Published in final edited form as:

Gynecol Oncol. 2007 September ; 106(3): 453–460.

Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III

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Abstract

Objectives—SGN-00101 (HspE7, Nventa, San Diego, CA) is a novel therapeutic vaccine consisting of a fusion protein containing an *M. bovis* BCG heat shock protein (Hsp65) covalently linked to the entire sequence of HPV 16 E7. This trial was designed to evaluate the efficacy and toxicities of HspE7 in women with CIN III.

Methods—HIV (–) women with biopsy-proven CIN III were eligible. Two cohorts were accrued; one cohort to establish efficacy and a second cohort with a longer follow-up period to improve the precision of the trial to estimate response rates. Each patient underwent 3 monthly subcutaneous vaccinations with 500 µg of HspE7 followed by monthly colposcopic follow-up for 1 month in cohort 1 and an extended observation period (2 months) in cohort 2. All patients then underwent a LEEP or cone biopsy of the cervix. A complete pathologic response (pCR) was defined as no evidence of CIN or CIN I (only HPV changes). A partial response (PR) was defined as colposcopic lesion regression of >50% in size. Cervicovaginal lavage samples were obtained at each visit for HPV typing using MY09/ MY11 HPV PCR.

Results—Seventy-two patients were registered and screened, of whom 64 were eligible. Fifty-eight patients completed the trial and were evaluable (31 in cohort 1, 27 in cohort 2). There were no significant epidemiologic or HPV type differences between the 2 cohorts so responses were combined for analysis. Of the 58 evaluable patients, 13 (22.5%) had a pCR; 32 (55%) had a PR and 11 (19%) had stable disease. Two (3.5%) patients in cohort 2 had microinvasive disease and were defined as progressive disease. Thirty-three of 58 (57%) of the patients were infected with HPV 16 prior to vaccination or in subsequent visits. There was no significant difference in regression in women infected with HPV 16 compared to those without HPV 16 infection (88% vs. 70%; $p=0.12$). Women who had a previous LEEP or ablation for CIN were 2.7 times more likely to have a complete response compared to patients without previous treatment, although the difference was not statistically significant (95% CI for rate ratio: 0.95–6.19, $p=0.10$). At a cellular level, there was a significant association between local inflammation and response; lower grade of lesional inflammation correlated with a response to HspE7 ($p=0.04$ using Wilcoxon rank sum test).

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Conclusions—HspE7 appeared to demonstrate activity in women with CIN III and met a priori assumptions for efficacy; however, it is unclear whether this response was due to natural regression rather than treatment effect. HspE7, which targets the HPV 16 E7 oncoprotein, had efficacy in patients infected with HPV types other than 16, suggesting cross-reactivity. A larger randomized, controlled trial is needed to better define efficacy and to identify subsets of women most likely to benefit from immunotherapy.

Keywords

HPV; Immunotherapy; Heat shock protein; HPV E7 protein

Introduction

Carcinoma of the uterine cervix is a common malignancy in young and middle-aged women worldwide. It is associated with high-risk HPV infection, especially HPV type 16, which is the most common oncogenic type worldwide [1–4]. Low-grade lesions (CIN I) usually regress spontaneously, whereas highgrade CIN II and III often persist and may progress to carcinoma [5,6].

The inability of HPV-infected women to mount an effective HPV-specific T and B cell immune response as well as specific host-mediated molecular events contribute to the pathogenesis of CIN and invasive cancer [7–9]. Current treatment strategies such as a Loop Electrosurgical Excision Procedure (LEEP) or cone biopsy of the cervix are aimed at destroying visible lesions and may eliminate HPV-infected cells and associated disease. Such ablative procedures may activate cellular immune responses to the associated HPV antigens. However, patients with CIN who have undergone ablative surgery or even a hysterectomy may still have HPV infection [10], which may result in recurrent or persistent CIN. Recently, there has been a number of new immunotherapeutics that have been in clinical trials for the treatment of HPV and cervical disease. Preliminary results of some of the more promising treatments are reviewed in a recent report [11].

Prophylactic HPV L1 vaccines are highly effective in preventing type-specific HPV 16 and 18 infection, but it will likely be decades before the true impact of prophylactic HPV vaccines will be fully appreciated [12]. Vaccination rates in the US and developed countries will vary markedly by birth cohort and depend on the percent of eligible women who will be vaccinated. Furthermore, HPV L1 vaccines will reduce but not eliminate cervical cancer, since many oncogenic HPV types are not included. Thus, these vaccines will reduce but not eliminate the need for screening and additional therapeutics for women who develop cervical cancer precursor lesions. Due to a trend of an increased rate of preterm birth in women with a history of LEEP or cone biopsy of their cervix, there is a need for alternatives to standard extirpative therapies [13].

There have been several recent reviews of therapeutic cervical cancer vaccines [8,14–17]. Nventa Biopharmaceuticals Corp. (San Diego, CA) has produced an immunotherapeutic vaccine called SGN-00101 (HspE7) that consists of the *M. bovis* BCG heat shock protein (Hsp65), covalently linked at its C terminus to the entire sequence of the HPV 16 E7 protein. Heat shock proteins (Hsp) loaded with antigen elicit significant T and B cell responses against microbial pathogens and tumor antigens [18–20] which are assumed to be partly responsible for clinical regression in vaccinated subjects. Clinical responses to HspE7 immunotherapy have been observed in children with recurrent respiratory papillomatosis [21] and men and women with genital warts [22] and anal intraepithelial neoplasia (AIN) [23]. Since responses to HspE7 were seen in these other HPV-related diseases, we tested HspE7 in women with CIN III. The aims of the current study were to determine the effect of HspE7 administration in women with

CIN III over a 5- to 7-month time period and to assess the toxicity profile of HspE7 in this study cohort.

Materials and methods

This was a single-arm, open-label, phase II study conducted by the New York Cancer Consortium (an NCI-sponsored consortium) and the New York Gynecologic Oncology Group (NYGOG) to establish the response rate and toxicities of HspE7 in women with biopsy-proven CIN III. A secondary aim was to correlate clinical responses to HspE7 vaccination in women infected with HPV 16 compared to women infected with other HPV types. The protocol was approved by the IRB of each participating institution.

Patient eligibility

Adult women with colposcopically directed biopsies showing histopathologic changes consistent with CIN III (CIN II–III or CIN III) were eligible for this trial. Diagnoses reported from the referring institutions were then centrally reviewed by the study pathologist (ASK) for confirmation before initiation of therapy. Patients who were immunocompromised, had a history of chronic, uncontrolled disease or were on immunosuppressive drugs were excluded. All eligible registered patients had entry laboratory studies that had to be within the following ranges: white blood cell count ($>3,500/\mu\text{l}$), hemoglobin ($>10\text{ g/dL}$), platelets ($>150,000/\mu\text{l}$), lymphocytes ($>500/\mu\text{l}$), total bilirubin ($<2\text{ mg/dL}$), AST/ALT ($<2.5\times$ the upper limit of normal) and creatinine ($<2\text{ mg/dL}$). Patients who had endocervical curettings that revealed CIN were eligible as long as the endocervical lesion was directly extending from the primary lesion and was colposcopically visible in its entirety. Patients with recurrent CIN lesions were eligible for this trial. All patients gave written informed consent.

Patients underwent a screening visit (visit 1) that included baseline renal, liver and hematologic studies as well as HIV testing (see Fig. 1 for schema of clinical trial). A detailed history was taken which included known demographic, behavioral and clinical risk factors. A baseline colposcopy was performed and findings were documented on grid colposcopy forms and photodocumented at sites where the equipment was available. Eligible patients were registered and underwent treatment with HspE7 administered in three 500- μg subcutaneous doses on alternating thighs at one-month intervals (Fig. 1). Patients remained in the office for observation following each vaccination for 1 h. In cohort 1, the patients underwent a Pap smear and a colposcopy at visit 5 (month 4). Colposcopists were not blinded to the participation of patients in the study since those providers were also administering HspE7. At visit 6 (month 5), all cohort 1 patients underwent a LEEP or cone biopsy of the cervix. After documentation of minimal toxicity and apparent efficacy in the first cohort, an extended, longer observation period in the second cohort was approved by the NCI to improve precision of the trial to estimate response rates. In cohort 2, the patients underwent an additional 2 months of observation during which time they had a colposcopy. At visit 8 (month 7), all of the patients in cohort 2 had a LEEP or cone biopsy of the cervix. Urine pregnancy tests were performed at every visit. Patients with a positive test were removed from the study.

Cervicovaginal lavage (CVL) and cervical brushes for HPV testing were obtained at all visits. Samples were processed by precipitating the DNA and resuspending the pelleted DNA in buffer. HPV identification was done by polymerase chain reaction (PCR) with the MY09/MY11 L1 consensus primers including primers which amplify a 450-bp HPV DNA fragment and a control primer set which simultaneously amplifies a cellular beta-globin DNA fragment and serves as an internal control for amplification, as previously reported [24]. PCR products that were positive with the HPV generic probe were analyzed for HPV DNA types using biotinylated type-specific oligonucleotide probes for multiple HPV types.

Response evaluation

Responses to HspE7 vaccination were determined by the histopathology of post-vaccination LEEP specimens or by colposcopic impressions. A pathologic complete response (pCR) was defined as a post-vaccine LEEP specimen negative for CIN. Since the diagnosis of CIN I/koilocytosis is often difficult and subjective and may not represent a true intraepithelial neoplasia [25], but an acute HPV infection, the diagnosis of CIN I in the LEEP specimen was included as a pCR. Since the histologic distinction between CIN II and CIN III can be difficult, we did not include a CIN II on a LEEP as a classification of partial response (PR). However, if there was a 50% or more reduction of lesion size post-vaccination on colposcopy or colpophotographs based on gross colposcopic measurements made on grid colposcopy forms (Fig. 2), this was classified as a clinical partial response (PR), based on gross colposcopic measurements made on the grid colposcopy forms (Fig. 2). Such a strategy for defining partial response has been utilized in other clinical trials involving cervical dysplasia [26,27]. Stable disease (SD) was defined as persistent CIN III with less than 50% reduction of lesion size. Progressive disease (PD) was defined as any colposcopically measured increase in lesion size or invasion identified on any biopsy performed during the study or in the final LEEP pathology.

Sample size calculation

The spontaneous regression rate of CIN III in a 6-month time period is not definitively known. Nearly all patients undergo timely treatment, only being observed if on a clinical trial. Prior studies have often combined cases of both CIN II and CIN III [28] or had a short observation period [29]. One clinical trial has reported the regression rate of CIN III in a treated versus placebo group [29] and evaluated the effects of oral supplementation of β -carotene on the regression of high-grade CIN. The regression rate of CIN III lesions in the placebo group was 19% in 24 months ($n=32$ with CIN III). In a study by Trimble et al. [28], the spontaneous regression rate of high-grade CIN (both CIN II and CIN III) in a 15-week period of time was 28%. In that trial, the number of the patients with CIN II and those with CIN III were not specified [28].

The patients in cohort 1 had a follow-up period of 6 months from the screening visit. For calculation of sample size, we estimated the spontaneous regression rate of CIN III in 6 months based on extrapolations from the Keefe et al. [29] study and from unpublished data from an ongoing patient registry at Montefiore Medical Center/Albert Einstein College of Medicine to be 10%. The sample size of 31 evaluable patients was determined assuming a single-stage Phase II design. Since the 6-month spontaneous regression rate in an untreated CIN III population was estimated to be 10%, a response rate of less than 10% was considered to be clinically insignificant (P_0). The target response rate in the vaccinated subjects was estimated to be 35% (P_1). The vaccine was to be rejected for further study if ≤ 6 responses were observed among the 31 patients. Under these assumptions, the study design had the following operating characteristics. The probability of accepting the treatment for further study if the response rate was unacceptably low ($<10\%$) was no more than 5%. There was a 95% probability of accepting the treatment for further study if the response rate was at least 35%. To adjust for an anticipated 15% loss to follow-up rate, the target enrollment was determined to be 36 patients. We used the initial efficacy seen in cohort 1 to power cohort 2 for added precision to estimate the response rate where we predicted the maximum width of the 95% confidence interval to be $\pm 12\%$ with 27 additional subjects.

Local inflammation

All LEEP specimens were graded for lesional and non-lesional inflammation as well as thorough documentation of the presence of meganuclei, koilocytosis, cervicitis and squamous metaplasia by the study pathologists. The degree of inflammation was graded by the study pathologists as follows: grades 0–1 represented no to minimal inflammation; grade 2

represented increased inflammation; grade 3 represented moderate inflammation; and grade 4 represented severe inflammation.

Data analysis

Data for response were analyzed for all women who received the study drug and completed the trial. Toxicities were analyzed in all patients receiving at least one dose of vaccine. Between-group differences in categorical variables were evaluated for statistical significance by Fisher's exact test. Continuous variables were compared between groups using the Wilcoxon rank sum test. Further exploratory analyses were also performed using the logistic regression model to identify potential predictors of response and adjust for confounders. Associations between lesional inflammation and clinical response were correlated using the Wilcoxon rank sum test. Statistical significance was defined as a p -value <0.05 . Analyses were performed using SAS version 8.2 (SAS Institute, Cary, NC).

Results

Patient characteristics

Fig. 3 describes the patient registration and their disposition. Seventy-two women with colposcopically directed biopsies showing CIN III or CIN II–III were enrolled into this phase II study at two institutions in the New York Cancer Consortium between November, 2003 and November, 2005. Eight patients withdrew consent prior to vaccination and did not receive study drug. Three patients received at least one dose of study drug, but were removed from the trial due to poor follow-up. One patient became pregnant after completing the vaccine series, and her LEEP was delayed until after her full-term, uneventful pregnancy. One patient desired a LEEP immediately after the 3 doses of the vaccine were administered, without an observation period. Despite her complete response, these data are not included in the efficacy and response data due to her early withdrawal from the study. Fifty-eight patients received all three doses of drug, completed the study and underwent a LEEP or cone biopsy of the cervix; 31 in cohort 1 and 27 in cohort 2. One patient underwent a hysterectomy for leiomyomas and menorrhagia instead of a LEEP. Clinical characteristics of these patients and response rates are shown in Table 1. There were no significant differences between the groups for race, ethnicity, HPV type status and number of HPV types, so the groups were combined for subsequent analyses. There were no significant differences (data not shown) between the groups for epidemiologic HPV risk factors (i.e. number of lifetime sexual partners, smoking status, history of sexually transmitted diseases, etc.).

Response to treatment

Of the 58 evaluable patients, 13 (22.5%) had a pCR; 32 (55%) had a PR and 11 (19%) had stable disease. Two (3.5%) patients in cohort 2 had microinvasive disease and were defined as having progression. Of the 13 patients who had a complete pathologic response, 8 had no CIN and 5 had CIN I on the LEEP specimen. Seven patients did not come back to the study investigators for their 12-month visit and were seen by their primary providers so follow-up information was not verified. Two out of 51 (3.9%) patients had a documented recurrence at their 12-month follow-up visits (range of follow-up of all patients 12–32 months). One of these patients had a complete response on trial and one had a partial response, both had no evidence of recurrence after repeat LEEPs (follow-up times for the patients are 21 and 22 months).

Although it did not reach statistical significance, women who had a previous LEEP or ablation for CIN ($n=11$) were 2.7 times more likely to have a complete response compared to patients without previous treatment (pCR rates: $5/11=45\%$ for previous LEEP vs. $8/47=17\%$ for no LEEP; 95% CI for rate ratio: 0.95–6.19, $p=0.10$). Of note, none of the patients who reported

a prior LEEP before HspE7 vaccination has recurred since HspE7 therapy (median follow-up time of this subset of patients is 26 months).

Similar to results of other HspE7 trials for other HPV-associated neoplasms [21–23,30], there was no difference in responses (pathologic or colposcopic) to HspE7 in patients without HPV 16 infection at any visit compared to those who were infected with HPV 16 (22/25 or 88% vs. 23/33 or 70%, $p=0.12$). Also, there was no difference in the clinical responses of women who were infected with one HPV type compared to those infected with multiple HPV types (data not shown), but this may be due to the small sample size. There was a decline in HPV dot blot intensity and eventual loss of HPV 16 in two patients who underwent a complete pathologic response. All patients who had a partial response were documented to have colposcopic regression after only one HspE7 vaccination. Other demographic, behavioral, and clinical risk factors were not found to be significantly associated with complete or partial response rates in either bivariate or multivariate analyses.

All but five patients (91%) had a grade 1 local reaction to HspE7 administration at one or more vaccinations consisting of one or a combination of the following local signs and/or symptoms: rash, pain, pruritis and swelling. All reactions were self-limited and were not present within 1 week except in two patients who had local reactions that lasted longer than a week, but eventually resolved. One patient had a grade 3 adverse event (seizure) for which she was hospitalized. She had a history of a seizure disorder and was found to be non-compliant with her anti-seizure medication as documented by a sub-therapeutic serum level of her anti-seizure medication. This adverse event was determined to be unrelated to the vaccination as it occurred 2 weeks after vaccination.

Many patients who exhibited a colposcopic regression without completely resolving their disease (patients with a PR) had minimal histopathologic evidence of acute and chronic inflammation involving the remaining intraepithelial neoplasia compared to women with stable disease (See Fig. 4). There was a significant association between inflammation and clinical response; lower grade of lesional inflammation correlated with response to HspE7 ($p=0.04$ using Wilcoxon rank sum). Of note, most of the subjects undergoing complete regression had findings of squamous metaplasia in the cervical quadrants of their previous CIN III lesions. Squamous metaplasia is frequently associated with cervical inflammation. This may have been associated with the immunologic (T cell) responses thought to be associated with lesional ablation.

Discussion

Preliminary results in this small cohort of women suggest that HspE7 is effective in treating a subset of women with CIN III and warrants further investigation. However, without a control arm, it cannot be ascertained if these responses were due to natural regression alone. HspE7 vaccine treatment met the predetermined criteria of at least a 35% clinical response rate (CR + PR). The observed clinical response rate (78%) was more than the estimated spontaneous regression rate of CIN III alone which is estimated to be 10% in 6 months (95% C.I.=67–89%). Furthermore, no patient had a serious drug-related adverse event during the observation period supporting the safety and tolerability of HspE7 in this clinical setting. Only mild, self-limiting, injection site-related side effects were encountered in this small clinical trial.

Forty to 50% of women with CIN III have been shown to have detectable HPV 16 infection [31–33]. Thirty-three of 58 (57%) evaluable patients in this trial were either documented to have HPV 16 on the first or subsequent visits. Eleven of 58 (19%) women on this trial had a history of previous ablative treatment for high-grade CIN. Surprisingly, a high percentage (5/11 or 45%) of these women with recurrent CIN III had a complete pathologic response to HspE7

immunotherapy. One woman who had a PR had a history of four previous LEEP procedures prior to vaccination with HspE7. One could hypothesize that these women would be less likely to have a response because they already had recurrent disease. Heat shock proteins are known to be immunostimulants and these patients may have been 'primed' for immunologic responses by previous ablative therapies, more so than patients who have had a cervical biopsy alone. Of note, none of these women with a history of recurrent CIN have recurred since HspE7 therapy. This observation of higher response rates to immunotherapy in women with recurrent CIN needs to be studied in larger cohorts. An extended analysis of the durability of responses in the patients on this study is ongoing.

Two study patients were defined as having progressive disease due to microinvasive disease identified on the final LEEP specimen. Both were in cohort 2. It is possible that these women had foci of microinvasion prior to enrollment which were not detected in the entry biopsies. However, it is also likely that these two patients had no response to HspE7 and progressed during the trial period. Many prior studies have addressed the poor positive predictive value of diagnosing microinvasive disease with colposcopy, probably due to the absence of characteristic features one would see in an invasive cancer [34–36]. In a large meta-analysis, the positive predictive rate of colposcopic impression was only 56% for microinvasive disease [34]. Because of this, we suggest as an important safety issue that extended periods of follow-up (>5 months) in women with CIN III are not recommended in future studies.

Since the original design and implementation of this trial, the data from the Atypical Squamous Cells of Unknown Significance (ASCUS)-Low Grade Squamous Intraepithelial Lesion (LGSIL) Triage Study (ALTS) trial showing discrepancies between colposcopy images were published [27,37]. There were only fair rates of agreement among ALTS colposcopy quality control reviewers. Much of the difference was probably due to the image quality [27]. Despite interpretation differences using colposcopy, in this trial, there was a local, microscopic clinical correlate of colposcopic response. There was a significant association between lesional inflammation in the LEEP and clinical response; lower grade of lesional inflammation correlated with complete pathologic and colposcopic response to HspE7 ($p=0.04$ using Wilcoxon rank sum). Further studies of these cellular and immune biologic correlates of response are underway.

Regression of CIN III in this trial occurred in both women infected with HPV 16 and women without HPV 16 infection. A trend existed where responses were less likely in HPV 16-infected women. This could be due to the more aggressive nature of HPV 16-associated CIN III. Without a control arm, this cannot be overinterpreted. However, future studies using this agent should not restrict patients based on the HPV type. The lack of HPV type-specific treatment efficacy has been reported in other HspE7 trials [22,23,30].

The natural regression rate assumptions of this trial were made prior to recently published natural regression data [28]. Trimble et al. report a 28% spontaneous regression rate of high-grade CIN in a 15-week period of time. The patients in that trial differ in many ways. First, the Trimble trial had a heterogeneous mix of CIN II and CIN III patients, so direct extrapolations of the natural regression of CIN III alone cannot be made. The natural regression rate of CIN III is known to be lower than that of CIN II [38]. In addition, the rate of HPV 16 infection (57%) and the rate of recurrent CIN lesions (19%) were much higher in our study than that reported in the Trimble study and that reported in the general pool of CIN III patients so direct comparisons of clinical responses cannot be made between these two studies.

Removing CIN does not necessarily eliminate persistence of the HPV infection as shown by the frequency of recurrence of CIN after LEEP [39]. Administration of immunotherapies such as HspE7 may be an attractive alternative to ablative surgical intervention for a subset of women

with high-grade CIN yet identified. Placebo-controlled trials need to be performed to fully determine the treatment efficacy and to identify the appropriate subsets of women with high-grade CIN for whom immunotherapy may be useful.

Acknowledgements

National Cancer Institute (NCI) Protocol Number 5850. Clinicaltrials.gov unique identifier NCT00075569.

The authors would like to acknowledge the support of the New York Gynecologic Oncology Group and the New York Cancer Consortium PI, Dr. J. Sparano.

Funding/Support: The New York Cancer Consortium is funded through the National Cancer Institute (N01 CM17103, Dr. Wadler at the time of trial initiation, now the PI is Dr. J. Sparano). HPV DNA testing is funded by CTEP Translational Research Initiative (Contract Number 24SX S020, Dr. Kadish). Funding is also provided by the National Center for Research Resources (K12 RR017672, Dr. Einstein). Translational studies were supported in part by the Center for AIDS Research at the Albert Einstein College of Medicine and Montefiore Medical Center funded by the National Institutes of Health (NIH AI-51519).

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Cohort 1

↑ Vaccination with HspE7
 ↑ Cervico-vaginal lavage (CVL) for HPV typing

Cohort 2:

↑ Vaccination with HspE7
 ↑ Cervico-vaginal lavage (CVL) for HPV typing

Fig 1.

Schema of study design. Vaccination with HspE7 was given at months 1, 2 and 3. In cohort 1, all patients had a LEEP or cone biopsy of the cervix at visit 6, 5 months after screening. In cohort 2, all patients had a LEEP or cone biopsy at visit 8, 7 months after screening. All patients were also evaluated 6 months after their LEEP/cone for recurrence of CIN.

Fig 2.

This is an example of a patient (sequence number P5850-MMC01-035) who had a partial response. A1 shows the pre-vaccination colposcopy with the associated colposcopy diagram on the study colposcopy grid form (B1). AWE—acetowhite, M—mosaicism. A2 shows the post-vaccination colposcopy and the associated colposcopy diagram (B2). Note that there is a decrease in lesion size between A1 and A2. This patient underwent colposcopic regression of >50% and had evidence of CIN III on her final LEEP pathology. This corresponds with the definition of a partial response (PR) for this study.

Fig 3. Patient registration and evaluable patients. There were 58 evaluable patients; 31 in cohort 1 and 27 in cohort 2.

Fig 4. Inflammation and clinical response. 100× view of a vaccinated patient with (A) a complete response, (B) a partial response, (C) a stable disease and (D) a progressive disease. Note the lesional inflammatory response surrounding the CIN more prominent in the patients with stable disease and progressive disease.

Table 1
Racial, ethnic and HPV type differences between cohorts

	Cohort 1	Cohort 2	<i>p</i> -value
<i>Race</i>			
Caucasian	18/31 (58%)	16/27 (59%)	1.00
African American	12/31 (39%)	11/27 (41%)	
Other	1/31 (3%)	0/27 (0%)	
<i>Ethnicity</i>			
Hispanic	14/31 (45%)	16/27 (59%)	0.31
Non-Hispanic	17/31 (55%)	11/27 (41%)	
<i>HPV type</i>			
HPV 16	15/31 (48%)	14/27 (52%)	0.64
Not HPV 16	12/31 (39%)	7/27 (26%)	
No HPV	2/31 (6.5%) ^a	4/27 (15%) ^b	
No Sample ^c	2/31 (6.5%)	2/27 (7%)	
<i>Number of HPV types</i>			
One HPV Type	14/27 (52%)	12/21 (57%)	0.78
Multiple HPV Types	13/27 (48%)	9/21 (43%)	

^aOne patient had HPV 83 consistently detected at later visits.

^bOne patient had HPV 18 consistently detected at later visits.

^cAll four patients with no sample at the first visit had HPV 16 detected at later visits and were defined as HPV 16 positive for subsequent analyses.