Baseline findings and safety of infrequent vs. frequent screening of human papillomavirus vaccinated women

Karolina Louvanto[1], Tiina Eriksson[2], Penelope Gray[2,3], Dan Apter[4], Iacopo Baussano[5], Anne Bly[2], Katja Harjula[2], Kaisa Heikkilä[1,2], Mari Hokkanen[2], Leila Huhtinen[2], Marja Ikonen[2], Heidi Karttunen[2], Mervi Nummela[2], Anna Söderlund-Strand[6], Ulla Veivo[2],Joakim Dillner[3], Miriam Elfstrom[3], Pekka Nieminen[7], Matti Lehtinen[2,3]

[1]Turku University Hospital, University of Turku, Finland

[2]University of Tampere, Finland

[3]Karolinska Institute, Stockholm, Sweden

[4]Family Federation Finland, Helsinki, Finland

[5]International Agency for Research on Cancer, Lyon, France

[6]SkåneUniversityHospital, Lund, Sweden

[7]University of Helsinki, Finland

Corresponding author: Karolina Louvanto, MD, PhD, Associate Professor; Department of Obstetrics and Gynecology; Turku University Hospital, University of Turku

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Kiinanmyllynkatu 4-8, 20521 Turku, Finland; e-mail: karolina.louvanto@utu.fi; phone: +358 231339201

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Abbreviations used: ASC-H: atypical squamous cells- cannot exclude high-grade lesion, ASCUS: atypical squamous cells of undetermined significance, AGC: atypical glandular cells, CIN: cervical intraepithelial neoplasia (the number denotes the grade), HSIL: high grade intraepithelial lesion, HPV: Human papillomavirus, hrHPV: high risk (carcinogenic) HPV types, lrHPV: low risk (non-carcinogenic) HPV types, MALDI-TOF: matrix-assisted laser desorption time-of-flight

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Novelty and Impact:

First cohorts of human papillomavirus (HPV)-vaccinated women are reaching the screening ages and there is an increasing need to redesign cervical cancer screening programs. Less frequent screening in HPV-vaccinated birth-cohorts could produce considerable savings without increasing cervical cancer incidence. We report here baseline findings and interim results of safety of infrequent screening among HPV16/18-vaccinated females. Cervical high-grade lesions showed to be equally rare findings in all the study-arms indicating no safety concerns on infrequent screening.

ABSTRACT

Less frequent cervical cancer screening in human papillomavirus (HPV) vaccinated birth cohorts could produce considerable savings without increasing cervical cancer incidence and loss of life-years. We report here the baseline findings and interim results of safety and accuracy of infrequent screening among HPV16/18 vaccinated females. The entire 1992-94 birth-cohorts (30139 females) were invited to a community-randomized HPV16/18-vaccination trial. A total of 9,482 female trial participants received HPV16/18-vaccinationin 2007-09 at age of 13-15. At age 22, 4273 (45%) of these females consented to attend a randomized trial on frequent (ages 22/25/28)(Arm 1: 2073females) vs. infrequent screening (age 28)(Arm 2: 2200females) in 2014-2017. Females (1329), who had got HPV16/18 vaccination at age 18 comprised the safety Arm 3. Baseline prevalence and incidence of HPV16/18 and other high-risk HPV types were: 0.5% (53/1000 follow-up years, 10^4) and 25% (2530/10^4) in the frequently screened Arm 1; 0.2% (23/10^4) and 24% (2,413/10^4) in the infrequently screened Arm 2; and 3.1% (304/10^4) and 23% (2284/10^4) in

the safety Arm 3.Corresponding prevalence of HSIL/ASC-H and of any abnormal cytological findings were:0.3% and 4.2% (Arm 1), 0.4% and 5.3% (Arm 2), and 0.3% and 4.7% (Arm 3).Equally rare HSIL/CIN3 findings in the infrequently screened safety Arm A3 (0.4%) and in the frequently screened Arm 1 (0.4%)indicate no safety concerns on infrequent screening despite the up to 10-times higher HPV16/18 baseline prevalence and incidence in the former.

INTRODUCTION

Prophylactic human papillomavirus (HPV) vaccines have proven to be safe and efficacious against cervical cancer as other anogenital and oral infections with low risk (lr) - and high-risk (hr) -HPV types, and associated neoplasia [1-4]. HPV vaccines are being implemented in vaccination programmes in both developing and developed countries. They have reported significant reductions in the prevalence of hrHPV infections and related cervical lesions, provided that generally high coverage has been achieved [4-6]. Rapid changes in the screening performance, especially deterioration of the positive predictive values of all screening tests, are imminent. [7] At the same time the overuse of cervical screening with obvious harms is a concern. [8] Now, that the first cohorts of HPV vaccinated women are reaching the screening

ages, there is an increasing need to redesign cervical cancer screening programs, to reduce the number of screening visits, and to synchronize tools for future cervical cancer prevention.

The superior sensitivity of molecular HPV-DNA testing as compared to Pap-testing in screening for cervical cancer precursors has been verified in multiple randomized trials. [9-12] Many countries have changed or are changing their screening programs towards HPV-Primary Screening. [13] Consequently cervical screening practices are rapidly changing for both unvaccinated and vaccinated birth cohorts. Mathematical models suggest that with high HPV vaccination coverage (80% or above) the screening interval can be increased up to 20 years with no change in cervical cancer incidence. [14] Integration of the vaccination and screening programs has, however, received relatively little attention. [15]

Nordic screening programs, which apply 3 to 5-year intervals resulting in10 or more life-time screening visits with reasonably high overall coverage, have not been able tackle HPV-disease burden associated with epidemic spread of HPV16. [16,17] Furthermore, despite e.g. most young Finnish women attend opportunistic cervical screening every second year since the start of oral contraception, the incidence of cervical cancer in Finland between 25-39 years of age is now higher than at the start of cervical screening 50 years ago. [17-19] At the same time, opportunistic screening tests which comprise over 60% of all tests, identify mostly lesions that would have regressed spontaneously. [18] Furthermore, the costly over-diagnostics, follow-up

and treatment of the mostly (90%) spontaneously regressing HPV DNA positive cytological findings also reduces the quality-of-life. [20]

Prophylactic HPV vaccination program provision of up to 93% vaccine efficacy against CIN3+ is crucial in reducing the number of screening visits in vaccinated women. [21] Increase of both the number of quality-assured life-years gained, and improved quality-of-life by subsequent vaccination and reduced number of screening and follow-up visits of the vaccinated women are now within reach. In the following, we describe baseline findings of a randomized trial launched to assess how the number of screening visits can be reduced from three visits to one visit in young adult females vaccinated as early adolescents. Also, the interim safety analysis of the trial is reported.

METHODS

Procedures

HPV16/18 vaccination was performed at the health care facilities in all the 250 municipal junior high schools of the 33 trial communities in 2007-2009 (Figure 1). [22] All the vaccinated participants in the 1992-94 birth cohorts received Cervarix® (AS04-HPV-16/18) –vaccine at age 13-15 (Arms A1 and A2) or at age 18 (Arm A3). Altogether 9482 HPV16/18 vaccinated and 3872HPV16/18 cross-vaccinated female participants, who had received hepatitis B-virus vaccine in the community-randomized trial, were eligible at age 22 to attend the trial.Virtually all (99.4% and 86.3% of those vaccinated at the ages of 13-15 years old and 18 years old, respectively) of them received three vaccine doses.

All 1992-94 born females, resident in the trial communities (Supplementary Table 1), were eligible to attend and were invited to the randomized screening trial at ages 22, 25 and 28 years, provided that they had received HPV16/18 vaccination. After obtaining informed consent pelvic examination, cytological Pap-smear and a cervicovaginal self-sample for HPV and *Chlamydia trachomatis* DNA-testing were obtained at the first and second screening visits at ages 22 and 25.

Study design, formation of randomized cohorts and study events in the screening of HPV-vaccines trial are outlined in Figure 1. All participants in Arms A1 and A2 will attend three visits during which cervical samples are taken. In the frequently screened arm A1, the participants will receive all information on cytological findings at ages 22, 25 and 28. In the infrequently screened arm A2, the participants will receive only information indicative of colposcopy (ASC-H, HSIL, AGC) at ages 22, 25 and 28. In the safety arm A3 the participants, who had received HPV16/18 vaccine as

cross-vaccination at age 18, attend two visit only. At age 22 they receive only information on cytological findings indicative of colposcopy. At the trial end (Arms 1 and 2, age 28, Arm 3, age 25), all cytological findings and HPV DNA findings will be conveyed to all the trial participants.

Laboratory analyses

All samples are analyzed for genotypes specific HPV-DNA using matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry for the detection of HPV6/11/16/18/ 31/33/35/39/45/51/52//56/58/59/66/ and 68. [23, 24] MGP consensus primers are used followed by a mass extension reaction with type-specific primers that each have a unique molecular weight for a specific type. Following completion of the mass extension reaction, unextended primers demonstrate the absence and extended primers show the presence of each specific genotype. Confirmative testing, using Luminex was performed on all positives for HPV11/68 due to a cross-reaction between the primers for HPV11/89 and HPV68/70.*Chlamydia trachomatis* DNA analysis is performed with the Abbott Real Time CT/NG assay.

Statistical analysis and Study power

The interim analyses were performed for the first screening visit observations in the 1992, 1993, 1994 -born participants under 11-month time-windows during 2014, 2015-2016 and 2016. Safety was analyzed in the 1992 and 1993 born participants during 2014-18. Descriptive statistics and prevalence/incidence (/10,000 follow-up years between 18 and 22 years of age) estimates of the baseline HPV DNA and/or cytological findings were estimated STATA (Stata Corp, LLC, US).

Analyses or non-inferiority end-point findings between the different trial arms, and estimates for screening sensitivity, specificity and predictive values will be estimated by STATA.

The lowest detectable sensitivity (71.6%) to exclude non-inferiority in the identification of CIN2+ in 7000 frequently vs. infrequently screened 30-year-old women assuming 95% specificity of screening in cumulative CIN rate has 80% power (p=0.05).Data available on request from the authors.

Ethics

Our trial (NCT02149030) has been approved by the Pirkanmaa Hospital District Ethical Review Board in 2013.

Data availability

After completion of the trial in 2025, anonymous data is available with the principal investigator at FICAN-Mid.

RESULTS

Our two randomized trial arms A1 (frequently screened) and A2 (infrequently screened) comprise consented 22-year-old female attendees (2073 and 2200, respectively), who got the HPV16/18 vaccine as early adolescents (ages 13 to 15) in 2007-2009. Females (1329) cross-vaccinated with the HPV16/18 vaccine at age 18 in 2010-2013 consented to participate in a separate safety arm A3 for infrequent screening. Almost all (97%) of the consented females participated the 1st screening visit with cervical sampling (Figure 2).

Stepwise trial enrolment at age 22 was identical in each birth cohort (Figure 3). There was no difference in the attendance whether HPV16/18 vaccination had taken place already in early adolescence at ages 13 to 15 or at the age of 18 (cross-vaccination) (Figure 3). As for the 1992and 1993 birth cohorts which have already attended the 2nd screening round visits, 89% and 86% of the 1st screening round participants have participated (Figure 4).

There were no material differences in the demographic or sexual risk-behaviour characteristics of the screening trial participants (Table 1). The number of individuals with five or more sexual partners by the age of 22 was somewhat higher in those, who had received HPV16/18 vaccination as early adolescents (42.5% both Arms 1 and 2), as compared to those who received HPV16/18 vaccination at the age of 18 (38.6%, Arm 3). Corresponding *C. trachomatis* prevalence at age 22were 2.4% and 2.3% (Arms 1 and 2), and 1.5% (Arm 3).

HPV16/18 DNA prevalence and incidence at the first screening visit (at age 22) were notably rare in both the frequently and infrequently screened Arms A1 and A2 (0.5% and 0.2%, and $53/10^4$ and $23/10^4$) as compared to the safety Arm A3 (3.1% and $304/10^4$) which had received HPV16/18 vaccination at age 18 (Table 2). Also, the prevalence (and incidence) of vaccine-covered HPV types 31/33/45 was about two times lower among Arm 1 and Arm 2 participants, who had been vaccinated between 13-15 years of age as compared to Arm 3 participants (A1 2.8%, A2 2.3%, A3 5.3%, Table 2).Corresponding prevalence (and incidence) of overall hrHPV types were materially equal between the three arms (A1 27%, A2 26%, A3 27%).

Abnormal cytological findings at the 1st screening visit (at age 22) showed no major differences between the different screening arms. Prevalence of ASCUS findings varied from 3.1% to 4.5%, between the three arms (Table 3). The prevalence of LSIL findings was highest in Arm 1 (Table 3), but the overall prevalence of these mild cytological findings varied within a narrow range of 5.1% to 5.9%. The prevalence of ASC-H/HSIL findings were comparable in the frequently

screened Arm A1 (0.3%) and the infrequently screened Arms A2 (0.2%), and the safety Arm A3 (0.3%).

Interim safety analysis of infrequent screening among the cross-vaccinated females was performed at the end of 2018 when virtually all participants from the 1992 and 1993 birth cohorts had attended the 2nd screening visit (Figure 4). There were three (0.4%) HSIL/CIN3 cases found in 824 infrequently screened, cross-vaccinated Arm A3 participants as compared to six (0.4%) HSIL/CIN3 cases in 1280 frequently screened Arm A1 participants, who had received HPV16/18 vaccination early adolescents (Table 4). One of the Arm A3 participants with a HSIL/CIN3 diagnosis was HPV33 positive already at the time of HPV16/18 vaccination.

DISCUSSION

We report on successful enrolment of three female birth cohorts (1992-94), vaccinated in 2007-2009 as early adolescents, into a randomized trial to compare frequent vs. infrequent cervical screening of HPV vaccinated females at ages 22, 25 and 28. Concomitant enrolment of a sizeable cohort of 22-year-old females cross-vaccinated with the HPV16/18 vaccine at age 18 was done.

The sizeable HPV-vaccinated birth cohorts, now at the screening age from the community-randomized trial that was launched in Finland in 2007 to identify the HPV vaccination strategy with the highest impact. [22]. Homogeneous cytological findings in the different trial arms at

baseline assure that enrolment and randomization of the HPV vaccinated females has been successful. On the other hand, the impact of getting vaccinated between ages 13 to 15 is seen in the very low occurrence of cervical HPV vaccine types, HPV16/18, infections at the first screening round (age 22) in the trial Arms A1 and A2 as compared to the similarly-aged safety Arm A3 which comprises females later cross-vaccinated at age 18. The HPV16/18 occurrence and HPV31/33/45 occurrence (prevalence and incidence) were respectively up to 10-fold and 2-fold higher in the latter, which fits with relatively early exposure to major hrHPVs of adolescent Finnish females and also with the relatively broad cross-protectivity of the HPV16/18 vaccine. [21,22]

Overall, safety in our trial is being guaranteed by sampling all participants on every visit with referral of the study participants to diagnosis and treatment pertinent to the cytological results according to mandatory local standard of care (HSIL and ASC-H findings), and by the safety interim analysis. The safety of infrequent screening could be further confirmed by identical occurrence of HSIL/CIN3 lesions in the infrequently and frequently screened females, even if the former had received HPV16/18 vaccination at age 18. The similar low occurrence of HSIL/CIN3 findings in both arms A3 and A1 indicates that continuing infrequent screening in Arm A2 participants, who were vaccinated as early adolescents, up to 28 years of age appears safe. The study setting will enable assessment of the accuracy of frequent cervical screening (at ages 22, 25 and 28) vs. infrequent screening (at the age of 28). In addition to safety, randomized trial evidence on comparable accuracy of the two screening modes should help to minimize number of screening visits in vaccinated young adult women. However, screening results between ages 22 to 25/28 may

not be directly related to cervical cancer risk [25] and surveillance of the screening cohorts will be warranted also after closing the trial in 2026 – 20 years after it was started.

To the best of our knowledge this is the first randomized trial on the performance of frequent vs. infrequent screening of women who have received HPV vaccination as early adolescents. Over time infrequent organized screening of HPV vaccinated women would mean considerable (up to 10-fold) savings compared to the present situation or running two preventive measures (HPV vaccination and screening) concomitantly without synchronization. Completing trial enrolment with altogether 6995 participants from 1992-1995 birth cohorts we are amply powered to deliver randomized-trial evidence on the performance and impact of infrequent screening in HPV vaccinated women by the end of 2023.

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References

1. Lehtinen M, Dillner J. Clinical trials of human papillomavirus vaccines and beyond. *Nature Rev Clin Oncol* 2013;10(7):400-10.

2. Kash N, Lee MA, Kollipara R, Downing C, Guidry J, Tyring SK.Safety and Efficacy Data on Vaccines and Immunization to Human Papillomavirus. *J Clin Med* 2015;4(4):614-33.

3. Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J, Moreira ED Jr, Ngan Y, Petersen LK, Lazcano-Ponce E, Pitisuttithum P, Restrepo JA, Stuart G, Woelber L, Yang YC, Cuzick J, Garland SM, Huh W, Kjaer SK, Bautista OM, Chan IS, Chen J, Gesser R, Moeller E, Ritter M, Vuocolo S, Luxembourg A; Broad Spectrum HPV Vaccine Study. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *New Engl J Med*2015;372(8):711-723.

4. Herrero R, González P, Markowitz LE. Present status of human papillomavirus vaccine development and implementation. *Lancet Oncol* 2015; 16(5):e206-16.

5. Drolet M, Bénard T, Boily MC, Ali H, Baandrup L, Bauer H, Beddows S, Brisson J, Brotherton JM, Cummings T, Donovan B, Fairley CK, Flagg EW, Johnson AM, Kahn JA, Kavanagh K, Kjaer SK, Kliewer EV, Lemieux-Mellouki, Markowitz L, Mboup , Mesher D,

Niccolai L, Oliphant J, Pollock KG, Soldan K, Sonnenberg P, Tabrizi SN, Tanton C, Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: A systematic review and meta-analysis. *Lancet Infect Dis* 2015;15(5):565-80.

6. Mariani L, Vici P, Suligoi B,Checcucci-Lisi G, Drury R. Early direct and indirect impact of quadrivalent HPV (4HPV) vaccine on genital warts: a systematic review. *Adv Therapy* 2015;32(1):10-30.

7. El-Zein M, Richardson L, Franco EL. Cervical cancer screening of HPV vaccinated populations: Cytology, molecular testing, both or none. *J Clin Virol* 2016;76:S62-8.

8. Alber JM, Brewer NT, Melvin C, Yackle A, Smith JS, Ko LK, Crawford A, Glanz K. Reducing overuse of cervical cancer screening: A systematic review. *Prev Med* 2018; 116(11):51-9.

9. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, Rådberg T, Strander B, Forslund O, Hansson BG, Hagmar B, Johansson B, Rylander E, Dillner J. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J Natl Cancer Inst* 2009;101(2):88-99.

10. Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJ; International HPV screening working group.Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*2014;383(9916):524-32.

11. Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, Gilham C, Baysson H, Roberts C, Dowie R, Desai M, Mather J, Bailey A, Turner A, Moss S, Peto J. HPV

testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *Lancet Oncol* 2009;10(7):672-82.

12. Isidean SD, Mayrand MH, Ramanakumar AV, Gilbert L, Reid SL, Rodrigues I, Ferenczy A, Ratnam S, Coutlée F Franco EL; CCCaST Study Group. Human papillomavirus testing versus cytology in primary cervical cancer screening: End-of-study and extended follow-up results from the Canadian cervical cancer screening trial. *Int J Cancer* 2016;139(11):2456-66.

13. Tota JE, Bentley J, Blake J, Coutlée F, Duggan MA, Ferenczy A, Franco EL, Fung-Kee-Fung M, Gotlieb W, Mayrand MH, McLachlin M, Murphy J, Ogilvie G, Ratnam S. Introduction of molecular HPV testing as the primary technology in cervical cancer screening: Acting on evidence to change the current paradigm. *Prev Med*2017;98:5-14.

14. Baussano I, Ronco G, Segnan N, French K, Vineis P, Garnett GP.HPV-16 infection and cervical cancer: modeling the influence of duration of infection and precancerous lesions. *Epidemics*2010;2(1):21-8.

15. Franco EL, Coutlee F, Ferenczy A. Integrating human papillomavirus vaccination in cervical cancer control programmes. *Public Health Genomics*. 2009;12(5-6):352-61.

16. Hortlund M, Elfstrom M, Sparen P, Almstedt P, Strander B, Dillner J.Cervical cancer screening in Sweden 2014-2016. *PLoS One* 2018;13(12):e0209003.

17. Harper D, Nieminen P, Paavonen J, Lehtinen M. Cervix cancer incidence can increase despite HPV vaccination. *Lancet Infect Dis* 2010; 10:594-5.

18. Salo H, Leino T, Kilpi T, Auranen K, Tiihonen P, Lehtinen M, Vänskä S, Linna M, Nieminen P. The burden and costs of prevention and management of genital disease caused by

HPV in women: a population-based registry study in Finland. I*nt J Cancer*. 2013;133(6):1459-69.

19. NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries [Internet]. Association of the Nordic Cancer Registries. Danish Cancer Society. . [cited 13/04/2017].

20. Eriksson T, Torvinen S, Woodhall SC, Lehtinen M, Apter D, Harjula K, Hokkanen M, Rissanen P, Paavonen J, Lehtinen M. Impact of HPV16/18 vaccination on quality of life: a pilot study. *Eur J Contraception & Reproductive Health Care*2013;18(5):364-71.

21. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsague X, Skinner SR, Apter D, Naud P, Salmerón J, Chow SN, Kitchener H, Teixeira JC, Hedrick J, Limson G, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, De Carvalho NS, Germar MJ, Peters K, Mindel A, De Sutter P, Bosch FX, David MP, Descamps D, Struyf F, Dubin G; HPV PATRICIA Study Group et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol* 2012;13(1):89-99.

22. Lehtinen M, Luostarinen T, Vänskä S, Söderlund-Strand A, Eriksson T, Natunen K, Apter D, Baussano I, Harjula K, Hokkanen M, Kuortti M, Palmroth J, Petäjä T, Pukkala E, Rekonen S, Siitari-Mattila M, Surcel HM, Tuomivaara L, Paavonen J, Nieminen P, Dillner J, Dubin G, Garnett G. Gender-neutral provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: Results of a community randomized trial (III). *Int J Cancer*2018; 143:2299-310.

23. Söderlund-Strand A, Carlson J, Dillner J.Modified general primer PCR system for sensitive detection of multiple types of oncogenic human papillomavirus.*J Clin Microbiol* 2009; 47:541-6.

24. Söderlund-Strand A, Dillner J.High-throughput monitoring of human papillomavirus type distribution. *Cancer Epidemiol Biomarkers Prev* 2013;22:242-50.

25. Castanon A, Sasieni P. Is the recent increase in cervical cancer in women aged 20-24 years in England a cause for concern? *Prev Med.* 2018;107:21-8.

Table 1. Characteristics of HPV16/18 vaccinated women[a] participating cervical screening and/or answering a questionnaire at baseline visit by trial arm: A1 = frequent screening at 22/25/28, A2 = infrequent screening at [22/25] /28, and A3 = safety arm, women[b] screened at age [22]/ 25.

Variable		A1 (N=2073) n/mean[c] (%/SD[c])	A2 (N=2200) n/mean[c](%/SD)	A3 (N=1329) n/mean[c] (%/SD)
Chlamydia trachomatis	pos.	49 (2.4)	51 (2.3)	20 (1.5)
	neg.	1961 (94.6)	2083 (94.7)	1269 (95.5)
	missing	63 (3.0)	66 (3.0)	40 (3.0)
	total	2073 (100)	2200 (100)	1329 (100)
Age at sexual debut		16.4 (1.9)	16.5 (1.8)	16.6 (1.8)
No. of lifetime partners	0	76 (4.7)	75 (4.4)	40 (3.8)
	1	269 (16.6)	263 (15.4)	201(19.1)
	2	191 (11.8)	203 (11.9)	133 (12.6)
	3	194 (12.0)	205 (12.0)	124 (11.8)
	4	185 (11.4)	220 (12.9)	128 (2.2)
	≥5	689 (42.5)	726 (42.5)	406 (38.6)
	missing	17 (1.1)	18 (1.1)	20 (1.9)
	total	1620 (100)	1710 (100)	1052 (100)
Smoking	never	932 (57.5)	1015 (59.4)	686 (65.2)
	quit	208 (12.8)	192 (11.2)	113 (10.7)
	current	462 (28.5)	484 (28.3)	244 (23.2)
	other than cig.	13 (0.8)	9 (0.5)	4 (0.4)

missing	6 (0.4)	10 (0.6)	5 (0.5)
total	1620 (100)	1710 (100)	1052 (100)

[a]HPV16/18 vaccinated as early adolescents between 12 to 15 years of age
[b]HPV16/18 cross-vaccinated at 18 years of age
[c]mean age (standard deviation) at sexual debut by the baseline
[22/25] ASCUS and LSIL findings are not communicated to Arm 2 and Arm 3 participants

Table 2. Baseline HPV DNA findings (prevalence, n_1 / incidence ($/10^4$), n_2) in 22 year-old HPV16/18 vaccinated women[a] participating cervical screening by trial arm: A1= frequent screening at ages 22/25/28, A2=infrequent infrequent screening at age [22/25] /28, A3=safety arm vaccinated women[b], screening at age [22] /25.

	A1 (N=1332)		A2 (N=1314)		A3 (N=889)	
HPV type	n_1 (%)	n_2	n_1 (%)	n_2	n_1 (%)	n_2
HPV6/11	80 (6.0)	601	61 (4.6)	464	25 (2.8)	281
HPV16	4 (0.3)	30	3 (0.2)	23	22 (2.5)	248
HPV18	3 (0.2)	23	0 (0.0)	0	5 (0.6)	56
HPV31	8 (0.6)	60	9 (0.7)	69	21 (2.4)	236
HPV33	20 (1.5)	150	15 (1.1)	114	19 (2.1)	214
HPV45	9 (0.7)	68	6 (0.5)	46	7 (0.8)	79

Other hrHPV	337 (25)	2530	317 (24)	2413	203 (23)	2283
Total hrHPV	365 (27)	2740	339 (26)	2580	242 (27)	2722
Total HPV	406 (31)	3048	365 (28)	2778	254 (29)	2857
Missing from HPV DNA analysis	106		139		50	

[a]HPV16/18 vaccinated as early adolescents between 12 to 15 years of age
[b]HPV16/18 cross-vaccinated at 18 years of age
[22/25], [22] ASCUS and LSIL findings are not communicated to Arm 2 and Arm 3 participants

Table 3. Baseline cytological findings in 22 year-old vaccinated women[a] participating cervical screening trial arm: A1 = frequent screening at ages 22/25/28, A2 = infrequent screening at age [22/25] /28, A3 = safety arm vaccinated women[b], screening at age [22]/ 25.

Finding	A1 (N=1438) n (%)	A2 (N=1453) n (%)	A3 (N=939) n (%)
ASCUS	45 (3.1)	66 (4.5)	40 (4.3)
LSIL	23 (1.6)	13 (0.9)	8 (0.9)
ASC-H	1 (0.1)	5 (0.3)	2 (0.2)

AGC/HSIL	5 (0.3)	3 (0.2)	3 (0.3)
Total abnormal	74 (5.1)	87 (5.9)	53 (5.6)

[a]HPV16/18 vaccinated as early adolescents between 12 to 15 years of age
[b]HPV16/18 cross-vaccinated at 18 years of age
[22/25], [22] ASCUS and LSIL findings are not communicated to Arm 2 and Arm 3 participants

Table 4. Interim findings in HPV16/18 vaccinated women[a,b] of high-grade squamous intraepithelial neoplasia (HSIL/CIN3) diagnosed during a maximum 10 years of post-vaccination follow-up when participating frequent (Arm 1[c], n=1280) or infrequent (Arm 3[d], n=824) cervical screening at ages 22 and/or 25 years.

Birth	Vaccination	Cervical HPV DNA findings at	Diagnosis/

Arm	year	year	Vaccination	1st Screening	2nd Screening	year
A1	1992	2008	not available	HPV51/56/66	pending	HSIL/2018
A1	1993	2008	not available	HPV58/68	not available	CIN3/2015
A1	1993	2008	not available	HPV51	not available	CIN3/2016
A1	1994	2009	not available	HPV52/56	not available	HSIL/2018
A1	1994	2009	not available	HPV33	not available	HSIL/2016
A1	1994	2009	not available	HPV35	not available	HSIL/2016
A3	1993	2012	negative	HPV31/56/66	pending	CIN3/2017
A3	1993	2012	negative	negative	pending	CIN3/2017
A3	1993	2012	HPV33	HPV33	not available	CIN3/2014

CIN3 = cervical intraepithelial neoplasia grade 3
[a]HPV16/18 vaccinated as early adolescents between 12 to 15 years of age
[b]HPV16/18 cross-vaccinated at 18 years of age
[c]A1 = frequent screening at ages 22/25/28, [d]A3 = infrequent screening (safety arm) at age 25

Figure Legends

Figure 1. Study design, formation of randomized cohorts and study events in the screening of HPV-vaccines trial.

Figure 2. Flow chart of a randomized trial on the accuracy and safety of infrequent vs. frequent screening in women who got HPV16/18 vaccination as early adolescents in 2007-2009.

Figure 3. Participation to the first round of cervical cancer screening at the age of 22 years by birth cohort (1992, 1993, and 1994 -born).

Figure 4. Participation to the second round of cervical screening at the age of 25 years by birth cohort (1992 and 1993 -born).