended to decrease over time in both Arms, but the difference reached statistical significance only for HPV16 in Arm A ($p=0.03$), which had a larger number of participants compared with Arm B. There were no significant differences between Arms A and B in memory IgG B cell responses to HPV16 or HPV18 in the mixed effects model analysis (Table S3).

The correlation analyses of the memory IgG B cell responses to HPV16 and HPV18 with demographic and HIV disease characteristics at each visit showed sporadic significant correlations without a discernible pattern (Table S4). Moreover, memory IgG B cells did not correlate with antibody or T cell responses to the vaccine at any visit (Table S5).

DISCUSSION

We determined that IgG memory B and T cell responses to HPV16 and HPV18 were generated by QHPV and could be detected for up to 5 years after the last dose of vaccine administered to HIV-infected children. The persistence of the memory responses after QHPV is of interest, because the circulating antibodies generated by the vaccine decrease over time and become undetectable in some cases as we also showed in this cohort of HIV-infected vaccinees [29]. When this happens, the host presumably may use innate immunity and anamnestic adaptive responses when exposed to the HPV genotypes in the vaccine as

previously described for hepatitis B virus^[31]. Although a few studies examined short- and long-term B and T cell memory in HIV-uninfected women and children after QHPV, this is the first study of B cell memory generated by QHPV in HIV-infected vaccinees and the only study that examined the long-term persistence of their B and T cell responses^[32-35].

B cell memory to HPV16 and HPV18 in our study tended to decrease between 2 and 5 years after the last dose of QHPV. This contrasts with results in HIV-uninfected women whose HPV16 and HPV18 B cell memory responses remained stable between 1 and 4 years after QHPV^[32]. There are several differences between the two studies other than the immune competence of the vaccine recipients. These include differences in reagents and some technical aspects of the laboratory assays. In addition, the number of participants contributing data in the study of immune competent women decreased by 50% from 1 to 4 years and a formal statistical analysis of the kinetics of their HPV16 or HPV18 B cell memory was not presented.

We did not observe significant differences in B cell memory to HPV16 or HPV18 between the 3- and 4-dose immunization regimens and no correlations with the age of the vaccinee. In contrast, two studies of immune competent QHPV recipients (children with two doses and young women with two or three doses) showed differences in short-term memory B cell responses to HPV18 by age and number of doses^[33, 34]. Specifically, women with two doses did not develop B cell memory to HPV18 and women with three doses developed lower B cell memory compared with children with two doses. The differing results between our study and those in HIV-uninfected participants might be explained by the following: 1) the number of doses of vaccine (three vs. four in our study and two vs. three in the immune competent host studies); 2) time points in the kinetics of the memory B cell responses (long-term persistence in our study and peak response in the studies in immune competent hosts); 3) the age and immune competence of the hosts (children in our study and children and young women in the immune competent host studies); and 4) reagents and laboratory assay characteristics.

We showed in this HIV-infected cohort that HPV16- and HPV18-specific T cell responses, adjusted for the decline in global T cell function, persisted over time, while T cell functionality declined over this same time period. In contrast, Einstein et al. investigated persistence of B and T cell memory in young women for up to 4 years after HPV vaccination and showed fluctuations in their T cell responses over time without an obvious trend^[32].

Our interpretation that the decrease in T cell functionality over time reflected the progression of the HIV-associated immune deficiency is supported by the significant positive correlations of the HPV16 and HPV18 T cell responses with CD4+ T cell percentages and/or numbers and the negative correlations with HIV plasma RNA load and with the CD8+ T cell percentages and/or numbers at each visit. There was also a decrease in CD4+ T cells over time that did not reach statistical significance ($p=0.08$ in the mixed model analysis). Interestingly, the HPV-specific ELISPOT responses at 4 weeks after the 3rd dose of vaccine did not correlate with plasma HIV RNA, CD8+ T cells or nadir CD4+ T cells and only weakly correlated with CD4+ T cells at the time of vaccination^[30]. We avoided potential

technical confounders introduced by changes in the quality of the PBMC or of the assay characteristics over time by setting a viability criterion that we have previously shown to be appropriate for ELISPOT [36] and by testing samples from all time points for each participant in the same run. Moreover, a study-specific control consisting of PBMC from a healthy vaccine recipient was tested in every run and verified that the assay characteristics were constant across runs.

We did not find significant differences in the magnitude and persistence of T cell immunity to HPV16 and HPV18 between treatment Arms. These results also differed from the results of the study referenced above that compared the immunogenicity of two vs. three doses of QHPV in immune competent children and young women [33, 34, 37]. The study in immune competent hosts showed significant differences in IFN γ ELISPOT responses to two- compared with three-dose immunization regimens for both HPV16 and HPV18. Collectively, our results and those in immune competent hosts suggest that three doses of vaccine may be sufficient to reach maximum T cell responses to the vaccine.

The correlation analyses of B and T cell memory responses with serum antibody concentrations showed that higher T cell responses measured both by IFN γ and IL2 were associated with higher antibody concentrations. In addition, both antibody responses and T cell-mediated immunity were higher against HPV16 than 18. In contrast, B cell memory responses did not correlate with T cell ELISPOT or cLIA-measured antibody results and did not differ between HPV16 and 18. The correlation between T cells and antibodies is consistent with the T cell dependence of the antibody response to QHPV. Antibody responses to T cell dependent vaccines can be stimulated by Th1 (IFN γ), as well as Tfh (IL21) and Th2 (IL13) cytokines [37]. Of these, only IFN γ was measured in this study. Serum antibodies are produced by long-lived plasma cells. Memory B cells are important for anamnestic responses, but do not have a direct effect on serum antibody concentrations. However, since both long-lived plasma cells and memory B cells differentiate in the germinal center of the lymphoid tissues under the same stimulating conditions, the lack of correlation of memory B cells with T cell responses or antibody concentrations is surprising.

A few cases of break-through infections and low grade dysplasia have been reported in HPV-vaccine recipients [19, 38]. Persistent infections may represent low grade disease or deposition from an infected partner. Low grade disease due to vaccine types after HPV vaccination in those not infected prevalently are rarely seen and are presumably cleared by cytotoxic T cells [19, 38]. The potential role of vaccine-induced cell-mediated immunity in these situations is unknown and deserves further investigation.

This study was limited by the small samples size of the three-dose treatment group and by the premature closure of the study, which resulted in missing visits. The clinical significance of the decline in B cell memory responses to HPV and the general decline in T cell function over time in HIV-infected vaccinees is not known. However, the observations made in this study will apply to the nonavalent HPV vaccine, which is immunogenically noninferior to QHPV. It is also unclear if the current recommendations to start ARV at diagnosis of HIV infection will change the B and T cell responses of HIV-infected individuals including those to HPV vaccines or if a two-dose immunization schedule may be efficacious even if ARV

are initiated at diagnosis. Immunogenicity and efficacy studies of two doses of the HPV vaccines are needed before implementing this regimen in the HIV-infected group and effectiveness studies utilizing a large registry of HIV-infected individuals are needed to determine the protection conferred by the current three-dose regimen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Kinetics of T cell-mediated immunity between 2 and 5 years after the last dose of QHPV administration

Data were derived from 97 HIV-infected vaccine recipients, including 74 who received 4 doses of QHPV (Treatment A) and 23 who received 3 doses (Treatment B). T cell responses were measured by IFN γ and IL2 fluorospot and were expressed as SFC/ 10^5 PBMC (Y axis). In an unadjusted analysis, significant ($p < 0.05$) or marginally significant ($0.05 \leq p < 0.1$) response decreases over time were observed for IFN γ responses to PHA in treatment arm B, for IL2 responses in both treatment arms, and for IL2 responses to HPV16 in treatment arm A.

Figure 2. Kinetics of B cell-mediated immunity between 2 and 5 years after the last dose of QHPV administration

Data were derived from 97 HIV-infected vaccine recipients, including 74 who received 4 doses of QHPV (Treatment A) and 23 who received 3 doses (Treatment B). B cell responses were measured by IgG ELISPOT and were expressed as SFC/ 10^5 PBMC (Y axis). In an unadjusted analysis, significant ($p < 0.05$) response decreases over time were observed for HPV16 IgG in treatment arm A.

Table 1

Characteristics of Study Population at Enrollment in The Long-Term Follow-up Study

	Treatment Arm		
Category	A (N ¹ =74)	B (N=23)	P-value
Age			
Median (years)	12	12	0.426 ²
12 years	43 (58%)	13 (57%)	1.000 ³
Race/Ethnicity			
White (non-Hispanic)	5 (7%)	2 (9%)	
Black (non-Hispanic)	37 (50%)	6 (27%)	0.097 ³
Hispanic	31 (42%)	12 (55%)	
Gender (M/F)	32/42 (57%)	10/13 (57%)	1.000 ³
CD4 count (cells/μL)			
Median	710	749	.435 ²
<200	1 (1%)	0 (0%)	0.835 ²
200-1000	56 (76%)	17 (74%)	
>750	17 (23%)	6 (26%)	
CD4%			
Median	35	36	.699 ²
HIV RNA (copies/ml)			
Median	98	85	.472 ³
<400	9 (12%)	4 (17%)	.450 ³
400-5000	54 (73%)	16 (70%)	
5000	11 (15 %)	3 (13%)	
Antiretroviral (ARV)			
No ARV	1 (1%)	2 (9%)	0.006 ³
1 class of ARV only	2 (3%)	4 (17%)	
2 classes of ARVs	71 (96%)	17 (74%)	
cLIA antibody titers⁴			
HPV 16	1,922 (1331;2774)	376 (165;855)	<0.001 ²
HPV 18	198 (121;322)	39 (18;84)	<0.001 ²
LIA antibody titers⁴			
HPV 16	1,967 (1,303;2969)	357 (161;790)	<0.001 ²
HPV 18	230 (143;368)	45 (22;91)	<0.001 ²

¹ N= number of subjects² Wilcoxon test³ Fisher's Exact test

⁴ At 2 years after the last dose of vaccine; data missing from one participant in each group. LIA= luminescence immune assay; cLIA=competitive LIA

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Table 2

A. Comparison of IFN T Cell Responses to HPV16 and HPV18 within Arms

		Treatment Arm A			Treatment Arm B		
		HPV 16	HPV 18	P-value ¹	HPV 16	HPV 18	P-value
2 Years	N	54	54	<0.0001 ²	12	12	0.0278
	Median (Q1-Q3)	5 ³ (2-17)	3 (2-9)		5 (4-15)	2 (1-3)	
3.5 Years	N	43	43	0.0119	9	9	0.7188
	Median (Q1-Q3)	5 (3-10)	4 (2-8)		2 (1-8)	2 (1-6)	
4-5 Years	N	51	51	0.0605	12	12	0.0957
	Median (Q1-Q3)	4 (1-12)	3 (1-9)		1 (1-2)	2 (0-5)	
B. Comparison of IL2 T Cell Responses to HPV16 and HPV18 within Arms							
		HPV 16	HPV 18	P-value ¹	HPV 16	HPV 18	P-value
2 Years	N	54	54	0.0004 ²	12	12	0.0259
	Median (Q1-Q3)	28 ³ (8-59)	15 (6-38)		16 (8-46)	9 (2-23)	
3.5 Years	N	43	43	0.0219	9	9	0.4453
	Median (Q1-Q3)	25 (7-50)	13 (5-45)		12 (2-48)	19 (1-40)	
4-5 Years	N	51	51	0.1559	12	12	0.0332
	Median (Q1-Q3)	17 (2-36)	17 (3-33)		3 (1-13)	2 (0-10)	

¹ Paired Wilcoxon signed rank Test

² Bold-faced p-values indicate significant or marginally significant differences

³ SFC/10⁵PBMC

Table 3**A. Correlation of HPV16 cLIA Antibody Concentrations with T Cell ELISPOT**

		Treatment Arm A			Treatment Arm B		
Visit Year	Covariate	N	Spearman Corr. Coef.	P-value	N	Spearman Corr. Coef.	P-value
2 Years	HPV-16 T-cell IFN γ	52	0.33740	0.0166	12	0.03150	0.9225
	HPV-16 T-cell IL-2	52	0.52490	<.0001	12	0.59540	0.0411
3.5 Years	HPV-16 T-cell IFN γ	40	0.29860	0.0613	8	0.14290	0.7358
	HPV-16 T-cell IL-2	40	0.38300	0.0147	8	0.26190	0.5309
4-5 Years	HPV-16 T-cell IFN γ	48	0.34650	0.0158	9	0.48310	0.1877
	HPV-16 T-cell IL-2	48	0.44790	0.0014	9	0.70170	0.0351
B. Correlation of HPV18 cLIA Antibody Concentrations with T Cell ELISPOT							
Visit Year	Covariate	N	Spearman Corr. Coef.	P-value	N	Spearman Corr. Coef.	P-value
2 Years	HPV-16 T-cell IFN γ	52	0.27820	0.0504	12	-0.01630	0.9600
	HPV-16 T-cell IL-2	52	0.50260	0.0002	12	0.39040	0.2096
3.5 Years	HPV-16 T-cell IFN γ	40	0.19790	0.2210	8	0.04940	0.9076
	HPV-16 T-cell IL-2	40	0.25670	0.1099	8	0.73630	0.0372
4-5 Years	HPV-16 T-cell IFN γ	48	0.51710	0.0002	9	-0.09380	0.8104
	HPV-16 T-cell IL-2	48	0.73720	<.0001	9	0.29280	0.4445