



# Human papillomavirus-based screening at extended intervals missed fewer cervical precancers than cytology in the HPV For Cervical Cancer (HPV FOCAL) trial

Anna Gottschlich<sup>1,2</sup>   | Lovedeep Gondara<sup>3</sup> | Laurie W. Smith<sup>1,4</sup> | Darrel Cook<sup>5</sup> | Ruth Elwood Martin<sup>2</sup> | Marette Lee<sup>2,6</sup> | Stuart Peacock<sup>4,7</sup>  | Lily Proctor<sup>1,2,6</sup> | Gavin Stuart<sup>2</sup> | Mel Krajden<sup>2,5</sup> | Eduardo L. Franco<sup>8</sup>  | Dirk van Niekerk<sup>2,6</sup> | Gina Ogilvie<sup>1,2</sup> 

<sup>1</sup>BC Women's Hospital and Health Service, Women's Health Research Institute, Vancouver, Canada

<sup>2</sup>Faculty of Medicine, University of British Columbia, Vancouver, Canada

<sup>3</sup>Department of Data and Analytics, BC Cancer Agency, Vancouver, Canada

<sup>4</sup>Cancer Control Research, BC Cancer Agency, Vancouver, Canada

<sup>5</sup>BC Centre for Disease Control, Vancouver, Canada

<sup>6</sup>Cervix Screening Program, BC Cancer Agency, Vancouver, Canada

<sup>7</sup>Simon Fraser University, Faculty of Health Sciences, Vancouver, Canada

<sup>8</sup>Division of Cancer Epidemiology, McGill University, Montreal, Canada

## Correspondence

Anna Gottschlich, University of British Columbia, Faculty of Medicine, Vancouver, Canada.

Email: [anna.gottschlich@cw.bc.ca](mailto:anna.gottschlich@cw.bc.ca)

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## Abstract

While cervix screening using cytology is recommended at 2- to 3-year intervals, given the increased sensitivity of human papillomavirus (HPV)-based screening to detect precancer, HPV-based screening is recommended every 4- to 5-years. As organized cervix screening programs transition from cytology to HPV-based screening with extended intervals, there is some concern that cancers will be missed between screens. Participants in HPV For Cervical Cancer (HPV FOCAL) trial received cytology (Cytology Arm) at 24-month intervals or HPV-based screening (HPV Arm) at 48-month intervals; both arms received co-testing (cytology and HPV testing) at exit. We investigated the results of the co-test to identify participants with cervical intraepithelial neoplasia grade 2 or higher (CIN2+) who would not have had their precancer detected if they had only their arm's respective primary screen. In the Cytology Arm, 25/62 (40.3%) identified CIN2+s were missed by primary screen (ie, normal cytology/positive HPV test) and all 25 had normal cytology at the prior 24-month screen. In the HPV arm, three CIN2+s (3/49, 6.1%) were missed by primary screen (ie, negative HPV test/abnormal cytology). One of these three misses had low-grade cytology findings and would also not have been referred to colposcopy outside of the trial. Multiple rounds of cytology did not detect some precancerous lesions detected with one round of HPV-based screening. In our population, cytology missed more CIN2+, even at shorter screening intervals, than HPV-based screening. This assuages concerns about missed detection postimplementation of an

**Abbreviations:** Aptima, Aptima HPV assay; ASCUS, atypical squamous cells of undetermined significance; BC, British Columbia; CADTH, Canadian Agency for Drugs and Technologies in Health; CIN2+, cervical intraepithelial neoplasia grade 2 or higher; CIN3+, cervical intraepithelial neoplasia grade 3 or higher; cobas, cobas 4800 HPV test; HC2, Digene Hybrid Capture 2 High-Risk HPV DNA Test; HPV, human papillomavirus; HPV FOCAL, HPV For Cervical Cancer randomized controlled trial; LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; WHO, The World Health Organization.

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extended interval HPV-based screening program. We recommend that policymakers consider a shift from cytology to HPV-based cervix screening.

#### KEYWORDS

cervical cancer, extended screening intervals, HPV-based screening

#### What's new?

Human papillomavirus (HPV)-based testing for cervical cancer is more sensitive than cytology in screening programs. However, there is persisting concern that HPV-based cervix screening at 4- to 5-year intervals could miss cancers otherwise detected by cytology at 2- to 3-year intervals. This is the first study to use co-testing 4 years after primary cytology- or HPV-based screening to identify precancers that would be missed by cytology- or HPV-based screening alone. Over eight times more precancers were missed by cytology-based screening every 2 years compared to HPV-based screening every 4 years, supporting an extended shift from cytology to HPV-based cervix screening.

## 1 | INTRODUCTION

The World Health Organization (WHO) recently launched the Global Strategy to Accelerate the Elimination of Cervical Cancer, which proposes that, if all countries worldwide achieve 90% vaccination coverage, 70% screening coverage, and 90% access to treatment for cervical precancer and cancer for eligible women and individuals with a cervix by 2030, elimination of cervical cancer as a public health problem is feasible by the end of the century.<sup>1</sup> To meet the screening coverage goal, decision-makers across the world are considering how to optimize cervix screening programs in their respective settings.

Nearly all cervical cancers result from a human papillomavirus (HPV) infection.<sup>2</sup> HPV-based screening for cervical cancer is more sensitive than cytology in the setting of a screening program<sup>3</sup> and thus a negative test provides greater assurance than cytology against the presence of precancerous lesions.<sup>4</sup> Accordingly, many national health programs have transitioned<sup>5,6</sup> or plan to transition<sup>7,8</sup> to organized HPV-based screening programs. For example, the Canadian Partnership Against Cancer released an Action Plan for the Elimination of Cervical Cancer in Canada, which includes the target of screening 90% of eligible individuals with an HPV test by 2030.<sup>9</sup> Some countries, such as the United States, have used co-testing with both HPV and cytology for the past 20 years; however, preliminary data has suggested that cytology may not significantly improve precancer detection in primary HPV-based screening programs.<sup>10</sup>

Although there is improved sensitivity with HPV-based screening, there is reduced specificity compared to cytology to identify clinically meaningful lesions.<sup>4</sup> There is general consensus that some proportion of lesions identified by any approach to cervix screening would spontaneously regress without treatment.<sup>11</sup> To improve specificity of HPV-based screening and thus minimize unnecessary follow-up testing and treatment, it is recommended that HPV-based screening programs use triage strategies, such as partial genotyping and/or reflex cytology, to minimize unnecessary referrals to colposcopy<sup>12-15</sup> and to extend intervals between negative screens.<sup>16-19</sup> Extended screening intervals are particularly appropriate in the context of cervical cancer,

which has a considerable lag time between exposure to the carcinogen and development of disease.<sup>20</sup> Precancerous lesions develop 1 to 5 years after HPV infection, and, if precancer is untreated, invasive cancer can take 15 to 20 years to develop.<sup>21</sup> This is similar to lung cancer, where smoking exposure can begin in teenage years but cancer does not develop until age 50 to 60,<sup>22</sup> or liver cancer where infection often occurs in early adulthood and disease in middle ages.<sup>23</sup>

However, there remains some concern that extended screening intervals will lead to missed detection of cervical cancer.<sup>24</sup> For example, multiple studies have provided evidence that women have concerns about the safety of extended intervals with HPV-based screening, even after being provided with the information currently available about HPV-based screening.<sup>25-28</sup> It is thus critical to provide clear evidence that even with extended intervals, HPV-based screening misses fewer detections of precancer and cancer than cytology.

HPV FORe CervicAl Cancer (HPV FOCAL) was randomized controlled trial (2008-2016, N = 25 223) in British Columbia (BC) that compared HPV-based screening to cytology by calculating the cumulative incidence of high-grade precancer 48 months after screening.<sup>3</sup> The trial consisted of an HPV Arm, which received two rounds of extended interval HPV-based screening (spaced 48 months apart), and a Cytology Arm, which received three rounds of cytology at the intervals recommended at the time (spaced 24 months apart). In both arms, the 48-month (exit) screen consisted of cytology and HPV co-testing, providing a complete census of events.

The goal of organized screening programs is to detect the majority of precancer cases while minimizing harms caused by screening (either directly from the screen or indirectly from treatment or emotional distress). There has been concern that with the less frequent intervals used in HPV-based cervix screening there will be more missed cancers. Using data from HPV FOCAL, we examined which precancers would have been missed by HPV- or cytology-based screening, respectively, at trial exit. We assessed whether more precancers would be missed with cytology-based screening every 24 months or HPV-based screening every 48 months. The objective of this analysis is to obtain evidence that could reassure programs

adopting primary HPV-based screening that the extended interval recommended with an HPV-based approach would not result in missed detections of precancer and that co-testing is not necessary in a primary HPV-based cervix screening program.

## 2 | MATERIALS AND METHODS

In this descriptive analysis, we investigated the number of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and grade 3 or worse (CIN3+) that would not have been detected if participants received only primary HPV-based screening or cytology at exit, corresponding to their baseline primary test, instead of the co-test that was received per study protocol. We included the subset of participants from HPV FOCAL who had (a) a negative result on their trial arm's respective primary screen at exit screening and (b) high grade precancer detected at the exit co-test screening.

### 2.1 | Sampling and design: The HPV FOCAL trial

HPV FOCAL has been extensively described in the literature.<sup>3,18,29-37</sup> Briefly, HPV FOCAL recruited women between the ages of 25 and 65 from Metro Vancouver and Greater Victoria, BC. HPV FOCAL included three arms: the intervention arm (HPV Arm), the control arm (Cytology Arm) and the safety arm (not included in this analysis). The HPV Arm received HPV-based screening at baseline and co-testing (HPV testing and liquid-based cytology [LBC]) at 48-month exit, while the Cytology Arm received LBC testing at baseline and 24-months and co-testing at 48-month exit (Figure 1). We chose to co-test at exit to have a complete census of events in both arms. As a result, we assume that all CIN2+ lesions were detected.

Women who had positive screen results were referred to follow-up testing according to the trial protocol (Figure 2). Women in the HPV Arm who were HPV positive at baseline were triaged with LBC and if their LBC result was "atypical squamous cells of undetermined significance" (ASCUS) or worse, were referred for immediate colposcopy. If their LBC results were negative for intraepithelial lesion or malignancy (NILM), they were asked to re-screen at 12 months with HPV and LBC. If at 12 months both HPV and LBC were negative, they were asked to return at 48 months for exit co-testing, while if either were positive, they were referred for immediate colposcopy. Women in the Cytology Arm who had baseline results with low-grade squamous intraepithelial lesion (LSIL) or greater were immediately referred to colposcopy. If baseline cytology results were ASCUS, the sample received HPV triage testing and if positive, the person was referred to immediate colposcopy. Women who had ASCUS with HPV negative triage results were asked to return at 12 months for repeat LBC testing. Women who had ASCUS or worse at 12 months were immediately referred for colposcopy, while those with NILM were asked to return at 24-month LBC testing and if NILM, at 48-months for co-testing.

### 2.2 | Primary lab analyses

HPV testing was performed with Digene Hybrid Capture 2 High-Risk HPV DNA Test (HC2), an assay that used DNA signal amplification and gave binary positive/negative results for 13 types of high-risk HPV. To perform LBC, the participant's sample was placed on a slide using the ThinPrep 2000 processor.<sup>38</sup> Smears were manually screened by program cytotechnologists who referred all abnormal results to a cytopathologist for final interpretation and reporting.

### 2.3 | Adjunct lab analyses

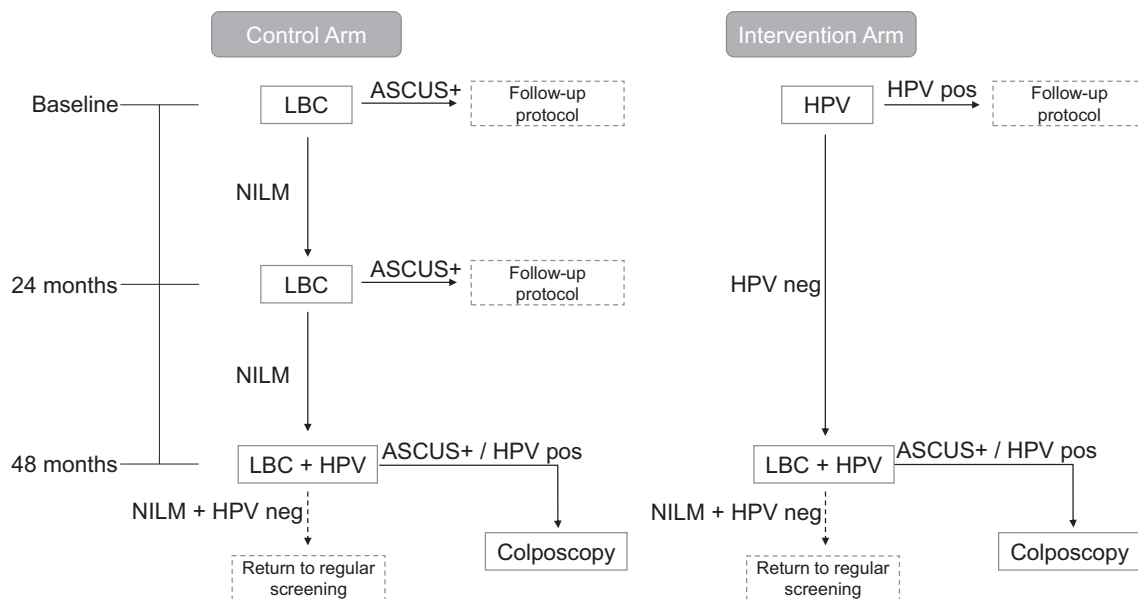
As part of adjunct studies to understand the efficacy of other HPV assays, we used two other assays in the trial. The results obtained by these assays were blinded until the conclusion of the trial and participants were managed on the results from HC2 alone. The first additional assay, cobas 4800 HPV Test (cobas), used PCR DNA amplification and gave positive/negative results for HPV16, HPV18 and other high-risk HPV (11 types) and the second, Aptima HPV Assay (Aptima), targeted E6/E7 mRNA and gave positive/negative results for HPV16, HPV18/45 and other high-risk HPV (11 types).

### 2.4 | Participant cervix screening history prior to HPV FOCAL

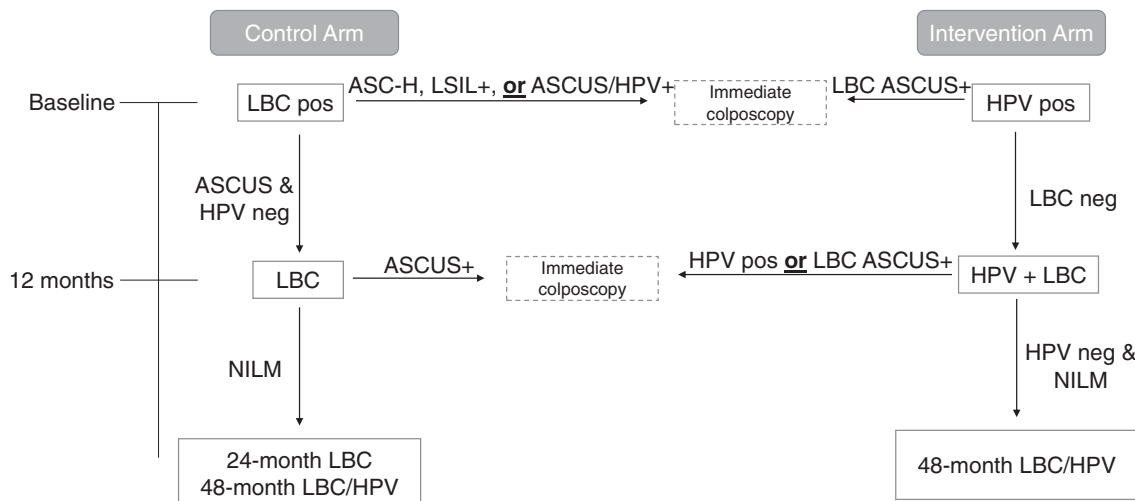
In British Columbia, cervix screening is provincially managed by the BC Cancer Agency.<sup>39</sup> The program maintains provincial screening guidelines and coordinates the reminder system used to notify primary care providers when their patients are due for screening. Additionally, there is one centralized laboratory where all screens and tests are processed and one centralized registry where the results of all screens and follow-up management are maintained. We linked trial data for HPV FOCAL participants to their screening data from the Cervix Screening Program, creating a cohort containing a complete record of each HPV FOCAL participant's screening history in the province before and after participation in HPV FOCAL (the FOCAL-DECADE cohort). Using the data from the FOCAL-DECADE cohort, we included the number of screens that participants had received prior to entry into HPV FOCAL in the analysis.

### 2.5 | Variable creation and statistical analyses

This analysis included women from the HPV FOCAL trial who had a CIN2+ that was considered not detected by the primary test in the screening arm to which she was assigned. We defined "not detected by primary test" as detections of CIN2+ that would not have been detected if they had only received their arm's respective primary test at trial exit. For instance, women from the HPV Arm who were HPV negative/LBC positive at exit and had CIN2+ detected at colposcopy as well as women from the Cytology Arm who were LBC negative/



**FIGURE 1** HPV FOCAL trial testing flow chart. Women in the Control Arm received liquid-based cytology (LBC) at baseline and 24-month testing and co testing with HPV and LBC at 48-month exit testing. Women in the Intervention Arm received HPV-based screening at baseline and co-testing at 48-month exit testing. Women who had abnormal results were referred to further testing and/or repeat screens based on study follow-up protocol



**FIGURE 2** HPV FOCAL trial follow-up protocol. At baseline, if women in the Control Arm had an abnormal screen and had “atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesions” (ASC-H) or “low-grade squamous intraepithelial lesions” (LSIL) or worse were referred for colposcopy. Additionally, women with abnormal screens who had “atypical squamous cells of undetermined significance (ASCUS) AND were HPV positive at a triage test were referred for colposcopy, while those with ASCUS who were HPV negative at triage were asked to return for repeat testing at 12 months. At 12 months, those who had ASCUS or worse were referred to colposcopy, while those with normal screens were asked to return for testing at 24 and 48 months. Similarly, at baseline, women in the Intervention Arm who were HPV positive were given a LBC triage test. Those who were ASCUS or worse were referred to colposcopy, while those who had a normal LBC were asked to return at 12 months. At 12 months, women were co-tested and if either test was abnormal were referred to colposcopy, and if both were normal were asked to return for testing at 48 months

HPV positive at exit and had CIN2+ detected at colposcopy were considered to be “not detected by primary test.”

We assessed all test results received throughout the HPV FOCAL trial for each study participant. Additionally, we calculated the number

of screens study participants had received in the province prior to entry into the HPV FOCAL trial, time since their last screen prior to HPV FOCAL and the result of their most recent screen prior to HPV FOCAL.

To allow for comparison across arms, we summarized the number of missed detections in each arm, as well as calculated the proportion of missed detections out of total CIN2+ detected in each arm. Finally, we calculated the number of missed detections by baseline age, using 10-year age groups (25-29, 30-39, 40-49, 50-59 and 60-65) in each study arm.

### 3 | RESULTS

HPV FOCAL randomized 9552 and 9457 to the HPV and Cytology Arms, respectively. Of those, 9540 (99.9%) and 9408 (99.5%) completed baseline screening and 8296 (86.9%) and 8078 (85.4%) completed exit screening in the HPV and Cytology Arms, respectively. Those who did not complete exit screening were either lost to follow-up (HPV Arm N = 1097; Cytology Arm N = 1202) or exited the trial early due to CIN2+ detection through baseline screening (HPV Arm N = 147; Cytology Arm N = 90) or 24-month screening (Cytology Arm N = 38).<sup>3</sup> At the 48-month exit co-test screen, in the HPV Arm 49 CIN2+ were detected and in the Cytology Arm 62 CIN2+ were detected.

#### 3.1 | Not detected by cytology

In the Cytology Arm, there were a total of 62 CIN2+ (33 CIN2, 29 CIN3+) detected at exit screening (Table 1). Of these, 25 (40.3%) were not detected by cytology, 17 CIN2 and 8 CIN3+. The distribution of CIN findings among missed cases by age group can be found in Table 2. The highest percentage of nondetected CIN3+, which are the least likely to spontaneously regress, out of total missed detections was in the youngest age group.

Furthermore, in the Cytology Arm, the youngest age group (25-29) had the highest burden of missed detections, with 0.72% of total population screened at exit having a missed CIN 2+ detection (Table 3). This percentage decreased as age group increased. Notably, nearly 60% of the total CIN2+ detections in the 40 to 49 and 50 to 59 age group were not detected by cytology.

The 25 women who had CIN2+ not detected by cytology had between 3 and 13 cervix screens prior to entry into HPV FOCAL (mean = 5.9). Over 75% (19/25) had never had an abnormal screen prior to entry into HPV FOCAL, and all had a normal screen directly prior to HPV FOCAL entry. The average time between their most recent screen prior to HPV FOCAL and baseline HPV FOCAL screening was 1.94 years. At baseline, 96% (24/25) of the missed detections had normal cytology results. One had LSIL and was referred to colposcopy, with normal colposcopy results. At 24-months, all participants who received testing (22/25) had normal cytology results. At exit, all had normal cytology and were HPV positive.

#### 3.2 | Not detected by HPV

In the HPV Arm, there were a total of 49 CIN2+ (27 CIN2, 22 CIN3+) detected at exit screening (Table 1). Of these, three were not detected

**TABLE 1** Summary of precancers not detected by primary test<sup>a</sup> at exit testing in the cytology and HPV arms

	Cytology Arm	HPV Arm
	Total	Total
	CIN2+ = 62	CIN2+ = 49
During HPV FOCAL		
Precancers not detected by primary test <sup>b</sup>	25	3
% of total CIN2+ at 48 month co-test	40.3	6.1
Missed CIN2	17	2
Missed CIN3+	8	1
% neg at previous protocol screen <sup>c</sup>	100.0	100.0
% neg at baseline screen <sup>d</sup>	96.0	66.7
Pre-HPV FOCAL		
% neg at last conventional cytology screen prior to HPV FOCAL as part of screening program	100.0	100.0
Average no. screens prior to HPV FOCAL	5.9	2.0
% ever abnormal screen prior to HPV FOCAL	24.0	0.0
Years since last screen prior to HPV FOCAL	1.9	1.5

<sup>a</sup>“Not detected by primary test”: CIN2+ that would not have been detected if they had only received their arm's respective primary test at exit testing.

<sup>b</sup>Cytology for control arm; HPV test for intervention arm.

<sup>c</sup>24-month (N = 22) or baseline (N = 3) cytology for control arm; baseline (N = 2) or 12-month (N = 1) HPV test for intervention arm.

<sup>d</sup>Cytology for control arm; HPV test for intervention arm.

by HPV, two of which were CIN2 and one CIN3+. The distribution of CIN findings among missed cases by age group can be found in Table 2.

Two of the three missed detections were age 30 to 39 at baseline and the third was in the 40 to 49 age group (Table 3). Two had screens recorded in the provincial screening registry prior to HPV FOCAL (mean number of screens = 2.0), none of which had abnormal results. The average time between their most recent screen prior to HPV FOCAL and baseline HPV FOCAL screening was 1.52 years. At HPV FOCAL baseline, two were HPV negative. The third was HPV positive and had normal cytology at triage so was referred to re-screen at 12-months, where she was HPV negative but had abnormal cytology. However, the colposcopy was normal, so she was referred to 48-month exit testing. At exit, all three were HPV negative using HC2. Two had HSIL results from their LBC (one had CIN2 and the other CIN3+) and one had ASCUS (CIN2). Notably, the one with ASCUS would not have been referred to colposcopy even outside of the trial, due to the low-grade findings. The adjunct lab analysis showed that all three were HPV negative with cobas, however the two with HSIL were positive with Aptima. The Roche Linear Array HPV Genotyping Test (LA) was used to genotype these two cases and

	Cytology Arm					HPV Arm				
	Total	CIN2		CIN3+		Total	CIN2		CIN3+	
Age <sup>b</sup>	N	N	%	N	%	N	N	%	N	%
25-29	6	3	50.0	3	50.0	0	0	NA	0	NA
30-39	7	6	85.7	1	14.3	2	1	50.0	1	50.0
40-49	7	4	57.1	3	42.9	1	1	100.0	0	NA
50-59	4	3	75.0	1	25.0	0	0	NA	0	NA
60+	1	1	100.0	0	0.0	0	0	NA	0	NA

**TABLE 2** Not detected by primary test<sup>a</sup> by CIN finding and age group at baseline

<sup>a</sup>“Not detected by primary test”: CIN2+ that would not have been detected if they had only received their arm's respective primary test at exit testing.

<sup>b</sup>Age at entry into HPV FOCAL (participants were ~4 years older at the exit co-test).

**TABLE 3** Proportions not detected by primary test<sup>a</sup> out of total by age group at baseline

Age <sup>b</sup>	Cytology Arm					HPV Arm				
	Missed	CIN2+	Total pop <sup>c</sup>	% of CIN2+	% of Total	Missed	CIN2+	Total pop <sup>c</sup>	% of CIN2+	% of Total
25-29	6	18	834	33.3	0.72	0	14	829	0.0	0.00
30-39	7	22	2349	31.8	0.30	2	17	2425	11.8	0.08
40-49	7	12	3026	58.3	0.23	1	9	3075	11.1	0.03
50-59	4	7	2464	57.1	0.16	0	7	2448	0.0	0.00
60+	1	3	784	33.3	0.13	0	2	775	0.0	0.00

<sup>a</sup>“Not detected by primary test”: CIN2+ that would not have been detected if they had only received their arm's respective primary test at exit testing.

<sup>b</sup>Age at entry into HPV FOCAL (participants were approximately 4 years older at the exit co-test).

<sup>c</sup>Total = all participants who received exit co-test in age group.

gave results that one was positive for HPV types 6 and 67, and the other for HPV type 84.

## 4 | DISCUSSION

This analysis of data from the HPV FOCAL trial found that at trial exit, over eight times more high-grade CIN lesions would have been missed by cytology (25 out of 8296 screened, 0.301%) than by HPV-based screening (3 out of 8078 screened, 0.037%). In the Cytology Arm, three rounds of cytology at 24-month intervals (baseline, 24-month and exit) did not detect the 25 lesions that were detected by one HPV-based screen (at exit). Furthermore, all 25 women who had CIN2+ not detected by cytology at exit had at least one negative screen through the BC provincial screening program immediately prior to HPV FOCAL, reinforcing the evidence that cytology can continue to miss cervical lesions over many rounds of screening.<sup>40</sup>

In comparison, in the HPV Arm, only three high-grade lesions were missed by HPV-based screening but detected by cytology after one prior negative HPV-based screen (baseline or 12-month), one of which had low-grade cytology findings and would not have been referred to colposcopy in the provincial screening program either. Furthermore, it is noteworthy that with other widely used clinically validated HPV assays, two of these three lesions would have been detected. These results are in line with other studies that have shown that the risk of cervical precancer after one negative HPV-based

screen is significantly lower than after a negative Pap test.<sup>19,41,42</sup> As previously reported,<sup>3</sup> HPV testing at baseline detected more CIN2+ earlier (147 CIN2+ detected in the HPV Arm compared to 90 in the Cytology Arm), leading to a smaller total number of precancers in the HPV Arm at exit screen, 48 months later (49 CIN2+ detected by exit co-testing in the HPV Arm vs 62 in the Cytology Arm). Similarly, prior literature has shown that HPV-based screening identifies precancerous lesions earlier than cytology.<sup>41,43,44</sup>

In the Cytology Arm, undetected precancers were most prominent among women in the younger age groups, specifically those in their late-20s at baseline testing and early to mid-30s at exit. HPV infection and low-grade lesions are common in women around sexual debut (usually in their early 20s) and many of these lesions regress spontaneously without treatment.<sup>11,45</sup> Because of this, HPV-based screening is often not recommended until women are at least 30 years old. Here we saw that, among the six missed detections in participants who were less than 35 years old at exit testing, three (50%) had CIN3+, which is less likely to regress without treatment.<sup>46</sup> This highlights the importance of appropriate and effective screening among women in their late 20s and early 30s.

Many studies have compared the ability of primary HPV-based screening and cytology to detect cervical precancer. This analysis is one of the first of its kind to explore the precancers that would not have been detected by a primary screening test. Study participants came from the population of a randomized controlled trial, and thus it is assumed that the participants in the two arms are interchangeable



and no confounding is present.<sup>3,34</sup> Additionally, due to the co-testing that was conducted at the exit screen in the HPV FOCAL trial, we were uniquely able to identify all cervical precancers to assess which lesions would not have been identified without the co-test. Furthermore, the Cervix Screening Program and Registry, maintained by BC Cancer, ensured consistency of screen and test results with its centralized laboratory, and allowed us to identify the complete screening history of each study participant, both before and after their participation in the HPV FOCAL trial.

The results of this analysis must also be interpreted in the context of its limitations. The HPV FOCAL population was generally well screened, even before participation in the trial (we saw that most women with a missed detection had a screen within the 2 years prior to HPV FOCAL entry). Thus, our results may not be generalizable to other populations. However, we did see that HPV-based screening increases CIN2+ detection even in a population that is already engaged in cytology screening. Additionally, the HPV FOCAL trial used an assay for HPV-based screening that gave only a binary positive/negative result for 13 types of high-risk HPV. This assay is no longer commonly used, in part due to its inability to discriminate among HPV genotypes. We saw that among the three missed detections in the HPV Arm, two were positive using one of the other HPV assays that provide such genotype discrimination. While no screening test will perform perfectly, it is possible that as technology continues to develop, HPV assays will miss even fewer precancers.

In fact, current literature has demonstrated that triage strategies that use partial or extended genotyping,<sup>47</sup> as well as those that use ki-67/pi16 dual staining,<sup>48</sup> increase the sensitivity of the screening strategy to detect precancerous lesions. These strategies will likely grow the gap in performance between cytology and HPV-based cervix screening and will minimize unnecessary referrals to colposcopy that are a concern with HPV-based screening.

The findings from this analysis support prior evidence that the higher sensitivity of HPV-based screening enables better safety for subsequent CIN2+ detection in screening programs. Furthermore, our findings provide strong evidence that HPV-based screening programs will miss fewer precancers than cytology, even with extended intervals between screens. In the United States, co-testing at 5-year intervals is one of the recommended strategies for primary cervical cancer screening.<sup>49</sup> However, in our study, the addition of cytology to HPV testing led to very few additional detections, adding to prior evidence that co-testing may not add value to primary HPV-based cervix screening programs.<sup>40,50</sup> Furthermore, with improved triage strategies after a positive HPV test, including partial/extended genotyping and ki-67/p16 dual staining, unnecessary referrals to colposcopy can be minimized. Given these results, we recommend that policymakers consider a shift from cytology or co-testing to primary HPV-based cervix screening. Future research should investigate the most effective ways of communicating the improved safety of primary HPV-based screening to prevent cervical cancer compared to cytology, even with the extended intervals recommended with an HPV-based approach to screening.

## AUTHOR CONTRIBUTIONS

Anna Gottschlich: Formal analysis, writing - original drafting. Lovedeep Gondara: Formal analysis, writing - review & editing. Laurie W. Smith: Conceptualization, funding acquisition, writing - review & editing. Darrel Cook: Data curation, methodology, writing - review & editing. Ruth Elwood Martin: Conceptualization, funding acquisition, writing - review & editing. Gavin Stuart: Conceptualization, funding acquisition, writing - review & editing. Marette Lee: Methodology, project administration, writing - review & editing. Stuart Peacock: Conceptualization, funding acquisition, writing - review & editing), Lily Proctor: Project administration, writing - review & editing. Eduardo L. Franco: Conceptualization, funding acquisition, writing - review & editing. Mel Krajden: Conceptualization, funding acquisition, writing - review & editing. Dirk van Niekerk: Conceptualization, funding acquisition, writing - review & editing. Gina Ogilvie: Conceptualization, funding acquisition, writing - review & editing.

The work reported in the article has been performed by the authors, unless clearly specified in the text.

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## CONFLICT OF INTEREST

Mel Krajden was the principal investigator, and Gina Ogilvie, Dirk van Niekerk, Eduardo L. Franco and Darrel Cook were coinvestigators on investigator-led, industry-funded (Hologic Inc and Roche) adjunct studies to the HPV FOCAL trial, designed to compare the performance of different HPV assays. Funding for the adjunct studies was not applied to the operation of the HPV FOCAL trial results presented here. Funding for industry-funded studies was issued to the investigator institutions to conduct these adjunct studies and investigators did not personally benefit financially. Darrel Cook received speaker honoraria and travel expenses from Hologic Inc. Mel Krajden has received grants/contracts paid to his institution from Roche, Hologic Inc. and Siemens unrelated to the present work. Eduardo L. Franco does not have conflicts of interest but disclose the following: he received grants from CIRH, CCSRI, CRS and NIH to fund studies other than the one reported in this article. The following are disclosures about activities unrelated to the present article: personal fees from Merck; a patent related to the discovery "DNA methylation markers for early detection of cervical cancer", registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada. The remaining authors have no disclosures.

## DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

## ETHICS STATEMENT

Written informed consent was obtained for all participants and ethics approval was obtained from the University of British Columbia Clinical

Research Ethics Board (HPV FOCAL: H06-04032; FOCAL-DECADE: H18-02063). The HPV FOCAL trial is registered at [isrctn.org](https://www.isrctn.com) with the identifier: ISRCTN79347302.

## ORCID

Anna Gottschlich  <https://orcid.org/0000-0002-3095-2323>

Stuart Peacock  <https://orcid.org/0000-0002-8243-8721>

Eduardo L. Franco  <https://orcid.org/0000-0002-4409-8084>

Gina Ogilvie  <https://orcid.org/0000-0001-5783-4493>

## TWITTER

Anna Gottschlich  @annagottschlich

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