



Detection of high-grade cervical neoplasia using extended genotyping: Performance data from the longitudinal phase of the Onclarity trial

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HIGHLIGHTS

- 29,513 subjects had evaluable cytology and valid HPV test results at baseline and 4985 were followed for three years.
- Three-year risk of $\geq$ CIN3 in HPV-negative women was statistically indistinguishable from that for co-testing negative women.
- HPV16 and HPV31 carried the highest $\geq$ CIN3 baseline and cumulative incident (three-year) risk values regardless of cytology.
- Extended genotyping resulted in a good balance of disease detection and risk stratification.
- Genotype-specific persistence led to greater $\geq$ CIN3 risk stratification than genotype switch or new infections.

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ABSTRACT

Objectives. The Onclarity cervical cancer screening trial was designed to establish the clinical validity of the Onclarity HPV assay for extended genotyping (xGT) during detection of high-grade cervical neoplasia grades 2 or 3 ($\geq$ CIN2 or $\geq$ CIN3). Here, three-year follow up data is presented to evaluate the overall efficacy of these screening strategies, compared to the baseline data.

Methods. At baseline 29,513 women, $\geq$ 25 years, had evaluable cytology and valid high-risk HPV results. Women with atypical squamous cells-undetermined significance or worse cytology or a positive HPV test were referred for colposcopy/biopsy. Participants that did not reach the study end point (treatment for $\geq$ CIN2) continued into the longitudinal phase that included the same protocol as baseline.

Results. The three-year cumulative incident risk (CIR) for $\geq$ CIN3 in HPV-negative women was 0.15% [95%CI: 0.06, 0.26] and for HPV- and cytology-negative women was 0.12% [95% CI: 0.03, 0.23]. HPV16 carried the highest baseline and three-year $\geq$ CIN3 CIR, followed by HPV31 and HPV18. At least one year of genotype-specific persistence increased $\geq$ CIN3 risk for xGT results compared to genotype non-persistence, HPV clearance, or new infection over the same time period. Risk-based screening with immediate colposcopy for HPV16/18/31 and further xGT triage resulted in better $\geq$ CIN3 sensitivity (79.2% versus 72.3%; relative difference of 6.9 [95%CI: 3.3, 10.4]) and a lower colposcopy/ $\geq$ CIN3 ratio (9.2 versus 11.2; relative difference of -1.9 [95%CI: -2.6 , -1.3]) when compared to primary HPV16/18-based screening.

Conclusions. An HPV-negative result offers the same assurance of no disease over three years of follow up as that offered by a negative co-testing result. xGT facilitates risk-based screening and persistence tracking and can help optimize disease detection during screening without excessive colposcopic procedures.

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1. Introduction

Persistent infection by one or more high-risk human papilloma-viruses (HPVs) is necessary for the progression to high-grade cervical intraepithelial neoplasia (CIN) and the development of cervical cancer [1–3]. Molecular diagnostics for high-risk HPV genotypes result in a greater sensitivity for detection of cervical precancer and cancer compared to traditional screening procedures based on cytology alone [4–7]. This translates into early detection with a reduction in the future risk of high-grade CIN and cancer, greater assurance following a negative HPV test, and extension of the interval between screenings [8]. HPV testing also provides improved detection for women with cervical adenocarcinoma or adenocarcinoma in situ, both of which are difficult to detect through cytology-based screening alone [9]. Currently, the American Cancer Society prefers HPV primary screening; the United States Preventive Services Taskforce recommends the inclusion of HPV testing, in addition to cytology, in order to extend the screening interval from three to five years [10,11].

Although HPV detection provides improved sensitivity and negative predictive value for high-grade CIN and cancer, sensitive HPV primary screening without proper triage has a relatively high positive rate and a larger number of potentially avoidable colposcopies compared to cytology-based screening [12,13]. Results from prospective, randomized cervical cancer screening trials [14–17], and from the follow up of women enrolled in the Kaiser Permanente/NCI cohorts in Portland and Northern California, led the 2015 ASCCP interim guidelines to recognize HPV primary screening with cytology triage [12] as a valid screening strategy for women ≥ 25 years of age. Subsequent to the ASCCP guideline update, results from the three-year longitudinal analyses of the ATHENA trial were published and reported that HPV16/18 primary screening maintains a higher sensitivity for $\geq$ CIN3 detection compared to hybrid screening (i.e. atypical squamous cells-undetermined significance (ASC-US) triage by HPV in women 21–29 years of age and co-testing in women ≥ 30 years of age), with no sacrifice in specificity [7].

In 2019, results from the baseline phase of the Onclarity trial were published and demonstrated that HPV genotypes beyond HPV16 and 18 carry a risk for high-grade CIN in the negative for intraepithelial malignancies (NILM) cytology population that exceeds the current risk threshold for immediate referral to colposcopy [18]. Specifically, HPV31 was reported to carry a risk for $\geq$ CIN2 and $\geq$ CIN3 at baseline that is close to that of HPV16 and exceeds that of HPV18 [19]. Additionally, baseline Onclarity trial findings suggest that the lowest risk genotype results (35/39/68, 51, 56/59/66) carry a $\geq$ CIN3 risk in the ASC-US or low-grade squamous intraepithelial lesion (LSIL) cytology population so low as to warrant re-testing for women after a fixed period (e.g. after one year), rather than immediate colposcopy [20]. These data are supported by prospective, post-hoc, and retrospective analyses, from studies describing cervical cancer screening populations from numerous countries, including the United States, the United Kingdom, Denmark, and the Netherlands [21–28]. In addition, the use of extended genotyping to stratify risk supports the most recent ASCCP guideline update (2019), which advocated for a risk-based approach to identify high-grade CIN during screening in order to prevent cervical cancer [18,29].

In this paper, the three-year cumulative risk values of HPV extended genotyping results from the Onclarity clinical validation trial are presented. In addition, the potential of extended genotyping is analyzed to refine risk stratification estimates by genotype and to determine the impact of same-genotype persistence on $\geq$ CIN2 and $\geq$ CIN3 risk stratification during the three-year longitudinal follow up period.

2. Materials and methods

2.1. Study design and participants

2.1.1. Baseline phase

The BD Onclarity™ trial involved a baseline phase and a three-year longitudinal phase. Recruitment during baseline occurred in a consecutive manner, during routine cervical cancer screening, across 31 sites throughout the USA, between August 26, 2013 and June 12, 2015. The baseline inclusion/exclusion criteria, and selection algorithm for colposcopy referral and biopsy were described in detail, previously [30]. Briefly, all women enrolled at baseline ($N = 33,858$) had BD SurePath™ liquid-based cytology (LBC) (Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions) and/or BD Onclarity™ HPV testing (“Onclarity;” Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions). During baseline, all women with a co-test positive screening result were referred to colposcopy. The only co-test positive women that did not have a colposcopy/biopsy procedure were those that were lost-to-follow up or those that were removed due to exclusion criteria (see Fig. 1). Approximately 5% of co-test negative women were referred, at random, to colposcopy/biopsy. In addition approximately 10% of the co-test negative women were chosen, at random, to enter the longitudinal phase, with no further activity at baseline. All other co-test negative women (approximately 85%) exited the study with no further trial-related activity.

2.1.2. Three-year, longitudinal phase

The co-test positive and 5% of co-test negative women that were referred to colposcopy at baseline entered the longitudinal phase if the following criteria were met: (1) were not excluded (lost to follow up, withdrew consent, hysterectomy, etc.) during baseline; (2) had valid histology results (any result, including “unsatisfactory”); and (3) were not treated for high-grade cervical pathology. The longitudinal phase of the study involved annual visits at years one, two, and three. Follow up visits included examinations and collection of an LBC specimen for cytology and HPV testing. Any woman with an LBC result of $\geq$ ASC-US was referred to a colposcopy/biopsy. Participants that were not treated for high-grade cervical pathology carried on in the longitudinal phase. Cytology-negative participants were not referred to colposcopy/biopsy during years one or two of the longitudinal phase, but they proceeded to the next year. All remaining participants received a colposcopy/biopsy referral during year three of the longitudinal phase. All histology end points followed the same p16 enhanced adjudication procedure as at baseline. Specimen collection occurred from November 11, 2014 until November 11, 2018 during the longitudinal phase of the trial [30].

2.2. Extended genotyping and persistence tracking

The analyses involving risk stratification for individual HPV genotypes were based on a hierarchical rank order (HPV16, 18, 31, 33/58, 52, 45, 35/39/68, 51, and 56/59/66) similar to that described previously, using the Onclarity HPV assay [19]. This order was utilized to classify HPV-positive subjects by hierarchical genotype rank: HPV16, else HPV18, else HPV31, else HPV33/58, else HPV52, else HPV45, else HPV35/39/68, else HPV51, HPV56/59/66. Verification bias adjustment (VBA) was performed for normalization of the difference in the selection rate for colposcopy/biopsy [31]. Bootstrapping was employed to calculate confidence intervals (CI; 95%) for adjusted risk values. Lower and upper limits for the 95% CIs were determined using the 2.5 and 97.5 percentiles within the bootstrapped distribution. Kaplan-Meier methods were used for estimating risk of disease over 3 years.

Five screening strategies, based on differential integration of HPV and cytology results, were utilized to determine screening performance

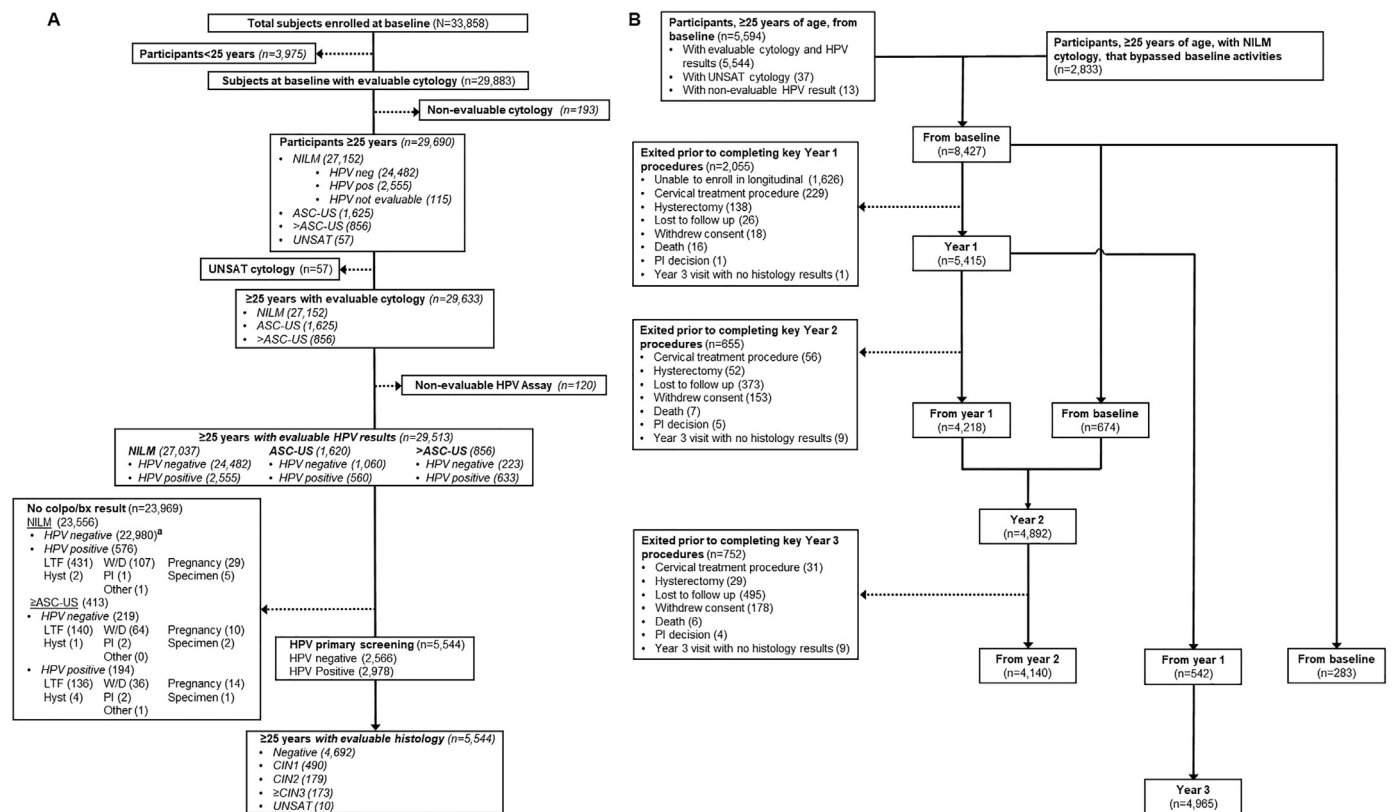


Fig. 1. Subject reconciliation during baseline (A) and follow up (B; years one, two, and three) phases of the Onclarity trial.

Abbreviations: NILM, negative for intraepithelial lesions or malignancies; HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; UNSAT, unsatisfactory; CIN, cervical intraepithelial neoplasia; PI, principal investigator.

for detection of ≥CIN3 during the baseline phase of the Onclarity trial for women ≥25 years of age ($n = 29,513$; please refer to Fig. 1). These strategies (see Fig. S1) were: (1) ASC-US triage: NILM and ASC-US/HPV-negative women return to routine screening; women with ASC-US cytology that are also HPV-positive and women with >ASC-US cytology are referred to colposcopy; (2) Hybrid strategy (“HS,” as utilized by Wright et al., 2014 in the ATHENA cervical cancer screening trial [7]; women age 25–29 years receive ASC-US triage screening as in (1), women ≥30 years receive co-testing, which involves a return to routine screening for cytology-negative/HPV-negative results and ASC-US cytology/HPV-negative results, a follow up screening in one year for a cytology-negative/HPV-positive result, and a colposcopy referral for a screen result of ASC-US cytology with an HPV-positive result or a cytology result of >ASC-US; (3) Primary HPV16/18 screening strategy (“PSS 16/18,” as utilized by Wright et al., 2014 and described by Huh et al., 2015) [7,12]: women with an HPV-negative result will return to routine screening, women with an HPV16 or 18 result receive an immediate colposcopy, regardless of cytology, and women with a positive HPV for any of the Other 12 genotypes receive a colposcopy if they have a cytology result of ≥ASC-US but a one-year follow up if they have negative cytology; (4) Primary HPV16/18/31 strategy (“xGT31”): women with an HPV-negative result will return to routine screening, women with an HPV16, 18, or 31 result receive an immediate colposcopy, regardless of cytology, and women with a positive HPV result for any of the Other 11 genotypes receive a colposcopy if they have a cytology result of ≥ASC-US but a one-year follow up if they have negative cytology; (5) Primary HPV16/18/31 xGT strategy (“xGT31+”): similar to the HPV16/18/31 primary screening strategy, the risk-based strategy refers HPV16-, 18-, or 31-positive women to immediate colposcopy. The remaining “Other 11” genotypes are triaged using LBC. From that pool, all >LSIL women receive an immediate colposcopy and all women with NILM

cytology return for testing at follow up. The remainder of the women (those with ASC-US or LSIL cytology) are managed based on the corresponding Onclarity assay results: those that are positive for HPV33/58, 45, or 52 will be referred to immediate colposcopy and those that are HPV35/39/68, 51, or 56/59/66 will return for testing at follow up. The formation and management of these strata are based on previous work from the Onclarity trial [19,20] and other studies, as described by Bonde et al. [32]

For persistence tracking, participants' HPV genotype prior to the baseline visit was unknown. Therefore, the baseline HPV result was employed only to establish genotype positivity. Same-genotype persistence was defined as a positive result for the same genotype at both baseline and year one visit. HPV genotype switching was defined by persistent high risk HPV infection in which the genotype was not the same from baseline to year one. HPV clearance was defined as an infection that cleared between baseline and year one. New infection was defined as a negative HPV result at baseline with a subsequent positive result at year one visit. Persistent negative was defined as a negative HPV result at both the baseline and year one visit.

The performance of screening strategies was modelled as follows. Participants with NILM cytology and an HPV-negative result at baseline were returned to routine screening under all strategies. The disease occurring in years 0–three was projected in this group using VBA. Note, by study design, these co-test negative participants were followed differentially than the rest of the cohort (i.e. only a 5% random subset were assigned to colposcopy at baseline and an additional 10% random subset were followed only during the three-year longitudinal phase). Within co-test negative subjects at baseline, all modelled strategies have 0% sensitivity and 100% specificity, and no colposcopies were modelled. For each strategy, subjects with baseline co-test positive results (HPV-positive and/or cytology ≥ASC-US) were split into three groups

according to HPV and cytology results at baseline; disease status over the three years was projected by VBA: (1) Participants who would have received baseline colposcopy; (2) Participants who would have been deferred for a one-year follow up; and (3) Participants who would return to routine screening. The modelled follow up after baseline for non-disease subjects in the first group and for all subjects in the second group was repeat yearly co-testing until either co-test negative or $\geq$ CIN2 discovered. In the follow up phase any co-test positive subject would receive a colposcopy under all modelled strategies. The co-test positivity over three years was estimated separately for disease and non-disease subjects, as observed during the study (note, all baseline co-test positive subjects received yearly HPV and cytology tests in the follow up phase). Bootstrapping with 200 replicates was used to calculate 95% confidence intervals for the differences in sensitivity and colposcopies per disease case detected between the PSS 16/18 strategy and the other modelled strategies.

This report is aligned with STARD reporting standards. The study protocol received approval from institutional review boards at each study site; written, informed consent was obtained prior to any trial-related activities. The principles set forth by the Declaration of Helsinki and Good Clinical Practice were observed during conduct of this study.

3. Results

3.1. Reconciliations and demographics, and cytology, HPV, and histology result distribution

3.1.1. Baseline phase

From the 33,858 subjects enrolled, 3975 were excluded from the analyses due to age (<25 years), leaving 29,883 individuals (Fig. 1A). The mean ($\pm$ SD) and median age of this group was 40.7 (10.9) and 39 years, respectively. The breakdown by race, ethnicity, smoking history, and other categories for this group is similar by percentage to the total study population (≥ 21 years of age) described previously (Table 1) [30]. Of the 29,883 women, ≥ 25 years of age, 90.9% ($n = 27,152$) were NILM cases, 5.4% ($n = 1625$) ASC-US, and 2.9% ($n = 856$) $>$ ASC-US; 86.4% ($n = 25,813$) were HPV-negative and 12.6% (3753) were HPV-positive.

Approximately one-third (35.1%; $n = 1319$) of HPV-positive participants were either HPV16, 18, or 31 positive (in hierarchical order) (Table 1). The prevalence values of HPV16, 18, 31, and the Other 11 genotypes within $\geq$ CIN2 and $\geq$ CIN3 are shown in Table S1; these results are shown across different cytology categories. As expected and shown previously [30], HPV16 had the highest prevalence within the $\geq$ CIN3 histology group, followed by HPV31 and then HPV18 (Table S1). Of the total age ≥ 25 population, 18.7% ($n = 5594$) participated in a colposcopy/biopsy procedure. From the population of 5594, there were 5544 participants that had evaluable cytology, valid HPV test results, and had a satisfactory histology: 93.5% ($n = 5182$) had $\leq$ CIN1 histology, 6.3% ($n = 352$) had $\geq$ CIN2 histology, and 3.1% ($n = 173$) had $\geq$ CIN3 histology; 0.2% ($n = 10$) had unsatisfactory histology (Fig. 1). During baseline, there were nine cases of adenocarcinoma in situ, three cases of squamous cell carcinoma, and one case of adenocarcinoma/adenosquamous carcinoma.

3.1.2. Three-year, longitudinal phase

Reconciliation during the three-year follow up period is shown in Fig. 1B. 8427 participants (from baseline) were eligible for year one activities associated with the longitudinal phase. From that group, 2055 exited prior to any activities at year one due to various factors (including inability to enroll in the longitudinal phase, cervical procedures, hysterectomy, loss to follow up, withdrawn consent, death, PI decision), 674 participants bypassed year one activities and participated in year two activities, and 283 participants bypassed activities at year one and year two and participated in activities at year three. From the 5415 participants at year one, 655 exited prior to activities at year two (for

Table 1

Baseline demographics and characteristics of subjects, ≥ 25 years of age, at baseline in the Onclarity trial.

Eligible subjects ($n = 29,883$)	
Mean age, yrs (SD)	40.7 (10.9)
Median age, yrs	39
Min age, yrs	25
Max age, yrs	83
Race	
Asian	1.4% (416)
African American	18.1% (5397)
White	78.9% (23,591)
Other ^a	1.6% (479)
Ethnicity	
Hispanic or Latino	19.8% (5916)
Not Hispanic or Latino	80.2% (23,964)
Other	$<0.1\%$ (3)
Smoking history	
Non-smoker	66.3% (19,815)
Current	15.2% (4545)
Past	18.5% (5523)
HPV-vaccinated	
Yes	5.5% (1637)
No	93.3% (27,877)
Unknown	1.2% (369)
Postmenopausal	20.1% (6000)
Immunocompromised	1.3% (375)
Abnormal cytology (past 5 years)	13.6% (4073)
Colposcopy (past 5 years)	8.7% (2597)
Cytology findings	
NILM	90.9% (27,152)
ASC-US	5.4% (1625)
$>$ ASC-US	2.9% (856)
LSIL	71.6% (613)
ASC-H	10.9% (93)
$\geq$ HSIL ^b	11.4% (98)
AGC ^c	6.1% (52)
Unsatisfactory	6.7% (57)
Non-evaluable	22.5% (193)
HPV results	
HPV(−)	86.4% (25,813)
Any HPV	12.6% (3753)
HPV16 (+)	19.8% (744)
HPV18 (+)	5.5% (205) ^e
HPV31 (+)	9.9% (370) ^f
HPV16/18 (+)	25.3% (949)
HPV16/18/31 (+)	35.1% (1319)
Other 11 genotypes (+)	64.9% (2434)
Invalid results	8.4% (317)
Histology	
No colposcopy	81.3% (24,289)
Colposcopy	18.7% (5594)
NEG	84.7% (4737)
CIN1	8.8% (493)
CIN2	3.2% (179)
$\geq$ CIN3 ^d	3.1% (173)
UNSAT	0.2% (12)

Abbreviations: SD, standard deviation; Min, minimum age; Max, maximum age; NILM, negative for intraepithelial lesions or malignancies; ASC-US, atypical squamous cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot rule out HSIL; HSIL, high-grade squamous intraepithelial lesions; SCC, squamous cell carcinoma; AGC, atypical glandular cells; AGC-neo, atypical glandular cells-neoplastic; AIS, adenocarcinoma in situ; HPV, human papillomavirus; NEG, negative; CIN, cervical intraepithelial neoplasia; UNSAT, unsatisfactory.

^a Includes American Indian, Alaska Native, Native Hawaiian, or other Pacific Islander, or other.

^b $\geq$ HSIL includes one case of SCC.

^c AGC includes one case of AGC-neo and one case of AIS.

^d $\geq$ CIN3 histology included nine cases of AIS, three cases of SCC, and one case of adenocarcinoma/adenosquamous carcinoma.

various reasons that are like those at year one) and 542 bypassed year two activities and participated in activities at year three. From the 4892 participants at year two, 752 exited the study for reasons like those described above. In total, 4965 participants were included in year three activities. During the longitudinal phase, there were two cases of adenocarcinoma in situ and no cases of invasive cervical carcinoma.

3.2. Baseline and three-year cumulative incidence risk values for individual Onclarity results

Onclarity assay results were utilized to determine risk values for $\geq$ CIN3 and $\geq$ CIN2 associated with HPV extended genotyping through hierarchical ranking that was assigned to the genotypes across all cytology results and in either the NILM, ASC-US/LSIL, or $>$ LSIL populations (Table 2). HPV16 carried the highest baseline and three-year CIR values, relative to the other Onclarity results, for both $\geq$ CIN3 and $\geq$ CIN2. HPV31 also carried a relatively high risk for both $\geq$ CIN3 and $\geq$ CIN2 across the three cytology categories. Most of the Other 12 individual genotype results had $\geq$ CIN3 and $\geq$ CIN2 risk values that were notably lower than HPV16 and 31 at baseline and after three years. In addition, the differential risk for high-grade CIN between baseline and three-year time points

Table 2

Baseline and three-year cumulative $\geq$ CIN3 risk for Onclarity assay results, stratified by cytology category^{a,b}.

Any Cytology	Baseline; % [95% CI]	Year 3; % [95% CI]
Any HPV	5.31 [4.54–6.10]	7.48 [6.49–8.51]
HPV16	14.15 [11.85–16.65]	18.74 [15.60–22.25]
HPV18	5.16 [2.19–9.12]	8.00 [3.48–11.97]
HPV31	9.84 [6.26–13.25]	13.18 [8.99–17.88]
HPV33/58	4.16 [2.06–7.08]	5.54 [2.91–8.81]
HPV52	3.19 [1.47–5.40]	4.94 [2.67–7.85]
HPV45	2.71 [0.57–5.07]	4.16 [1.15–7.33]
HPV35/39/68	1.45 [0.52–2.45]	3.01 [1.47–4.55]
HPV51	1.62 [0.43–4.08]	4.29 [1.29–8.69]
HPV56/59/66	0.21 [0.00–0.66]	0.21 [0.00–0.66]
HPV-negative	0.10 [0.03–0.20]	0.15 [0.06–0.26]
NILM		
Any HPV	3.15 [2.44–3.95]	4.40 [3.44–5.29]
HPV16	8.12 [5.57–10.83]	8.12 [5.57–10.83]
HPV18	2.88 [0.78–7.01]	4.42 [0.91–8.85]
HPV31	7.74 [4.40–11.48]	9.02 [5.30–12.75]
HPV33/58	2.85 [0.59–5.70]	2.85 [0.59–5.70]
HPV52	1.72 [0.41–3.78]	2.27 [0.46–4.56]
HPV45	0.74 [0.00–2.21]	2.77 [0.00–5.53]
HPV35/39/68	0.76 [0.00–1.59]	1.57 [0.42–3.10]
HPV51	0.95 [0.00–3.59]	0.95 [0.00–3.59]
HPV56/59/66	0.29 [0.00–0.91]	0.29 [0.00–0.91]
HPV-negative	0.08 [0.01–0.18]	0.12 [0.03–0.23]
ASC-US/LSIL		
Any HPV	5.14 [3.66–6.63]	8.62 [6.62–10.39]
HPV16	16.91 [11.75–23.26]	22.31 [15.72–29.82]
HPV18	3.77 [0.00–9.35]	6.60 [0.00–14.93]
HPV31	7.04 [2.22–13.25]	13.75 [5.88–23.88]
HPV33/58	1.40 [0.00–4.31]	3.70 [0.00–9.00]
HPV52	3.31 [0.00–8.00]	6.32 [1.23–12.86]
HPV45	2.06 [0.00–6.25]	2.06 [0.00–6.25]
HPV35/39/68	1.37 [0.00–3.21]	5.24 [1.08–9.96]
HPV51	1.41 [0.00–4.35]	8.59 [1.81–18.20]
HPV56/59/66	0.00 [0.00–0.00]	0.00 [0.00–0.00]
HPV-negative	0.40 [0.10–0.89]	0.82 [0.28–1.62]
$>$ LSIL		
Any HPV	39.77 [31.89–47.31]	51.15 [39.82–60.63]
HPV16	54.17 [40.59–67.17]	64.35 [48.12–76.98]
HPV18	28.57 [6.90–50.00]	46.43 [13.33–76.92]
HPV31	53.33 [27.53–75.00]	76.67 [31.25–88.89]
HPV33/58	33.33 [10.00–53.85]	66.67 [22.22–100.00]
HPV52	36.36 [9.40–66.67]	57.58 [16.67–82.50]
HPV45	30.00 [0.00–64.58]	30.00 [0.00–64.58]
HPV35/39/68	20.00 [0.00–40.00]	20.00 [0.00–40.00]
HPV51	20.00 [0.00–90.00]	20.00 [0.00–55.56]
HPV56/59/66	0.00 [0.00–0.00]	0.00 [0.00–0.00]
HPV-negative	0.00 [0.00–0.00]	0.00 [0.00–0.00]

Abbreviations: $\geq$ CIN3, cervical intraepithelial neoplasia, grade 3 or worse; HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies; ASC-US, atypical squamous cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

^a From women, $\geq$ 25 years of age, with evaluable cytology and valid Onclarity results.

^b Risk values calculated hierarchically; HPV16, else 18, else 31, else 33/58, else 52, else 45, else 35/39/68, else 51, else 56/59/66.

was largest for HPV16 across cytology categories and was also relatively high for HPV31 and HPV18 across cytology categories, compared to the other individual genotype results. The one-, two-, and three-year cumulative incident $\geq$ CIN3 and $\geq$ CIN2 risk values for HPV16, HPV31, and HPV18 are plotted in Fig. 2. Fig. 2 also shows the three-year cumulative incident $\geq$ CIN3 risk associated with an HPV-negative result (0.15% [95% CI: 0.06, 0.26]) and for an HPV-negative/NILM result (0.12% [95% CI: 0.03, 0.23]), which were not statistically different.

Baseline and three-year $\geq$ CIN3 risk results were also analyzed by different genotype groupings within different cytology categories. As shown in Fig. 3, the values are superimposed over risk thresholds that are associated with specific clinical actions (e.g. return to routine screening, three-year return for testing, one-year return for testing, immediate colposcopy, or immediate treatment). Baseline and three-year risk values for HPV16/18/31 genotypes, associated with NILM cytology, were above the colposcopy referral threshold. The baseline $\geq$ CIN3 risk associated with the “bottom 7” HPV genotypes (HPV35/39/68/51/56/59/66) within the ASC-US/LSIL cytology group fell below the threshold for immediate colposcopy but the three-year CIR for this group was high enough to warrant repeat testing after one year, at follow up.

3.3. Cervical cancer screening strategies

Five screening approaches were analyzed using the baseline and three-year follow up data from women $\geq$ 25 years: (1) ASC-US triage; (2) HS, which involved ASC-US triage for women 25–29 and co-testing for women $\geq$ 30 years; (3) PSS 16/18, which involved HPV16/18 primary screening and cytology triage for the Other 12 genotypes; (4) xGT31, which involved HPV16/18/31 primary screening and cytology triage for the Other 11 genotypes; and (5) xGT31+, which involved HPV16/18/31 primary screening and sent combinations of genotype and cytology results to colposcopy, based on risk values associated with genotype result groupings (Fig. S1).

As shown in Table 3, xGT31 detected the most cases of $\geq$ CIN3 (180) and had the highest sensitivity (80.7%) at baseline when compared to the other four strategies that were modelled. The xGT31+ strategy detected 177 $\geq$ CIN3 cases and had a sensitivity of 79.2%, which was statistically greater than that for PSS 16/18 but not statistically different from xGT31. Notably, the xGT31+ strategy was associated with a colposcopy/ $\geq$ CIN3 ratio (9.2) lower than both the PSS 16/18 (11.2) and xGT31 (11.4) strategies.

After baseline screening and colposcopy/biopsy, participants that were not removed from all baseline screening strategies were modelled with the same follow up screening, which resulted in a colposcopy referral following a positive cytology result and/or a positive HPV result. Per protocol, only subjects who tested positive by cytology received a colposcopy at visit years one and two, while all subjects regardless of results received a colposcopy at year three. The modelled three-year cumulative performance values for all five strategies are shown in Table 3. Relative to xGT31, the missed $\geq$ CIN3 cases at baseline for HS, and to a lesser extent for PSS 16/18 screening and xGT31+, were largely detected at years one through three of follow up. One assumption used for modelling performance of strategies was that baseline discovered $\geq$ CIN2 cases (deferred one year by a modelled strategy) would remain co-test positive at year one. Therefore, the cumulative sensitivities of those three strategies at year three approached that of xGT31. Overall, however, xGT31+ provided the best balance of sensitivity of $\geq$ CIN3 detection while minimizing the number of colposcopies and screening tests across the entire study.

3.4. Risk of high-grade CIN based on HPV genotype persistence

Risk of incident disease in subjects without baseline disease was estimated according to HPV results at baseline and at each follow up year. By category, cumulative incident $\geq$ CIN3 and $\geq$ CIN2 risk values were highest in the genotype-specific persistence group (Table 4). The

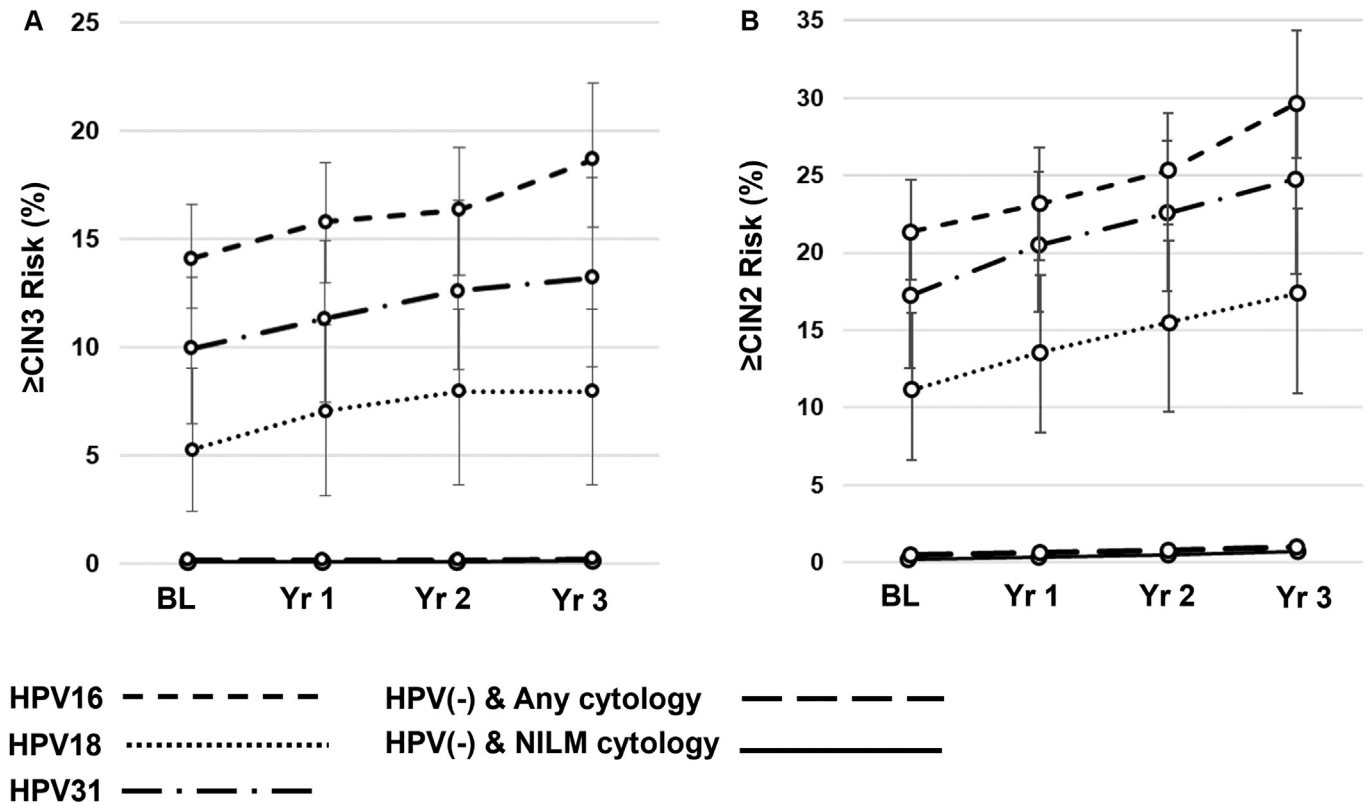


Fig. 2. Baseline and three-year cumulative incident risk (verification bias-adjusted) for (A) $\geq$ CIN3 and (B) $\geq$ CIN2 disease stratified by baseline screening results. **Abbreviations:** CIN, cervical intraepithelial neoplasia; BL, baseline; Yr, year; HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies.

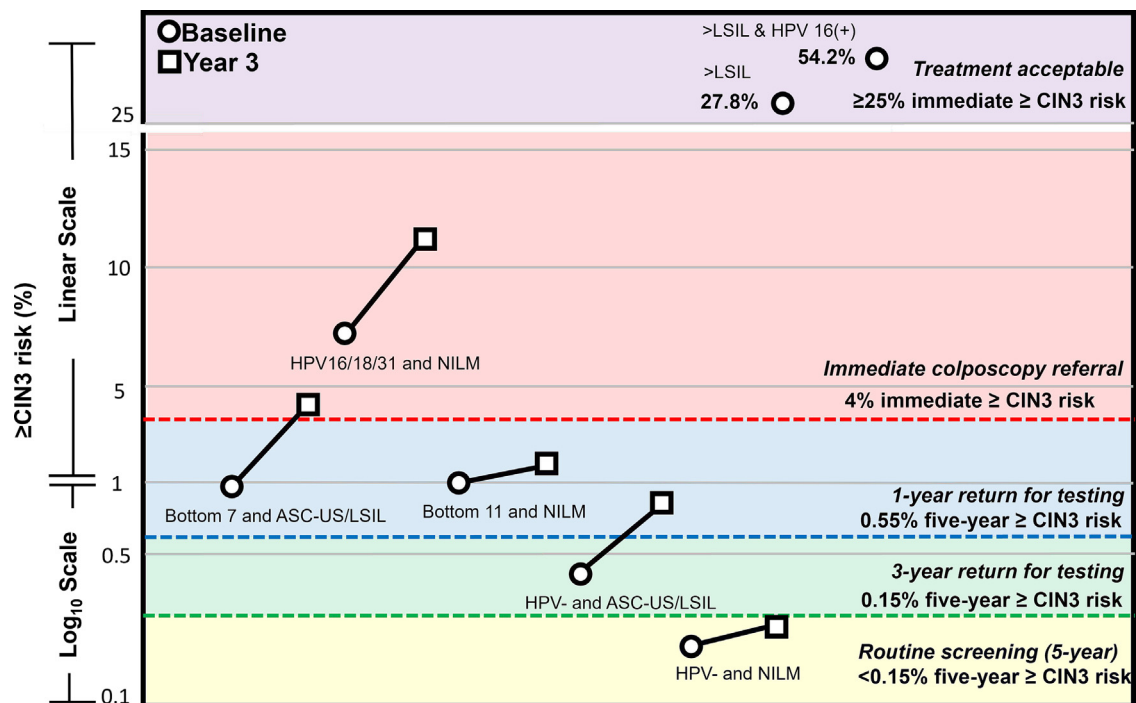


Fig. 3. Baseline and three-year cumulative $\geq$ CIN3 risk values are plotted over thresholds associated with clinical actions (e.g. immediate colposcopy, return to routine screening, one-year return for testing, three-year return for testing, etc.). Groupings associated with the top three genotypes (HPV16/18/31), the bottom seven genotypes (HPV35/39/51/56/59/66/68) and the bottom 11 genotypes (all except HPV16) are shown with different combination of cytology triage results. Zones in yellow, green, blue, red and purple indicate risk bands that result in differential clinical management depending on baseline and five-year risk of the screening result. Immediate colposcopy and treatment are governed by baseline risk, whereas one-, three-, and five-year return are governed by the women's five-year risk (if their immediate risk is below 4%).

Abbreviations: HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; NILM, negative for intraepithelial lesions or malignancies; LSIL, low-grade squamous intraepithelial lesions.

Table 3Performance associated with alternative baseline^a screening strategies and three years^b of co-testing follow up.

	Sensitivity	Relative difference in CI Sensitivity	Screening tests	Colposcopies	≥CIN3 detected	Colposcopies/≥CIN3	Relative difference in CI colpos/≥CIN3
PSS 16/18							
Baseline	72.3	ref	32,313	1802	162	11.2	ref
Year 3	85.9	ref	44,055	5035	275	18.3	ref
ASC-US Triage							
Baseline	53.8	−18.6 [−23.6, 13.5] ^c	31,133	1416	120	11.8	0.6 [−0.4, 1.6]
Year 3	52.2	−33.6 [−38.7, −27.6] ^c	35,173	2521	167	15.1	−3.2 [−5.0, −1.8] ^d
HS							
Baseline	53.8	−18.6 [−23.6, −13.5] ^c	53,990	1416	120	11.8	0.6 [−0.4, 1.6]
Year 3	73.1	−12.6 [−16.7, −9.0] ^c	63,957	4154	234	17.7	−0.6 [−1.6, 0.5]
xGT31							
Baseline	80.7	8.4 [5.1, 12.0] ^e	31,944	2049	180	11.4	0.2 [−0.4, 0.8]
Year 3	85.9	0.1 [0.1, 0.2] ^e	43,625	5252	275	19.1	0.8 [0.7, 0.9] ^f
xGT31+							
Baseline	79.2	6.9 [3.3, 10.4] ^e	31,994	1636	177	9.2	−1.9 [−2.6, −1.3] ^d
Year 3	85.9	0.1 [0.0, 0.2]	43,679	4866	275	17.7	−0.6 [−0.8, −0.5] ^d

Abbreviations: ASC-US, atypical squamous cells-undetermined significance; ≥CIN3, cervical intraepithelial neoplasia, grade 3 or worse; HPV, human papillomavirus.

^a Baseline refers to analyses performed using the population of $n = 29,513$ (please refer to Fig. 1).^b Values are cumulative from across the three-year follow up period.^c Indicates a sensitivity value that is significantly lower than the HPV16/18 partial GT strategy.^d Indicates a colposcopy/≥CIN3 value that is significantly lower than the HPV16/18 partial GT strategy.^e Indicates a sensitivity value that is significantly higher than the HPV16/18 partial GT strategy.^f Indicates a colposcopy/≥CIN3 value that is significantly higher than the HPV16/18 partial GT strategy.

cumulative ≥CIN3 risk at the three-year time point for the genotype-specific persistence group (4.2% [95%CI: 2.7, 6.0]) was close to three times higher than that observed for the genotype switch group (1.5% [95%CI: 0.0, 4.1]). An even greater difference was observed between genotype-specific persistence and new infection (1.1% [95%CI: 0.0, 2.9]), clearance (0.2% [95%CI: 0.0, 0.5]), and no infection (0.03% [95%CI: 0.0, 0.1]). Cumulative risk values for ≥CIN2 showed a similar trend to those associated with ≥CIN3 (Table 4). By Onclarity result groupings, same-genotype persistence led to a significantly higher ≥CIN2/3 risk than for cases in which the HPV infection cleared (Table S2).

4. Discussion

From the results presented above, it is clear that the genotype-specific data obtained via extended genotyping improves risk stratification of women with positive screening tests and facilitates risk-based screening. The three-year data reinforces this concept and

demonstrates that disease missed at baseline is better identified by genotype-specific HPV testing than cytology. The longitudinal data set also demonstrates the impact of genotype-specific persistence and shows that this type of persistent infection can greatly increase the risk for ≥CIN3 and ≥CIN2. In addition, this study included a comparison of three-year ≥CIN3 risk for HPV-negative and HPV- and cytology-negative women, and found that there was no difference between the two. This is important as these results further demonstrate no added assurance for a negative co-testing screening result when compared to a negative HPV screening result.

Our results demonstrate that implementation of extended genotyping, to facilitate risk-based cervical cancer screening, can improved high-grade CIN risk stratification and help optimize the trade-off between identifying disease and potentially unwarranted colposcopies. As previously demonstrated in the ATHENA trial, PSS 16/18 was able to detect more high-grade CIN at baseline when compared to hybrid screening [7]. This was because cytology-driven strategies miss disease that is actually prevalent at baseline that is present in the NILM cytology population. Here we determined the risk values associated with all individual genotyping results reported by Onclarity. HPV31 was associated with ≥CIN3 risk that was second only to HPV16 in the primary screening population and when stratified by cytology result. As expected, there were Onclarity results associated with intermediate ≥CIN3 risk values (HPV33/58, 52, and 45) and those associated with relatively low ≥CIN3 risk values (HPV35/39/68, 51, and 56/59/66). We leveraged these baseline risk values to model risk-based primary HPV screening using extended genotyping and cytology triage in order to determine colposcopy or return for re-testing. The xGT31 approach detected approximately 8% more ≥CIN3 cases (corresponding to HPV31-positive individuals with NILM cytology) at baseline compared to PSS 16/18. The modelled xGT31+ strategy's sensitivity was similar to the xGT31 strategy but was associated with a much lower colposcopy/≥CIN3 ratio at baseline when compared to PSS 16/18 or xGT31. Although women that are positive for genotypes carrying risk values above the clinical action threshold (e.g. HPV31 in addition to HPV16 and 18) should be considered for immediate colposcopy, cytology may be useful as a triage for intermediate-risk genotypes such as HPV33/58, 52, and 35, and the combined risk estimates can determine whether colposcopy or re-testing after one year is the preferred management. Clinical

Table 4

Changes in incident ≥CIN3 and ≥CIN2 risk, based on HPV genotype-specific persistent infection.

	≥CIN3; % [95% CI]	≥CIN2; % [95% CI]
No infection ($n = 3351$)		
Year 1	0.03 [0.0, 0.1]	0.1 [0.03, 0.2]
Year 3	0.03 [0.0, 0.1]	0.5 [0.2, 0.7]
Clearance ($n = 835$)		
Year 1	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]
Year 3	0.2 [0.0, 0.5]	0.9 [0.3, 1.7]
New infection ($n = 227$)		
Year 1	0.0 [0.0, 0.0]	2.2 [0.4, 4.2]
Year 3	1.1 [0.0, 2.9]	4.8 [2.0, 8.1]
GT switch ($n = 133$)		
Year 1	1.5 [0.0, 4.1]	1.5 [0.0, 4.1]
Year 3	1.5 [0.0, 4.1]	6.2 [2.5, 11.1]
GT persistence ($n = 799$)		
Year 1	1.6 [0.8, 2.6]	4.8 [3.5, 6.4]
Year 3	4.2 [2.7, 6.0]	11.3 [9.2, 14.1]

Abbreviations: HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; GT, genotype.

management for those genotypes with the lowest risk for high-grade CIN as reported here and in previous studies, like HPV56/59/66, might also be stratified after cytology triage. These genotypes have a low enough risk, even in women with ASC-US/LSIL cytology, to be excluded from colposcopy in favor of a follow up visit for re-testing at an appropriate interval (one or possibly three years) [18–20,32]. As with the HS and PSS 16/18 strategies, the extended genotyping approaches met the level of $\geq$ CIN3 disease detection shown by xGT31 during the follow up period (the majority at year one). This highlights an advantage of these risk-based approaches—colposcopies for lower-risk women can be avoided at screening, with an assurance that these robust algorithms will catch the large majority of disease at follow up. Ultimately, the clinical outcomes associated with risk-based management will reflect a balance between improved disease detection and the theoretical costs and harms incurred by additional colposcopy referrals.

Genotype persistence imposes a higher risk value for high-grade CIN than risk following HPV positivity associated with a genotype switch or with genotype clearance [33–38]. This supports a tiered-stratification approach for follow up of individuals who have a prior HPV-positive result, especially for women that did not receive an immediate colposcopy or women being re-tested following a colposcopy or treatment. Of the high-risk genotypes, HPV16 is the strongest viral carcinogen and is most likely to persist [36,38–40]. In addition, HPV16 tends to persist for a longer period of time compared to most other HPV types—likely contributing to its high prevalence [38]. Although HPV16 persistence is associated with the highest absolute progression rate to high-grade cervical disease, HPV31, 33, 18, and 45 all progress at an accelerated rate to high-grade CIN when associated with persistent, same-genotype infection [33–38]. Here, genotype-specific persistence infection more than doubled the risk for high-grade disease when compared to those with cleared infection. Genotype-specific persistence may not change the clinical management for some genotype results (e.g. HPV16, 31, or 18; which have a high enough baseline risk for immediate colposcopy); however, genotype-specific persistence would be important information for those with moderate or lower-risk genotypes that normally fall below the threshold for colposcopy. Future research is needed to determine how genotype persistence for lower-risk genotypes should be handled in women when considering a colposcopy referral. Knowing genotype information from the prior screen could help avoid an unnecessary colposcopy at the subsequent test for a woman who had an “apparent” persistent HPV infection that rather was associated with a genotype switch [41]. Finally, tracking of genotype persistence could have a major impact in low-resource settings where cytology triage may not be an option.

4.1. Strengths and limitations

Strengths of this study included the large group size and the application of VBA to account for negative results at screening that were not assessed by histology. This trial included a thorough composite pathology review for the reference method that ensured differential diagnosis of CIN3 from CIN2 through a number of steps, including the use of p16 immunohistochemistry. Limitations of this study included that only a three-year follow up occurred after baseline; however, NCI and ASCCP have published a validated methodology for converting three-year risk estimates to five-year risk estimates [42]. A significant amount of high-grade disease was detected and removed (due to treatment and study exit) during the baseline phase of the study. This likely skewed the true risk stratification associated with extended genotyping during follow up that would be expected in real-world settings. It was not possible to discern with precision the difference between disease that was missed at baseline and incident disease that occurred during the follow up period. The study size of this trial was not powered to specifically determine CIR associated with same-genotype persistence over the follow up period; therefore, the results are limited by low group numbers. Related to this limitation, the removal of the majority of disease at baseline

(as described above) made it difficult to obtain large numbers at follow up time points for persistence tracking. It should be noted that the Onclarity trial capped the vaccination rate for the study population at 10%. Recent studies demonstrate a reduction in prevalence of genotypes targeted by the vaccine [43]. However, while it is true that overall positive predictive value for vaccine-targeted genotypes will decrease as vaccination coverage increases, the risk for precancer associated with a genotype-specific result, at an individual level, should be consistent with those values published in the last decade from populations with low vaccine coverage. A further limitation of this study was the inability to individually identify HPV35, distinct from HPV39 and HPV68. Recent evidence demonstrates that it has an elevated risk for pre-cancer and cancer in women of African descent [44]. Future studies should address the impact of genotype on disease outcomes in different ethnicities (including African, Latina and indigenous women) to better inform optimal triage using the current risk-based approach.

5. Conclusions

These results, from the baseline and longitudinal phases of the Onclarity trial, support the application of HPV primary screening for women ≥ 25 years, with a combination of triage involving extended genotyping results and cytology. The addition of HPV31, as part of the xGT31 and xGT31+ screening strategies, provided better $\geq$ CIN3 sensitivity than HPV16/18 primary screening and hybrid screening. The xGT31+ strategy provided a better $\geq$ CIN3 sensitivity and lower colposcopy/ $\geq$ CIN3 ratio than primary HPV16/18 screening. An HPV-negative/any cytology result provided similar assurance of no disease after three years when compared to an HPV-negative/NILM result, reinforcing that co-testing offers no advantages over HPV primary screening. Finally, genotype persistence for one to three years greatly increased the risk associated with high-grade CIN, which demonstrates a useful advantage of extended genotyping for clinical management of patients from screening through follow up management.

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Author contribution

All authors contributed to the interpretation of the data, critically revised the manuscript for important intellectual content, and approved the final version to be published. Becton, Dickinson and Company employees that are also authors, played the following roles during the study and development of the paper: VP facilitated data analyses and revision of the manuscript; KY facilitated conception and design of the study, data acquisition and interpretation, and drafting and revision of the manuscript; JAA and CKC facilitated study conception and design, and manuscript revision. MHS and TCW were primary investigators for the study and played critical roles in data collection, data analysis, and results interpretation. All authors provided significant writing/editing effort into the manuscript; all authors provided final approval of the manuscript and agree to be accountable for the accuracy and integrity of this work.

Declaration of Competing Interest

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

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

CKC was an employee of Becton, Dickinson and Company, the study sponsor, at the time of study conduct and manuscript preparation.

VP, KY, and JAA are employees of Becton, Dickinson and Company, the sponsor of the study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygyno.2023.01.004>.

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