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Stratified risk of high-grade cervical disease using onclarity HPV extended genotyping in women, ≥25 years of age, with NILM cytology

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HIGHLIGHTS

- This study utilized extended genotyping to stratify ≥CIN3 and ≥CIN2 risk in women, ≥25 years, with NILM cytology.
- HPV 16 and 31 carried the highest risk for ≥CIN3 and ≥CIN2.
- Individual genotype reporting revealed risk strata associated with genotype groupings for ≥CIN3 and ≥CIN2.
- Clinical management for risk-based screening is discussed in the context of extended genotyping results.

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ABSTRACT

Objectives. Increasing evidence suggests that extended human papillomavirus (HPV) genotyping (beyond 16/18) is effective for risk stratification in women with normal cytology. This report provides extended genotyping results, using the BD Onclarity HPV Assay, for individual genotypes HPV16, 18, 31, 45, 51, and 52 and three pooled genotype results for HPV33/58, 35/39/68, and 56/59/66.

Methods. 27,037 women with normal cytology, ≥25 years, were enrolled into the Onclarity HPV trial during routine screening. Women positive for any HPV genotype were referred to colposcopy/biopsy. Hierarchical-ranked prevalence and risk values, associated with cervical intraepithelial neoplasia, grade 2 or worse (≥CIN2) or ≥CIN3, were calculated based on extended genotyping results.

Results. HPV 16 and 31 carried the highest risk for ≥CIN2 (11.6% and 12.1%, respectively) and ≥CIN3 (8.1% and 7.5%, respectively); these genotypes were the most prevalent in both ≥CIN2 (29.6% and 19.3%, respectively) and ≥CIN3 (43.7% and 22.5%, respectively). Of the other 12 genotypes, HPV 18, 33/58, and 52 comprised an intermediate risk band (≥CIN2 risk range: 4.9–6.8%; ≥CIN3 risk range: 3.9–5.0%). Genotypes 45, 51, 35/39/68, and 56/59/66 constituted the lowest risk band for both disease grades (≥CIN2 value risk range: 1.7–3.0%; ≥CIN3 value risk range: 1.2–3.6%).

Conclusions. Extended genotyping stratifies risk for ≥CIN2/3 in the ≥25 year-old, normal cytology population. While baseline HPV 16/31 values exceeded the risk threshold for colposcopy referral, the management of women with normal cytology who were positive for the intermediate- or lower-risk genotypes may evolve based on refined risk estimates as well as clinical factors.

1. Introduction

Persistent infection with one or more high-risk human papillomaviruses (HPVs) is necessary for the development of high-grade cervical intraepithelial neoplasia (CIN; precancer) and cervical cancer [1–4]. Multiple clinical trials, both here and abroad, have demonstrated greater sensitivity for detection of cervical cancer and pre-cancer using clinically validated HPV testing compared to cytology-based screening. In addition, HPV testing improves the negative predictive

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value (NPV) for ≥CIN3, compared to cytology alone providing better assurance to the vast majority of screened women that they are at low-risk for cervical disease or cancer if they have a negative HPV result [5–8]. HPV testing also provides improved detection of invasive adenocarcinoma or adenocarcinoma in-situ, both of which are difficult to detect through cytology-based screening alone [9,10]. These data have established the enhanced effectiveness of HPV detection compared to cytology-based screening and current guidance in the USA includes cytology/HPV co-testing for women, ≥30 years of age, and HPV primary screening for women, ≥25 or ≥30 years of age, as acceptable alternative screening approaches to cytology-alone [10–13].

Although HPV detection provides greater screening sensitivity and interval extension between screenings [13], concern exists regarding the relatively high number (compared to cytology) of HPV positive results in primary screening algorithms that do not give rise to high-grade cervical histopathology [12,14]. Hence, all HPV primary screening algorithms include a second triage step for HPV positive women. In the US, HPV primary screening includes both stratification by partial genotyping for HPV 16 and 18, and cytology triage for the "other 12" genotypes, to optimize the balance between disease detection and colposcopy referral [12,15]. Theoretically, triage in screening is based on the principle of equal management for equal risk, with a 5–6% five-year risk of biopsy proven ≥ CIN3 being increasingly accepted as the threshold for colposcopic referral. Recent epidemiologic data has indicated that some of the HPV genotypes grouped in the "other 12" pool may be associated ≥CIN3 risk values that are higher than HPV18. For example, genotypes 31, 33, 52, and 58 have been shown in some studies to pose disease risk, regardless of cytology, at or above the threshold [16] for referral to colposcopy [17–23]. Finally, it has been suggested that women with ASC-US and LSIL cytology, who test negative for the eight lowest-risk genotypes, may be below the risk threshold for referral to immediate colposcopy [21].

The BD Onclarity™ HPV assay (Onclarity) is Food and Drug Administration (FDA)-approved for detection of HPV in the ASC-US triage (≥21 years of age), cotesting (≥30 years of age), and primary HPV (≥25 years of age) screening populations. FDA approval for Onclarity includes partial genotyping for 16/18/45 in the former two screening populations, and 16/18 for the latter population [24]. Although extended genotyping (genotyping beyond HPV 16/18) is not currently FDA-approved for any HPV assay, Onclarity is currently the only FDA-approved assay with a design that provides the capability of extended genotyping through individual detection of HPV 31, 51, 52 (in addition to 16, 18, and 45) and pooled detection of 33/58, 35/39/68, and 56/59/66 [25]. In this study, results are provided from analysis of data collected from the baseline phase of the Onclarity HPV trial, which was conducted in the USA and included over 33,000 women participating during routine screening. This analysis includes prevalence and risk values based on genotyping data generated from the six individual, and eight pooled genotypes (beyond the current FDA-approved genotyping claims), from the NILM cytology subpopulation. Overall, these results are intended to establish risk values associated with ≥CIN2 and ≥CIN3 for individual genotypes within a large USA-based screening population, and help determine whether risk stratification through extended genotyping could be an effective method for triage of HPV positive women in future clinical practice.

2. Materials and methods

2.1. Study design

The BD Onclarity™ trial involves a baseline and a three-year, longitudinal phase. The baseline design and selection algorithm for colposcopy referral and biopsy were described in detail previously [25].

Detection of ≥CIN2 lesions (CIN2, CIN3, adenocarcinoma in situ, and invasive cervical cancer), following biopsy, was the primary study endpoint. For histology, the Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions guidelines for adjudication of CIN2 cases, using $p16^{INK4A}$ (P16)-assisted immunohistochemistry to supplement hematoxylin and eosin (H&E) histopathology, and incorporating Bethesda terminology, is simplified to the CIN terminology. Here, CIN1 indicates LSIL, CIN2 here indicates HSIL (CIN2), and CIN3 indicates HSIL (CIN3) [26].

All H&*E*-stained biopsies and endocervical curettage (ECC) samples were initially reviewed by two pathologists who were provided the age of the subject but otherwise blinded to all other study information. When two of the three pathologists agreed on a diagnosis using eight disease criteria, consensus was reached: unsatisfactory, negative [including no significant pathological findings, reactive or inflammatory processes, atypical squamous cell or glandular changes, or squamous metaplasia], CIN1, CIN2, CIN3, adenocarcinoma in situ (ACIS), squamous cell carcinoma (SCC), and adenocarcinoma (AC) and adenosquamous carcinoma (ASC) (the latter two were compiled into one category). Discordance between all three diagnoses for a specimen prompted a review by all three pathologists, together, to achieve a consensus diagnosis.

This analysis includes subjects ≥25 years of age enrolled at baseline. From the 33,858 subjects enrolled (Fig. 1), 224 were excluded from this analysis due to non-evaluable cytology. From the remaining 33,634 subjects, those with ≥ASC-US (n = 3082) or unsatisfactory (n = 63) cytology were not utilized in this analysis. 30,489 subjects with NILM cytology remained, from which, 3337 subjects, <25 years of age (30,489 minus 27,152), were removed. Subjects with non-evaluable HPV results (n = 115) were also removed, which resulted in 27,037 subjects, 24,482 HPV negative, and 2555 of whom were HPV positive. Of these (total of 27,037 subjects included in the analysis), 23,556, subjects were omitted from this analysis for the following reasons: 22,725 subjects were HPV negative and were not randomly selected for colposcopy, 524 were lost-to-follow up, 254 withdrew consent, 44 were pregnant, 11 subjects provided specimens that were collected in error, 5 subjects exited due to a hysterectomy, and 2 subjects exited due to principal investigator decision. Of the 3481 subjects having colposcopies/biopsies at visit 2, 1502 subjects were HPV negative, and 1979 subjects were HPV positive. Six cases from this group had unsatisfactory histology, resulting in 3475 cases with evaluable histology that consisted of: 3131 negative, 209 CIN1, 64 CIN2, and 71 ≥ CIN3. The overall HPV vaccination rate in the study (≥1 dose) was restricted to ≤10%.

This report was prepared according to STARD reporting standards. The study protocol was approved by institutional review boards at all study sites, and written informed consent was obtained prior to any trial-related procedures. This study was conducted according to the principles set forth by the Declaration of Helsinki and Good Clinical Practice [25].

2.2. Statistical analysis

First, a Bayesian model was utilized for all subjects, ≥25 years, to hierarchically rank the 9 different genotype channels based on ≥CIN3 baseline risk. For a subject with co-infections, the Bayesian model assigned the risk of ≥CIN3 to be equivalent to the highest risk genotype. The risk estimates from the Bayesian model were derived by Markov-Chain Monte Carlo (MCMC) methods; the genotypes were then ordered, hierarchically, based on the final risk estimates, and that order was used in subsequent analyses. Uninformative prior uniform distributions were used for estimation of all risk values. Data analysis was accomplished using SAS/STAT® and R software.

Specifically, the analysis resulted in an order of risk of HPV 16, 31, 18, 33/58, 52, 45, 35/39/68, 51, and 56/59/66 (see Fig. 2 for Bayesian model results restricted to NILM subjects). This order was then utilized to classify HPV positive subjects by hierarchical genotype rank: HPV 16, else 31, else 18, else 33/58, else 52, else 45, else 35/39/68, else 51, else 56/59/66. Verification bias adjustment (VBA) was performed for normalization of the difference in the selection rate for colposcopy/biopsy

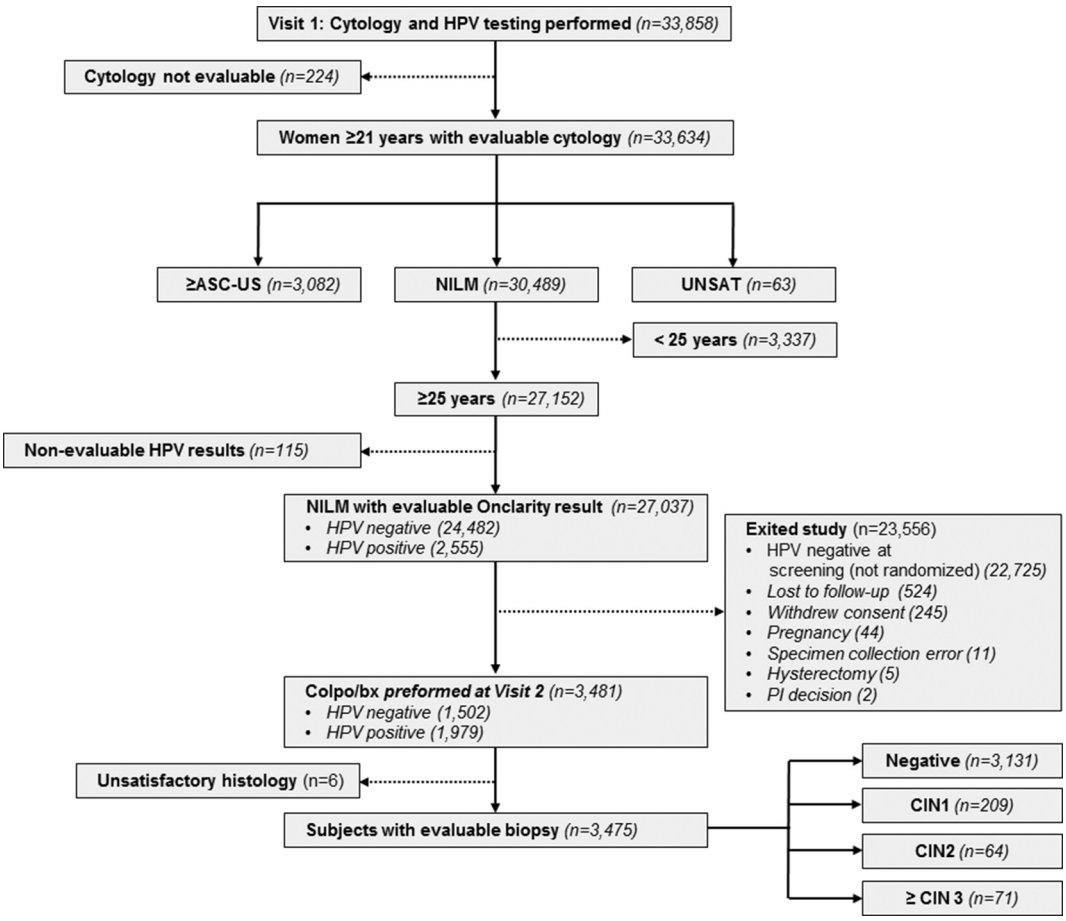

Fig. 1. Subject reconciliation during baseline enrollment and participation of subjects, ≥25 years of age, in this study. Abbreviations: NILM, negative for intraepithelial lesions or malignancies; HPV, human papillomavirus; ≥ASC-US, atypical squamous cells of undetermined significance or greater; UNSAT, unsatisfactory; PI, Principal investigator; colpo/bx, colposcopy and biopsy; CIN, cervical intraepithelial neoplasia.

[27]. Confidence intervals (CI; 95%) for adjusted risk values were calculated using bootstrapping. The lower and upper limits for the 95% CIs were determined using the 2.5 and 97.5 percentiles of the bootstrapped distribution.

3. Results

3.1. HPV prevalence and CIN histology in the age ≥ 25 and ≥30 year screening populations

The target study population included 27,037 and 22,284 women (with NILM cytology and valid HPV results), in the ≥25 year and ≥ 30 year age groups, respectively (Table 1). The mean and median ages for the ≥25 year age group were 41 and 40 years, respectively; for the ≥30 year age group they were 43.9 and 43, respectively. For the ≥25 year group, the racial make-up of the population in this study was 79.5% white, 17.5% African American, and 1.4% Asian; 20.1% of participants were Hispanic. The subjects were largely non-smokers with only 33.4% of subjects reporting to be current or former smokers. As the HPV vaccine was only available starting in 2006, and based also on capping the vaccine rate at 10% for the overall population, 93.4% of the study population was unvaccinated. Only 1.1% of the subjects were immunocompromised. 12.3% and 7.8% of the subjects reported having abnormal cytology or had a colposcopy procedure within 5 years prior to participation in this study. There were no meaningful differences in the ≥30 year age group with the exception that the frequency of vaccination was lower in the older women (5.4% vs 1.8%) (Table 1).

Any positive result for HPV was found in 9.5% and 7.9% of the ≥25 year and ≥30 year age groups, respectively. Individual and pooled genotype prevalence values were determined in a hierarchical manner, and are listed in Table 1. HPV 16, HPV 52, 35/59/68, and 56/59/68 were the four most prevalent genotyping results in both age groups. For histology, ≥CIN2 was diagnosed in 3.9% and 3.1% of the subjects that had a colposcopy/biopsy within the ≥25 year vs ≥30 year age group, respectively; ≥CIN3 was diagnosed in 2.0% and 1.8% of subjects that underwent a colposcopy/biopsy in the ≥25 year vs ≥30 year age group, respectively (Table 1).

3.2. HPV prevalence by CIN grade in subjects with evaluable histology

Overall HPV prevalence values, and individual and pooled genotype prevalence values (calculated hierarchically) are shown across CIN categories in Table 2. The prevalence of a qualitative positive HPV result increased in both ≥25 year and ≥30 year age groups with increasing CIN grade (≤CIN1 to ≥CIN3). In the ≥25 year age group, the prevalence increased for HPV 16 (from 7.1% to 43.7%), 31 (from 5.6% to 22.5%), and 18 (2.9% to 4.2%) as CIN grade became more severe (from ≤CIN1 to ≥CIN3)—known as enrichment. Prevalence values for the other 11 genotypes peaked at CIN2 and then declined for ≥CIN3. A similar pattern was observed for the ≥30 year age group except that HPV 33/58 and 45 also increased in prevalence from CIN2 to ≥CIN3, demonstrating enrichment. The prevalence of CIN across individual and pooled genotypes is shown in Table S1. ≥CIN3 was most prevalent in HPV 16 (11.2%), followed by HPV 31 (7.5%), HPV 18 (2.9%), and HPV 33/58 (2.8%), and the other nine genotypes (all of which had prevalence values below 2.0%). A similar pattern was observed in the ≥30 year age group.

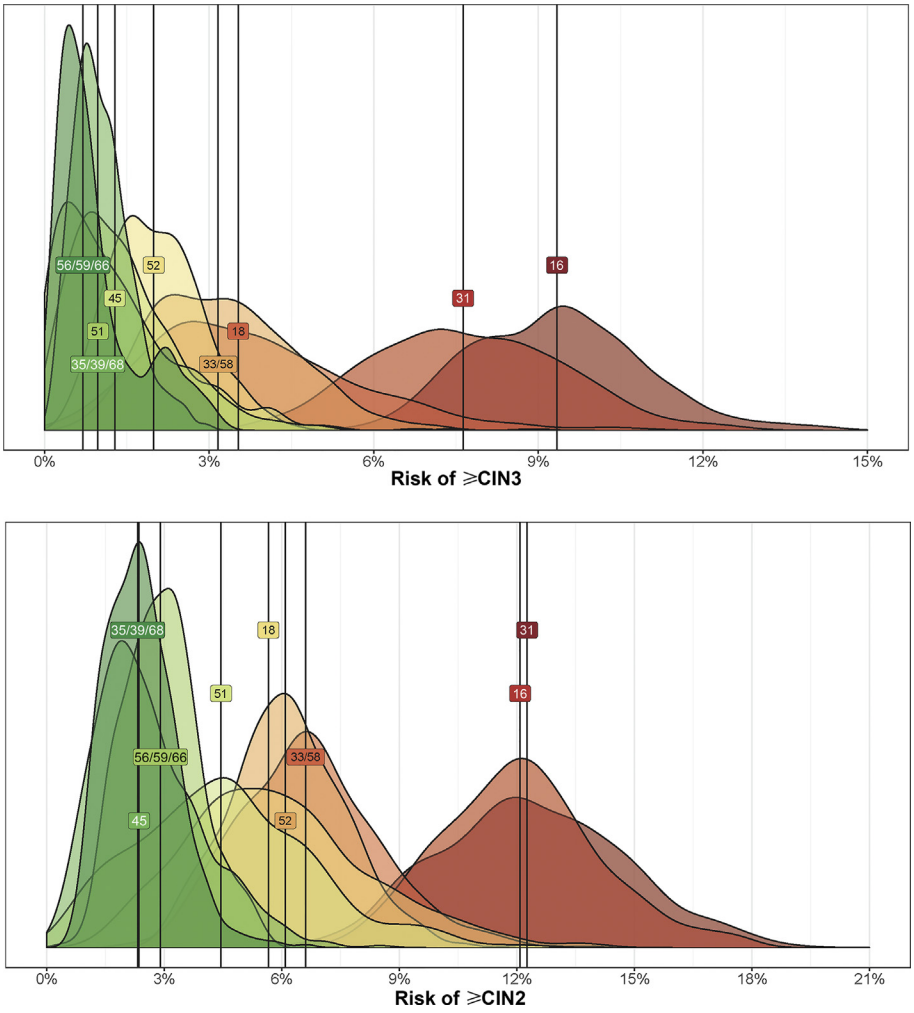

Fig. 2. Bayesian model for risk of ≥CIN3 and ≥CIN2 by HPV genotype using Markov-Chain Monte Carlo methods and including 95% confidence intervals. Abbreviations: HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia.

3.3. Risk for ≥CIN2 and ≥CIN3 associated with Onclarity results

Individual risk values for ≥CIN3 and ≥CIN2, associated with HPV genotyping (both individual and pooled genotyping results) using Onclarity, were determined by projecting disease status to all subjects based on women with evaluable histology (Table 3). Genotypes in Table 3 are listed in rows by descending order of ≥CIN3 risk, as determined by multivariate analysis (Fig. 2; see Methods). In Table 3, HPV 16 and 31 had the highest risk values for the ≥25 year (8.1% and 7.5%, respectively) and ≥30 year (6.9% and 8.4%, respectively) age groups. HPV 18, 33/58, 52, and 45 all had intermediate risk values for ≥CIN3. HPV 35/39/68, 51, and 56/59/66 had the lowest ≥CIN3 risk values for both age groups, the latter of which, was close to those for HPV negative individuals. ≥CIN2, risk values followed a similar pattern. HPV 16 and 31 had the highest risk values associated with ≥CIN2 in both ≥25 year (11.6% and 12.1%, respectively) and ≥30 year (9.3% and 11.7%, respectively) age groups. HPV 18, 33/58, and 52 all had similar intermediate risk values for ≥CIN2 in both age groups. HPV 33/39/68, 51, and 56/59/66 had similar, lower-risk values for ≥CIN2 in both age groups. HPV negative risk values were the lowest for both age groups (Table 3).

4. Discussion

This analysis demonstrates that genotypic information stratifies the risk of precancer/cancer for subjects who have NILM cytology that could comprise an HPV primary screening population. Individual genotypes HPV 16 and 31 carry baseline risk values for ≥CIN3 that are above the five-year risk threshold for referral to colposcopy (5–6%; based on risk values associated with LSIL cytology or ASC-US and HPV positive results) [13,16,20]. In addition, combined risk values for HPV 16/31/18/(33/58)/52 were at or above this risk threshold at baseline (5.2%; Table S2). Equal management for equal risk is a guiding principle for clinical decision making during cervical cancer screening and management [13,20]. Although additional factors may need to be considered (e.g., clinical action risk thresholds, total number of high-grade cervical disease cases detected, and total number of colposcopy referrals), this study provides evidence that, with NILM cytology, other genotypes (e.g., 31) may have similar risk to HPV 18 and may be considered for similar management.

Analysis of disease prevalence revealed a distribution in which genotype prevalence across ≥CIN2 and ≥CIN3 was highest for HPV 16 and 31, was intermediate for genotypes 18, 33/58, 52, and 35/39/68, and was lowest for genotypes 45, 51, and 56/59/66. The results here are consistent with previous studies conducted in population-based studies that reported the highest prevalence values from HPV 16, 31, 18, 33/58, and 52 within ≥CIN2 and ≥CIN3 for women with NILM cytology [19–23]. For example, in a sub-analysis from the ATHENA study, which involved genotyping from 34,823 samples (≥25 years, with NILM cytology), HPV16 and 31 represented 48.8% and 43.0% of ≥CIN2 and ≥CIN3, respectively (compared to 48.9% and 66.2%, respectively, here). Here, lower prevalence values within ≥CIN3 were found for

Table 1
Baseline age, Onclarity HPV result, and histology for subjects with NILM cytology[a,b].

	≥25 years (n = 27,037)	≥30 years (n = 22,284)
Age (years)		
Mean (SD)	41 (10.9)	43.9 (9.6)
Median	40	43
Min	25	30
Max	83	83
Race		
Asian	1.4% (390)	1.5% (336)
African American	17.5% (4729)	16.6% (3700)
White	79.5% (21,484)	80.4% (17,914)
Other[c]	1.6% (434)	1.5% (334)
Ethnicity		
Hispanic or Latino	20.1% (5440)	20.5% (4570)
Not Hispanic or Latino	79.9% (21,595)	79.5% (17,712)
Other	<0.1% (2)	<0.1% (2)
Smoking history		
Nonsmoker	66.6% (18,007)	66.1% (14,730)
Current	14.9% (4017)	14.3% (3197)
Past	18.5% (5013)	19.6% (4357)
HPV vaccinated		
Yes	5.4% (1451)	1.8% (399)
No	93.4% (25,263)	97.2% (21,668)
Unknown	1.2% (323)	1.0% (217)
Postmenopausal	20.6% (5571)	25.0% (5566)
Immunocompromised	1.1% (309)	1.3% (284)
Abnormal cytology (past 5 years)	12.3% (3333)	10.4% (2324)
Colposcopy (past 5 years)	7.8% (2114)	6.4% (1434)
Onclarity result		
Any HPV pos	9.5% (2555)	7.9% (1761)
HPV16	1.8% (481)[d]	1.5% (330)[e]
HPV31	0.9% (253)	0.8% (186)
HPV18	0.5% (122)	0.4% (89)[f]
HPV33/58	0.8% (224)	0.7% (157)
HPV52	1.1% (297)	0.9% (197)
HPV45	0.6% (152)	0.5% (104)[g]
HPV35/39/68	1.8% (494)	1.5% (342)
HPV51	0.5% (128)	0.3% (76)
HPV56/59/66	1.5% (404)	1.3% (280)
HPV neg	90.5% (24,482)	92.1% (20,523)
Histology		
No colposcopy	87.1% (23,556)	88.3% (19,687)
Colposcopy	12.9% (3481)	11.7% (2597)
NEG	89.9% (3131)	91.7% (2382)
CIN1	6.0%% (209)	5.0% (129)
≤CIN1	95.9% (3340)	96.7% (2511)
CIN2	1.8% (64)	1.3% (34)
≥CIN2	3.9% (135)	3.1% (80)
≥CIN3	2.0% (71)	1.8% (46)
UNSAT	0.2% (6)	0.2% (6)

Abbreviations: HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies; SD, standard deviation; Min, minimum age; Max, maximum age; pos, positive; NEG/neg, negative; CIN, cervical intraepithelial neoplasia; UNSAT, unsatisfactory; ACIS, adenocarcinoma in situ.
[a] Only includes subjects with valid (negative or positive) HPV results.
[b] HPV genotype prevalence determined hierarchically.
[c] Includes American Indian, Alaska Native, Native Hawaiian, or other Pacific Islander, or other.
[d] Includes 1 case of ACIS.
[e] Includes 3 cases of ACIS.
[f] Includes 1 case of ACIS.
[g] Includes 1 case of ACIS.

Table 2
HPV genotype prevalence within cervical disease in women with NILM cytology[a,b].

Onclarity result	≥25 years (n = 3475)			
	≤CIN1 (n = 3340)	CIN2 (n = 64)	≥CIN2 (n = 135)	≥CIN3[c] (n = 71)
Any HPV pos	55.6% (1857)	87.5% (56)	89.6% (121)	91.5% (65)
16	7.1% (237)	14.1% (9)	29.6% (40)	43.7% (31)
31	5.6% (188)	15.6% (10)	19.3% (26)	22.5% (16)
18	2.9% (98)	3.1% (2)	3.7% (5)	4.2% (3)
33/58	5.0% (168)	10.9% (7)	8.9% (12)	7.0% (5)
52	6.7% (225)	15.6% (10)	10.4% (14)	5.6% (4)
45	3.6% (119)	1.6% (1)	1.5% (2)	1.4% (1)
35/39/68	11.9% (397)	12.5% (8)	8.1% (11)	4.2% (3)
51	3.0% (100)	3.1% (2)	2.2% (3)	1.4% (1)
56/59/66	9.7% (325)	10.9% (7)	5.9% (8)	1.4% (1)
HPV neg	44.4% (1483)	12.5% (8)	10.4% (14)	8.5% (6)
Onclarity result	≥30 years (n = 2591)			
	≤CIN1 (n = 2511)	CIN2 (n = 34)	≥CIN2 (n = 80)	≥CIN3[d] (n = 46)
Any HPV pos	51.4% (1291)	79.4% (27)	87.5% (70)	93.5% (43)
16	6.5% (162)	11.8% (4)	27.5% (22)	39.1% (18)
31	5.4% (136)	14.7% (5)	22.5% (18)	28.3% (13)
18	2.9% (74)	2.9% (1)	3.8% (3)	4.3% (2)
33/58	4.9% (123)	5.9% (2)	6.3% (5)	6.5% (3)
52	6.1% (152)	17.6% (6)	10.0% (8)	4.3% (2)
45	3.3% (82)	0.0% (0)	1.3% (1)	2.2% (1)
35/39/68	11.0% (275)	11.8% (4)	7.5% (6)	4.3% (2)
51	2.2% (54)	2.9% (1)	2.5% (2)	2.2% (1)
56/59/66	9.3% (233)	11.8% (4)	6.3% (5)	2.2% (1)
HPV neg	48.6% (1220)	20.6% (7)	12.5% (10)	6.5% (3)

Abbreviations: HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies; CIN, cervical intraepithelial neoplasia; pos, positive; neg, negative; ACIS, adenocarcinoma in situ.
[a] Only includes cases with reportable HPV results and satisfactory histology.
[b] Prevalence values determined hierarchically.
[c] Includes 1 case of ACIS.
[d] Includes 5 cases of ACIS.

HPV 18 (4.2% versus 7.8%) and HPV 52 (5.6% versus 11.7%) compared to genotyping (using the LA-HPV assay) from the ATHENA trial; whereas prevalence values were elevated here within ≥CIN3 for HPV 33/58 (7.0% versus 3.9%). Importantly, prevalence values were determined in a hierarchical manner in ATHENA (similar to this study) and the overall disease prevalence found in the ATHENA for ≥CIN2 (3.7%) and ≥CIN3 (2.3%), was similar to that observed in this study (3.9% and 2.0%, respectively) [19].

Prevalence values for individual and pooled genotypes among the NILM cytology population were determined for both ≥25 year and ≥30 year age groups. HPV 16, 31, 18, (including 33/58 in the ≥30 age group only) showed a progressive increase in prevalence as disease severity increased from ≤CIN1 to ≥CIN3; with HPV 16 and 31 having the highest prevalence within ≥CIN2 and ≥CIN3 disease categories for both ≥25 year and ≥30 year age groups. All other genotypes either peaked in prevalence at CIN2 (HPV 52, 35/39/68, 56/59/66), or remained relatively flat (HPV 45, 51) as disease severity increased. This is consistent with prior results describing enrichment [28].

Presently in US clinical practice, it is often underappreciated that HPV positive women with NILM cytology fall below the ≥CIN2 risk threshold for colposcopy. Current US guidelines regarding co-testing for women ≥30 years of age and HPV primary screening for women ≥25 or ≥30 years of age recommend only HPV 16 and 18 positive women with NILM be referred for colposcopy based on the risk of precancer or cancer in these groups. [10–13] HPV 16 and 18 are the first and second most prevalent oncogenic genotypes in cervical cancer — accounting for 65–75% of cases [1,9,29]. In this study, HPV 16 was associated with a high baseline risk for high-grade cervical disease among women with NILM cytology [17–23]. However, HPV 18, was only the fifth highest genotype for ≥CIN2 and the third highest for ≥CIN3 by risk in the ≥25 year age group. Depending on several factors, including study design, geographic location, age of the study population, and length of follow-up, previous studies involving the NILM population have reported HPV 18 as the second-to-fourth highest in risk among the else-16 genotypes for ≥CIN3 [17–23]. For example, HPV 18 was reported as the third or fourth highest genotype (depending on age group) at baseline for ≥CIN3 in the ATHENA study [19], whereas it was the second highest in Kjaer et al. after 13.4 years of follow-up [18] and in Schiffman et al. after a 3-year follow-up [20]. It will be of interest to see if HPV 18 transitions to a higher risk of ≥CIN3 in the 3 year longitudinal analysis of the trial data.

Table 3
Risk of cervical disease by HPV genotype in women with NILM cytology[a].

Genotype[b,c]	≥CIN3	
	≥ 25 years (95% CI)	≥ 30 years (95% CI)
HPV pos	3.1% (2.5, 3.9)	3.0% (2.1, 3.9)
16	8.1% (5.8, 10.9)	6.9% (3.8, 10.2)
31	7.5% (4.1, 11.3)	8.4% (4.4, 13.7)
18	2.9% (0.0, 6.8)	2.6% (0.0, 6.7)
33/58	2.8% (0.6, 5.4)	2.3% (0.0, 5.3)
52	1.7% (0.4, 3.4)	1.2% (0.0, 3.3)
45	0.8% (0.0, 2.8)	1.2% (0.0, 4.1)
35/39/68	0.7% (0.0, 1.7)	0.7% (0.0, 1.9)
51	1.1% (0.0, 3.6)	1.8% (0.0, 5.9)
56/59/66	0.3% (0.0, 0.9)	0.4% (0.0, 1.3)
HPV neg	0.2% (0.0, 0.5)	0.1% (0.0, 0.3)
	≥CIN2	
HPV pos	6.0% (4.9, 7.1)	5.1% (3.9, 6.3)
16	11.6% (8.2, 15.3)	9.3% (5.6, 13.6)
31	12.1% (7.8, 16.7)	11.7% (6.5, 17.5)
18	4.9% (1.0, 9.5)	3.9% (0.0, 8.9)
33/58	6.8% (3.4, 10.5)	3.9% (0.8, 7.5)
52	5.9% (3.0, 9.3)	5.0% (1.9, 8.5)
45	1.7% (0.0, 4.4)	1.2% (0.0, 4.1)
35/39/68	2.7% (1.2, 4.2)	2.1% (0.7, 4.1)
51	3.0% (0.0, 6.8)	3.6% (0.0, 9.2)
56/59/66	2.4% (0.9, 4.4)	2.1% (0.4, 3.9)
HPV neg	0.6% (0.2, 1.0)	0.5% (0.1, 1.0)

Abbreviations: HPV, human papillomavirus; NILM negative for intraepithelial lesions and malignancies; CIN, cervical intraepithelial neoplasia; CI, confidence interval; pos, positive; neg, negative.

[a] Only includes cases with reportable HPV results and satisfactory histology.

[b] All genotypes listed in order determined according to ranking values obtained from ≥CIN3 calculations in the ≥25 year population (see Fig. 2).

[c] Genotypes were assigned hierarchically (16, else 31, else 18, else 33/58, else 52, else 45, else 39/68/35, else 51, else 59/56/66, else HPV neg); verification bias adjustment was applied with bootstrapping to determine 95% confidence intervals.

HPV 31 is among the most prevalent oncogenic genotypes (behind HPV 16, 18, and 45) in cervical cancer, worldwide [9,18,20,28]. In the current analysis, HPV 31 carried a risk for ≥CIN3 in the ≥25 year age group that was second only to HPV 16. Moreover, the risk value for ≥CIN3 posed by HPV 31 in the ≥30 year age group, and ≥CIN2 in both age groups, was higher than that for HPV 16, here. HPV 31 risk values vary to a degree in their relative ranking among the else-16 genotypes for ≥CIN3. Similar to the results here, genotyping at baseline from the ATHENA study resulted in the second-to-fourth highest ≥CIN3 risk associated with HPV 31—depending on age [19]. However, several studies, both USA-based and European, have reported HPV 33 to be the second highest genotype after HPV 16 for risk detection of ≥CIN3 [17,18,20,22,23]. It is important to note that previous work, showing a higher risk for HPV 33, included longitudinal analyses, which will be important to conduct for HPV 31 and HPV 33/58 from Onclarity trial data in future analyses.

The results here support hierarchical genotype groupings, or risk bands, that might be important to consider if and when guidelines for cotesting and primary HPV screening algorithms are reviewed and/or modified in the future. Schiffman and colleagues previously discussed the pros and cons of leveraging stratified genotype groupings as part of primary HPV screening triage to facilitate risk-based cervical cancer screening [21]. Specifically, four strata have previously been identified that represent risk bands to direct clinical actions ranging from immediate treatment to 3–5 year follow-up. Our data support this general outline for genotyping as part of risk-based screening (Fig. 3). However, additional analysis will be required to fine-tune the inclusion or exclusion of certain genotypes in particular bands that direct women to immediate colposcopy. For example, do HPV 31 or HPV 33 confer high-enough risk to be included with HPV 16 and 18 during primary screening to support a referral to colposcopy? In the ≥25 year screening population (n = 27,037) here, inclusion of HPV 31 with HPV 16/18 would detect approximately 16 additional cases of ≥CIN3. However, the identification of additional ≥CIN3 cases facilitated by the inclusion of HPV 31 into the HPV 16/18 primary screening algorithm would require approximately 200 additional colposcopies (approximately 12 more colposcopies per ≥CIN3 detected) (Fig. S1). As another example, does the prevalence of HPV 45 in adenocarcinomas (3rd after HPV 16 and 18) warrant elevating the overall risk band for HPV 45 above that determined by risk of ≥CIN3, similar to current clinical action based on a positive result for HPV 18.

Ultimately, the placement of genotypes into risk strata will require consensus from multiple prospective and observational studies and modeling studies that provide risk estimates and corresponding number of colposcopy referrals in order to provide an accurate risk-benefit analyses. Resource utilization and cost-effectiveness may also be considered for future guidelines. Including additional genotypes to HPV 16/18 for immediate colposcopy in HPV primary screening algorithms is ultimately a question of balance: do additional colposcopies represent a significant benefit to patients who would otherwise return in 1 year for a follow-up versus the risk of loss to follow-up and lack of treatment of prevalent CIN2/3. The same tensions already exist in all cervical cancer screening strategies that involve triage of a portion of patients.

At the other extreme, genotypes in the lowest risk band had values here (and reported elsewhere) that approached risk for ≥CIN3 associated with HPV negative in NILM cytology women. HPV 56/59/66, for example, had ≥CIN3 risks of 0.3% and 0.4% in the ≥25 year and ≥ 30 year age groups, respectively. Future analyses of additional longitudinal Onclarity data should help inform the discussion regarding the risk/benefits associated with a deferral of immediate colposcopy to a 1-year follow-up based on the low ≥CIN3 risk conferred by these genotypes in women with equivocal or worse cytology. Finally, additional reporting of individual genotypes, as part of primary screening, could potentially be beneficial as it will allow clinicians to track true genotype persistence during follow-up and to adjust patient risk for cancer and pre-cancer accordingly.

Strengths of this study included a large screening population from multiple centers across the USA. The NILM population described here yielded sufficient numbers of CIN2/3 to allow reliable calculations for prevalence and risk associated with individual genotypes. Colposcopy procedures were standardized across all sites and included cervical biopsy and/or ECC in the absence of visible lesions. All biopsy samples were subjected to a consensus pathology review by gynecologic pathologists who were blinded to all clinical/laboratory information (except age). In addition, substantial efforts were made to resolve equivocal CIN2 diagnoses through the use of p16 immunohistochemistry during adjudication. The data described here were obtained from first-take liquid-based cytology (LBC) samples which eliminates potential variability in viral concentration that can arise during analysis of samples from subsequent LBC specimen takes. In addition, this data was obtained from a validated, FDA-approved assay, with cutoff values that are relevant to clinical practice. Caution during interpretation of these findings should be taken since the results here represent baseline data; forthcoming longitudinal results should help alleviate this issue. Other potential confounding factors include interpretation of results when comparing pooled results (33/58, 35/39/68, and 56/59/66) here with individual results from genotyping in previous work. Finally, it should be noted that risk values associated with genotypes (e.g., HPV31 and HPV33) can vary in prevalence and virulence depending on the country or region in which screening is being conducted. While this trial is representative of genotype prevalence/virulence in the US, it may not represent the epidemiological characteristics for these same genotypes in other countries with the same accuracy; these results should be interpreted accordingly.

5. Conclusions

As further supportive data continues to emerge for risk-based strategies, genotype-based risk stratification (with or without cytology

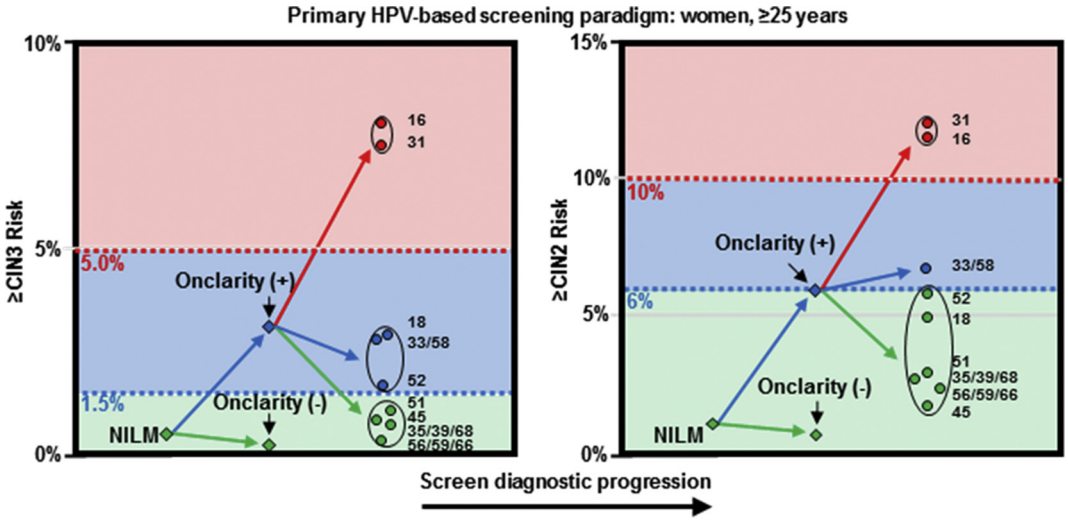

Fig. 3. Clinical management based on risk for high-grade cervical disease associated with individual HPV genotypes in women with NILM cytology. [a]The risk for ≥CIN3 (left graph) and ≥CIN2 (right graph) for women with NILM cytology is stratified by overall HPV result (based on Onclarity assay) and then by individual HPV genotype. At each level of stratification, the results are plotted by risk value (y-axis) into one of three zones: (1) green, which indicates a return to routine screening, (2) blue, [b,c] which indicates a return for repeat testing in 1 year and (3) red, [d,e] which indicates a referral to colposcopy. Abbreviations: HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies; CIN, cervical intraepithelial neoplasia. [a]The threshold values indicated in the figure serve an illustrative purpose only and are not the result of any systematic determination of consensus thresholds; they are also not specific to any particular country-based guideline. [b]The 1-year return threshold for ≥CIN2 (6%) is based on the NILM/HPV(+) ≥CIN2 risk value (5.1%) reported in the Onclarity trial co-testing population by Stoler et al., 2018 [30]. [c]The 1-year return threshold for ≥CIN3 (1.5%) is based on the NILM/HPV(16-/18-/45-) ≥CIN3 risk value (2.2%) reported in the Onclarity trial co-testing population by Stoler et al., 2018 [30] and the risk value (1.5%) for NILM/HPV (16-/18-) reported by Kahn et al., 2005 [31]. [d]The immediate colposcopy threshold for ≥CIN2 (10%) is based on the NILM/HPV (16+/18+) ≥CIN2 risk value (8.1%) reported in the Onclarity trial co-testing population by Stoler et al., 2018 [30] and the risk value (10.8%) for NILM/HPV (16+/18+/31+) reported here. [e]The immediate colposcopy threshold for ≥CIN3 (5.0%) is based on the NILM/HPV (16+/18+) ≥CIN3 risk value (5.9%) reported in the Onclarity trial co-testing population by Stoler et al., 2018 [30] and the risk value (5.2%) for LSIL cytology reported by Katki et al., 2013 [16].

triage) is expected to gain importance in the ongoing evolution of clinical management guidelines for cervical cancer screening. However, the impact of risk-based decision making will be dependent on the balance between sensitivity for disease detection and minimization of colposcopy procedures. Optimal stratification of genotypes into risk strata that mesh with clinical action thresholds is a promising advance and is feasible with clinically validated HPV assays that report extended genotyping information. Based on these results, the addition of HPV 31, (to HPV 16/18), as part of a primary HPV screening algorithm recommendation for colposcopy, offers potential benefit for detection of high-grade cervical disease in the NILM cytology population. More extensive analyses are currently in progress to determine how extended genotyping, reported by the Onclarity HPV assay, can be utilized in combination with cytology triage to optimize the balance of disease detection and colposcopy referrals.

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Potential conflicts of interest

MHS serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, Inovio Pharmaceuticals and Merck and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences.

TCW serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, and Inovio Pharmaceuticals and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems.

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