





INVITED REVIEW

Assessing the risk of cervical neoplasia in the post-HPV vaccination era

Matti Lehtinen^{1,2}  | Ville N. Pimenoff²  | Belinda Nedjai³ |
 Karolina Louvanto^{1,4} | Lianne Verhoef^{5,6}  | Daniëlle A. M. Heideman^{5,6} |
 Mariam El-Zein⁷ | Martin Widschwendter^{8,9,10,11} | Joakim Dillner² 

¹Medical Faculty, Tampere University, Tampere, Finland

²Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden

³Wolfson Institute of Population Health, Queen Mary University of London, London, UK

⁴Department of Obstetrics and Gynecology, Tampere University Hospital, Tampere, Finland

⁵Department of Pathology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

⁶Cancer Center Amsterdam, Imaging and Biomarkers, Amsterdam, The Netherlands

⁷Division of Cancer Epidemiology, McGill University, Montreal, Canada

⁸European Translational Oncology Prevention and Screening (EUTOPS) Institute, Universität Innsbruck, Hall in Tirol, Austria

⁹Research Institute for Biomedical Aging Research, Universität Innsbruck, Innsbruck, Austria

¹⁰Department of Women's Cancer, UCL EGA Institute for Women's Health, University College London, London, UK

¹¹Department of Women's and Children's Health, Division of Obstetrics and Gynecology, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden

Correspondence

Matti Lehtinen, Department of Lab Medicine, Karolinska Institute, Stockholm, Sweden.
 Email: matti.lehtinen@tuni.fi

Abstract

This review is based on the recent EUROGIN scientific session: “Assessing risk of cervical cancer in the post-vaccination era,” which addressed the demands of cervical intraepithelial neoplasia (CIN)/squamous intraepithelial lesion (SIL) triage now that the prevalence of vaccine-targeted oncogenic high-risk (hr) human papillomaviruses (HPVs) is decreasing. Change in the prevalence distribution of oncogenic HPV types that follows national HPV vaccination programs is setting the stage for loss of positive predictive value of conventional but possibly also new triage modalities. Understanding the contribution of the latter, most notably hypermethylation of cellular and viral genes in a new setting where most oncogenic HPV types are no longer present, requires studies on their performance in vaccinated women with CIN/SIL that are associated with nonvaccine HPV types. Lessons learned from this research may highlight the potential of cervical cells for risk prediction of all women's cancers.

KEYWORDS

cervical cancer, epigenetics, gynecological cancers, human papillomavirus, methylation

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; WID, woman's cancer risk identification.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *International Journal of Cancer* published by John Wiley & Sons Ltd on behalf of UICC.

1 | INTRODUCTION

This review is based on a recent EUROGIN main scientific session (12 April 2022) on assessing the risk of cervical cancer in the post-human papillomavirus (HPV) vaccination era. In keeping with those presentations, we wish to review the new demands and possibilities related to the management of cervical intraepithelial neoplasia (CIN): screening and triage of high-grade squamous intraepithelial lesion (HSIL)/cervical adenocarcinoma in situ (AIS) in HPV vaccinated and unvaccinated women.

The prevalence of vaccine-targeted oncogenic, high-risk (hr) HPV types is rapidly decreasing in countries with effective national vaccination programs.¹⁻⁵ Although the prevalence of nontargeted HPV types has not significantly changed the vaccination has led to changes in their relative proportions and in the overall ecological diversity of mucosal HPV types (Figure 1).^{5,9-11} Test performance, most importantly positive predictive value (PPV) of conventional screening tests (Pap-smear, HPV-tests) now faces new demands of the decreasing background of the HPV types with large oncogenic potential as the majority of positive findings threaten to be false positive findings as previously illustrated¹² and most recently demonstrated.^{13,14}

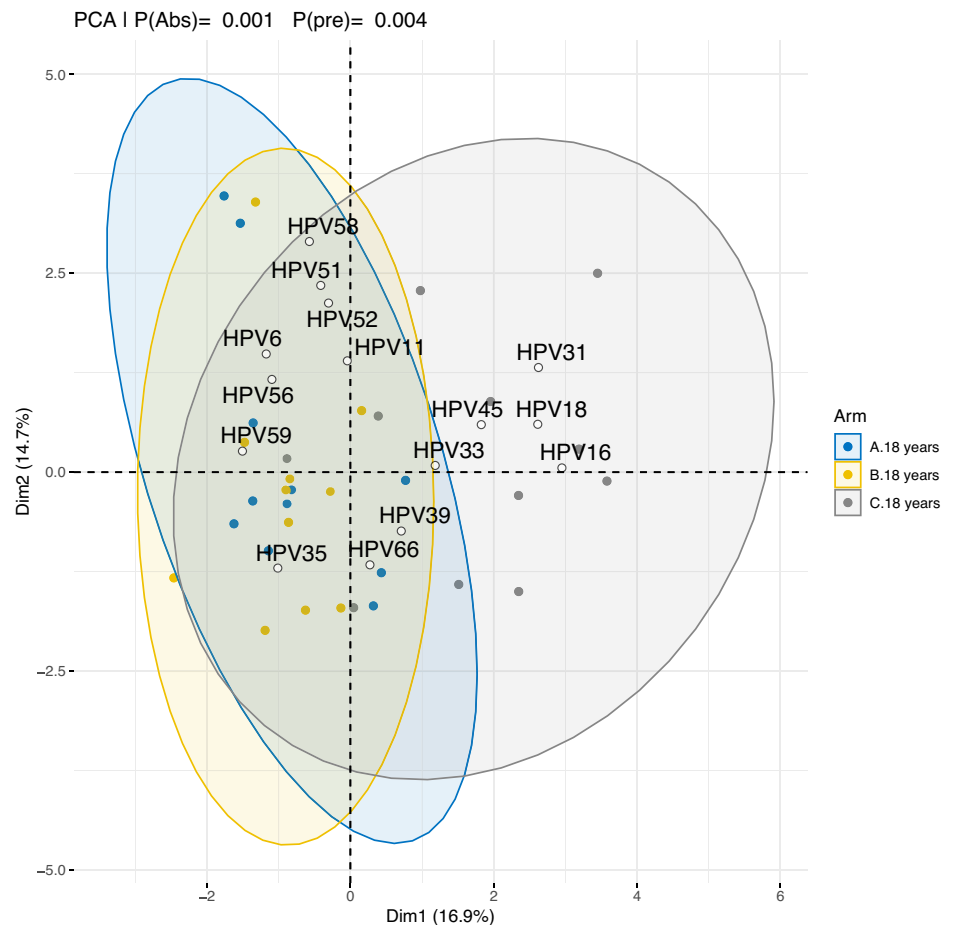
Increased understanding of the epigenetic changes (methylation) of both cellular and viral genes is now offering a new roadmap for cervical neoplasia triage of unvaccinated women¹⁵⁻¹⁷ who have the majority of severe cervical lesions that require triage and treatment.

In fact, early identification of a number of gynecological cancers is emerging via assessment of cervical cells' methylation status.¹⁸ Fortunately, the performance of the new risk-assessment measures can now be evaluated in women, who had been vaccinated against HPV 15 years ago as early adolescents. Even if among these women the necessary causes of cervical cancer HPV types 16/18 are abolished HSIL remains found (Figure 2), and validation of methylation markers here and now is pivotal to the future use of the new epigenetic measures.

2 | EVOLUTIONARY REPERCUSSION OF HPV VACCINATION ON DEFINING THE RISK OF CERVICAL NEOPLASIA

Papillomaviruses are one of the most oncogenic viruses infecting humans with a high viral diversity and a remarkably sustained common evolutionary human-pathogen interaction history.^{19,20} HPV vaccination and its current global implementation underline a quintessential need to systematically assess the likely changes in this deep evolutionary virus-host interaction. For the first time in postvaccinated populations a sizeable proportion of adolescent and early adults mostly women have developed a sustained strongly protective vaccine-induced immune response against the vaccine-targeted oncogenic hrHPVs. Moreover, with a readily achieved community-level

FIGURE 1 Community-level human papillomavirus (HPV) prevalence distribution visualized using ecological β -diversity analysis⁶ among young 18-year-old women 4 years after community-randomized gender-neutral (A) or girls-only (B) HPV vaccination, and control communities where hepatitis B-virus vaccination was implemented (C). Arm A/B communities cluster separately from the control arm C communities mostly due to depletion of vaccine-targeted HPV types 16/18/31/45 in the intervention A and B communities but also due to differential clustering driven by the not vaccine-targeted HPV types 51/58/59. White dots represent HPV types community-level prevalence distribution in two dimensions of the dissimilarity matrix with the blue (A), yellow (C) and gray (C) dots representing each of the 11 communities in each trial arm. The elliptic circles represent the overall diversity among the gender-neutral (A) or girls-only (B) HPV vaccinated and control (C) communities, respectively. Original HPV prevalence data has been previously described by Gray et al⁷ and Louvanto et al⁸



Trials on the impact of vaccination & screening strategies (ClinicalTrials.gov NCT00534639/NCT02149030)

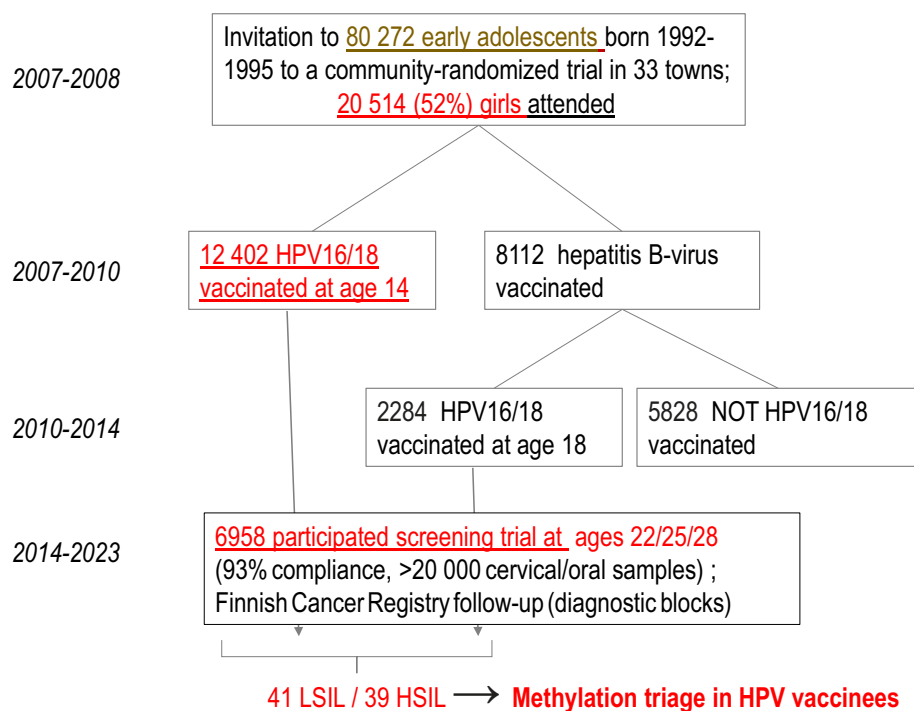


FIGURE 2 Finnish community and individually-randomized trial cohorts with population-based, country-wide human papillomavirus (HPV) vaccination and cervical screening of 1992 to 1995 birth cohorts since 2007

coverage of gender-neutral HPV vaccination the unvaccinated women and men have thus far been up to 15 years under herd protection against the targeted oncogenic HPVs.^{2,3,21} This direct and indirect protection gained from gender-neutral HPV vaccination has profoundly changed the community-level diversity distribution of vaccine-targeted and nonvaccine targeted HPV types (Figure 1).

Our recent work exploiting the population-based community-randomized HPV vaccination trial data from the vaccinated Finnish birth cohorts is demonstrating the powerful population-level effects of both gender-neutral and girls-only HPV vaccination on HPV type distribution (Figure 1).^{2,3,7,11,22-24} A subsequent question is: what will be the viral evolutionary response to the HPV vaccination? Rapid viral evolutionary responses have been observed most notoriously with SARS-CoV-2RNA-virus showing the emergence of new viral variants with escape mutants and higher transmissibility after vaccination. However, for DNA viruses with a slower rate of evolution and better proof-reading mechanisms such evolutionary responses are less likely and will require much more time.²⁵

The theory is that host immune recognition postvaccination will favor the selection of particular virus lineages. Proportional increase of immune individuals by vaccination enhances such evolutionary selection pressures.²⁶ Another fundament is that such evolutionary processes depend upon genetic diversity, which is high even for the most oncogenic hrHPVs both at species and strain level.^{11,19} Therefore, it has been important to systematically examine the available community-randomized HPV vaccine trial data for possible clearance patterns of vaccine-targeted HPVs ecological niche³ and search

signs of evolutionary responses of the nonvaccine targeted lower oncogenicity hrHPV types such as type replacement.^{7,23}

In the postvaccination era, it will be important to explore both the ecological and epigenetic variation in infection outcome at large for HPVs. Comprehensive understanding of the changes in virus-host interaction leading to differential lesion severity and cervical HPV types in vaccinated and unvaccinated women will likely pave the way for improved methods for future screening of cervical cancer.

3 | UNDERSTANDING TEST PERFORMANCE OF CERVICAL CANCER SCREENING IN THE POSTVACCINATION ERA

As alluded to earlier, with the high vaccination coverage, cross-protection and herd immunity, HPV transmission will ultimately be kept at a minimum so that cervical cancer screening must adapt to continue to provide benefit. Along with the postvaccination changes of viral genotypes prevalence distribution mentioned above, the impact on the epidemiology of cervical dysplasia in terms of reduction in cervical abnormalities has also been reported among HPV vaccinated women.^{4,27-29}

Because of the population-level impact of HPV vaccination and the decline in the prevalence of HPV-related outcomes, the pertinent question then arises: what would be the consequence on screening performance and practices as cohorts of HPV-vaccinated girls and adolescents reach the age to be screened for cervical cancer? We have previously illustrated the impact on the PPV of a future cervical

cancer screening test following reductions in precancerous lesion prevalence post-HPV vaccination.¹² We showed that even for the most optimistic scenario of test performance (99% specificity), the PPV will be so low when lesion prevalence falls below 0.16 per 1000 women (~0.02%); such positive test results will most likely be false triggering unnecessary diagnostic activities. Under such conditions, the harms from screening may then outweigh the pursued benefits. In a retrospective analysis of national datasets from 95 876 women (born 1998-1993) who attended cervical cancer screening in Scotland within 1 year of turning 20 years old, a significant reduction in the PPV of high-grade dyskaryosis for the detection of CIN2+ was observed among HPV vaccinated compared to unvaccinated women (65.7% vs 76.6%, respectively, P -value = .002).³⁰ Another ecologic study showed that, following the implementation of the HPV vaccination program in 2017 in Australia, the PPV of high-grade cytology in predicting high-grade disease decreased over time particularly for the younger age cohorts which is likely an effect of HPV vaccination.²⁷ Similarly, using data linkage between the Swedish National Cervical Screening Registry and the HPV vaccination registry, an 8% reduction in the PPV of high-grade cytology for CIN2+ was reported for vaccinated compared to unvaccinated women.¹⁴

The reduction in HPV prevalence and reduced performance of cytology as a consequence of HPV vaccination calls for rethinking of CIN triage and for new, better screening tests to improve risk stratification to triage women who are positive on screening for hrHPV types. High-risk prediction of HPV-driven cervical carcinogenesis will assist the transition to a more rational screening and management approach for cervical cancer, especially as molecular HPV testing has replaced cytology for cervical cancer screening in most high-income countries. One promising approach for the proper triage of HPV infections and associated lesions would be to rely on viral and cellular methylation markers to identify true progression potential.

Of utmost importance is the notion of screening conditional on vaccination status and the need for separate guidelines for vaccinated and unvaccinated women. Ideally integrated surveillance systems linking HPV vaccination, screening and disease outcomes would enable assessment of the impact of intervention programs and determination of the potential benefit-harm balance of these programs.

4 | METHYLATION OF COMBINED HOST AND HPV GENES AND RISK OF CERVICAL NEOPLASIA IN VACCINATED WOMEN

DNA methylation is a reproducible physical epigenetic change involved in a variety of cellular processes and plays an important role in cancer progression. Viral DNA methylation status is dynamic in the context of the viral life cycle and has been suggested as a host defense mechanism to silence viral transcription and replication. The association between hypermethylation of viral HPV genes and cervical precancer lesions and cancer has primed the development of HPV methylation biomarkers for diagnostic and triage purposes.³¹ Aberrant DNA methylation of not only HPV genes but also host-cell genes has been reported to increase along with the severity of cervical lesion progression, allowing this epigenetic event to be used as a biomarker, with the potential to predict whether HPV infection will lead to CIN2+ lesion or if the infection will resolve (Figure 3).³²

Combining the knowledge of methylation on host-cell and viral genes, the S5 classifier involves testing the levels of DNA methylation on CpGs from the host *EPB41L3* and viral genes: HPV16-L1, HPV16-L2, HPV18-L2, HPV31-L1 and HPV33-L2.³³ The *EPB41L3* gene codes for the membrane Band 4.1-like protein 3 which acts as a tumor suppressor inhibiting cell proliferation while promoting apoptosis.³⁴ Hypermethylation of CpG islands on the *EPB41L3* promoter leads to a decrease in gene expression, which was associated with the

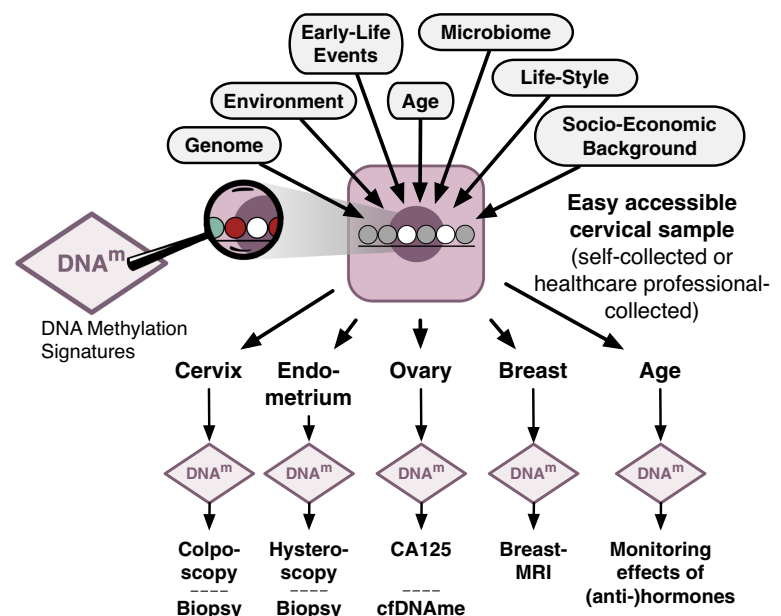


FIGURE 3 Utilizing DNA methylation signatures in easy to access epithelial cell containing cervical smear samples to predict the risk of (screen) all four women cancers

progression of multiple cancers including cervical and oropharyngeal, lung, gastric and esophageal cancer.³⁵⁻⁴⁰ A recent study by Banila et al highlighted the relevance of *EPB43L1* in cancer detection as 25 out of 26 hrHPV-negative cancers (tested with multiple hrHPV-genotyping assay) were positive by S5.¹⁷ At a cut-off of 0.80, S5 identifies more than 90% CIN3 cases and almost 100% of cervical cancers, independent of histology, FIGO stage hrHPV status.¹⁷ In examining S5 classifier components, Banila et al¹⁷ suggested that the relative proportion of the HPV methylation components of the S5-classifier decreased slightly with severity of lesion.¹⁷ HPV16 methylation had the highest weight out of all viral components; however, this was 1.8 times lower than the weight of *EPB41L3* methylation in advanced cancer (CSII+) specimens.¹⁷ This result is very important for the postvaccination era suggesting a key role for methylation analysis of host-cell genes, for example, *EPB41L3* in detecting high grade lesions and cancers. A meta-analysis on the performance of methylation assays indicated that S5 had a higher sensitivity for CIN2+ detection than considering *EPB41L3* methylation alone, without compromising specificity.³² This indicates that the combination of host cell and viral gene targets improves the accuracy for CIN2+ detection and this will certainly hold true for vaccinated women though the value of viral genes not included in the vaccines will still need to be considered.

A triage test will be required to distinguish hrHPV-positive (nonvaccinated and vaccinated) women with clinically relevant cervical lesions from those with transient infections. The S5 has been substantially evaluated as a triage test for hrHPV-positive nonvaccinated women and has demonstrated improved triage performance compared to hrHPV genotyping or cytology alone or combined.⁴⁰⁻⁴³ These observations suggest that the S5 classifier could help identify women with a high short-term risk of progression to cancer who need immediate treatment. Hernandez et al.⁴¹ suggested that the S5 classifier could reduce colposcopy referrals by 30% to 50% without affecting sensitivity for CIN2+ and CIN3+, therefore significantly improving cost-effectiveness to allow identification of women with a true risk of cancer. In addition, S5 had the ability to distinguish between <CIN2, CIN2 and CIN3+, a finding of importance for managing CIN2, given the complexity and uncertainty associated with this diagnosis.⁴³

The S5 classifier was also proven as a potential prognostic test, being able to identify women with progressive CIN2 in nonvaccinated women.⁴⁴ An improved predictive test could revolutionize the management of CIN2 as cases with progressive potential could be treated sooner and regressive cases managed expectantly. This is especially important for women in childbearing age as cervical treatments can increase the risk for preterm deliveries during pregnancies.

In the next decades, cervical cancer screening programs will have to cater for both vaccinated and nonvaccinated birth cohorts. When evaluating HPV methylation among HPV vaccinated women we need to remember the changes of HPV genotype distributions as the currently prevalent HPV genotypes among nonvaccinated women will not be detected in the future.⁸ The baseline results of infrequent vs frequent cervical screening trial among women vaccinated as early adolescents, it revealed that at the age of 22-year-old, the prevalence

of non HPV16/18 genotypes were extremely low (range 0.2%-2.5%) compared to the other hrHPV types with the range of 23% to 25%.⁹ The role of the other hrHPV genotypes and their role in cervical carcinogenesis remain to be determined. Given the preliminary genotyping prevalence in this cohort, it is most likely that S5-score will need to be adjusted with other HPV methylation sites from genotypes that are more prevalent in HPV-vaccinated women. It is foreseeable that both vaccinated and unvaccinated women will benefit from an expansion of hrHPV methylation sites in the current S5 classifier accounting for the shift in the prevalence of HPV genotypes.

5 | METHYLATION OF CELLULAR GENES AND RISK OF CERVICAL NEOPLASIA IN VACCINATED WOMEN

An aberrant DNA methylation pattern is a hallmark of cancer cells.⁴⁵ Hypermethylation is frequently observed in transcriptional regulatory elements, such as promoters and enhancers of host-cell (tumor suppressor) genes. These host-cell DNA methylation abnormalities are necessary for the ultimate progression to cervical cancer. Methylation levels of several host-cell genes have shown to increase with increasing CIN grade and are extremely high in cervical cancer.^{32,46,47}

For the well-studied host-cell methylation marker panel *FAM19A4* and *miR-124-2*, a very high methylation positivity rate was observed in cervical cancer (>98%), irrespective of histotype, FIGO stage, HPV status and geographical region of origin.⁴⁸ The high *FAM19A4/miR124-2* methylation positivity rates in cervical carcinomas were also found to be independent of hrHPV genotype,⁴⁸ suggesting that host-cell methylation analysis can similarly detect cervical cancers associated with nonvaccine targeted HPV types. Moreover, 94.7% (18/19) of hrHPV-negative cancers (as determined by multiple hrHPV assays) tested positive with the *FAM19A4/miR124-2* panel emphasizing its additional value.⁴⁸

Within the group of high-grade CIN lesions (CIN2/3) host-cell DNA methylation patterns are heterogeneous. About half of CIN2 and three-quarters of CIN3 have a cancer-like methylation pattern.⁴⁹ It was found that CIN2/3 lesions associated with a long-term (≥ 5 years) HPV infection (ie, so-called advanced lesions) have significantly higher methylation levels compared to CIN2/3 lesions with a more recently acquired (<5 years) HPV infection (ie, early or incident lesions).^{46,47} These findings suggest that cellular methylation positivity is characteristic of advanced cervical precursor lesions with a high short-term risk of progression to cancer.⁵⁰ This is further supported by the fact that methylation positivity of *FAM19A4* and *miR-124-2* in CIN2/3 lesions appears to be associated with increased p16^{INK4A}/Ki-67 immunoscores and low HPV-E4 expression^{51,52} underscoring the high specificity of the *FAM19A4/miR124-2* methylation test for nonproductive, transforming CIN2/3 lesions.⁵² In addition, in a prospective clinical cohort study, the absence of *FAM19A4/miR124-2* methylation was found associated with a high regression rate of CIN2/3 lesions⁵³ further corroborating the value of cellular methylation analysis as a biomarker that distinguishes

advanced from early lesions based on the level of epigenetic host-cell alterations. In reference to HPV vaccination, it was noted that the detection of CIN3+ by *FAM19A4/miR124-2* methylation is similar for lesions caused by HPV16/18 and those cause by other hrHPV types.⁵⁴

In light of the above, host-cell DNA methylation markers provide a specific molecular means to detect advanced CIN lesions in need of treatment, and may well serve the needs of cervical cancer screening in the postvaccination era (Figure 3). At present, these markers have been extensively evaluated in mainly nonvaccinated cohorts reporting on a good triage performance with a pooled methylation sensitivity for CIN3+ of 71.1% (95% CI: 65.7-76.0) at a set specificity of 70%.^{16,32,55-57} Retrospective longitudinal screening studies showed that HPV-positive but *FAM19A4/miR124-2* methylation-negative women had a 14-year CIN3+ risk equal to that of negative cytology triage outcome, and notably they had a lower risk for cervical cancer.^{16,55-57} Recent data show that additional risk-stratification of HPV-positive women with low-grade cytological abnormalities by *FAM19A4/miR124-2* methylation could substantially reduce direct colposcopy referral rate, while retaining high CIN3+ sensitivity.⁵⁸ Altogether, these findings support the use of cellular methylation markers as an interesting new molecular means for future cervical cancer screening, and the need to evaluate their performance in cohorts of vaccinated women. The premise is that host-cell methylation positivity is low in vaccinated screening cohorts, providing a modality to limit the false-positive rate of screening by specific detection of cervical lesions in need of treatment.

6 | UTILIZING DNA METHYLATION IN CERVICAL SAMPLES, THE FUTURE OF A HOLISTIC CANCER SCREENING APPROACH

HPV vaccination is an effective means of reducing the burden of cervical cancers in fertile-aged women as HPV infection is a necessary cause of cervical cancer.⁵⁹⁻⁶¹ However, even persistent HPV-infection alone is not sufficient for cervical carcinogenesis and therefore one can assume that another driver of this process would be an underlying cervical field defect that is not limited to immune surveillance of persistent HPV but includes factors intrinsic to epithelial stem/progenitor cells which serve as the cell of origin for cervical cancer. Such a field defect may, for example, be reflected by a reduced ability to induce apoptosis upon HPV persistence or a reduced ability of stem cells to differentiate. Independent cervical neoplasia risk factors like smoking,⁶² chlamydia,⁶³ long-term oral contraceptive pill use⁶⁴ or in utero exposure to specific drugs similar to Diethylstilbestrol⁶⁵ could trigger such a field defect.

It is noteworthy that cervical cancer is among the three most frequent cancers in women <44 years of age and but globally rare in women >45 years.⁶⁶ Upon oncogenic HPV infection, women harboring the field defect may be at a greater risk of developing a cervical cancer significantly earlier than they would do otherwise as up to 85% of 45-year-old women have had a genital HPV infection.⁶⁷

Hence, reducing the burden of the most common oncogenic HPV-infections with HPV vaccination might in the worst case scenario only result in pushing back the age of cervical cancer onset but not necessarily eliminating in all the overall burden of cervical cancer, assuming that the above-mentioned field defect is essential and can drive carcinogenesis in the presence of less oncogenic HPV subtypes that are not covered by current HPV vaccination strategies. Maybe 30 to 40 years after HPV-vaccination has commenced will we be able to assess this for invasive cervical cancer.

Ideal strategies utilizing would use an easy-to-access tissue sample, such as a cervical smear, and be capable of (a) monitoring the risk for cervical carcinogenesis irrespective of the presence of highly oncogenic HPV types and not reliant on morphological assessment of cervical cells: for example, we know that cytology is less informative in HPV vaccinated birth cohorts,²⁹ and (b) identifying women at risk for other cancers in order to guide primary and secondary preventive measures would be ideal.

We were the first to demonstrate that epigenetic analyses on self-samples are highly promising for cervical⁶⁸ and endometrial⁶⁹ cancer detection and have described epigenetic field defects preceding breast,⁷⁰ ovarian⁷¹ and cervical^{72,73} cancer. Very recently, we demonstrated that DNAm signatures derived in cervical smear samples are capable of detecting/predicting women with ovarian cancer, that is, the WID-OC test⁷⁴ and poor prognostic breast cancer, that is, the WID-BC test.⁷⁵ The WID-OC test was developed to identify/predict women with ovarian cancer, the majority of which arises from Müllerian Duct structures.⁷⁶ In line with the idea of an epigenetic field defect is the observation that the WID-OC test, which does not rely on the presence of tumor DNA in the sample, is able to identify endometrial cancer cases with a Receiver Operating Characteristic Area Under the Curve of 0.81 in samples with no detectable endometrial cancer DNA.⁷⁴ Finally, our yet unpublished data demonstrate that DNAm signatures can both detect and predict the future risk of cervical and endometrial cancer.

Aligned with the view that the cervical epithelial cells can capture and integrate risk factors at the level of the epigenome is the recent observation that the relative epithelial age (REA) assessed in cervical smear samples using the WID-REA test⁷⁷ allows the effects of hormones (ie, combined replacement therapy) and antihormones (ie, mifepristone) to be monitored. Modulation of the relative epithelial age is associated with the disease risk of organs distant to the cervix.

Cervical samples are likely to remain an essential component of screening in the post-HPV vaccination era. Various technologies (Figure 3) that do not rely on morphological assessments of cells, utilize self-samples and are able to identify women at risk of developing cervical as well as other prevalent or fatal cancers for which primary or secondary preventive measures are available, and can be implemented in the next 5 to 10 years.

7 | CONCLUSIONS

In the post-HPV vaccination era, the predictive values of currently used screening tests are declining as both cytology testing and broad

HPV testing will continue to test positive for lesions with nonvaccine HPV types with limited or even no oncogenic potential. Although the use of extended HPV genotyping that can focus on the most oncogenic HPV types may be helpful, DNA methylation can now provide an objective progression marker that can assist in predicting which lesions represent true precursors. This will be crucial for maintaining an acceptable balance between benefits and harms (sensitivity and specificity) of the screening. The fact that cervical cancer elimination is in sight does not imply that the cervical screening is about to be canceled. On the contrary, building on the effective, high attendance cervical screening program for assessing the risk also of additional cancer forms using methylation markers could open a new and innovative way for cancer prevention.

AUTHOR CONTRIBUTIONS

Matti Lehtinen: Introduction; Summary. **Ville N. Pimenoff:** Evolutionary repercussion. **Belinda Nedjai:** Methylation of HPV genes. **Karolina Louvanto:** Methylation of HPV genes. **Lisanne Verhoef:** Methylation of cellular genes. **Daniëlle A. M. Heideman:** Methylation of cellular genes. **Mariam El-Zein:** Understanding test performance. **Martin Widschwendter:** Utilizing DNAm in cervical samples. **Joakim Dillner:** Conclusions. The work reported in the article has been performed by the authors, unless clearly specified in the text.

ACKNOWLEDGEMENTS

This review is based on a presentation the co-authors gave at the EUROGIN 2022 Main Scientific Session in Dusseldorf, Germany on 12 April 2022. Support of the EU Horizon-2020 RISC Network is also gratefully acknowledged. DAMH reports grants from the Dutch Cancer Society (KWF 11337) and the Horizon 2020 Framework Programme for Research and Innovation of the European Commission through the RISC Network (grant. no. 847845). MW reports funding from the European Union's Horizon 2020 European Research Council Program under Grant Agreement No. 742432 (BRCA-ERC).

FUNDING INFORMATION

Matti Lehtinen and Joakim Dillner have received funding for their HPV vaccination studies through their employers from GSK Biologicals (ML) and Merck & Co. Inc (ML and JD).

CONFLICT OF INTEREST

Matti Lehtinen, Ville N. Pimenoff, Belinda Nedjai, Karolina Louvanto, Lisanne Verhoef and Joakim Dillner have no conflicts of interest to declare. Mariam El-Zein holds 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 (October 2018). Daniëlle A. M. Heideman is minority shareholder of Self-screen B.V., a spin-off company of VUmc; Self-screen B.V. develops, manufactures and licenses high-risk HPV and methylation marker assays for cervical cancer screening and hold patents of these tests. Martin Widschwendter is a shareholder of Sola Diagnostics GmbH, which holds an exclusive license to the intellectual property that protects the commercialization of the WID-tests.

ORCID

Matti Lehtinen  <https://orcid.org/0000-0002-9481-0535>
 Ville N. Pimenoff  <https://orcid.org/0000-0002-0813-7031>
 Lisanne Verhoef  <https://orcid.org/0000-0001-9877-0297>
 Joakim Dillner  <https://orcid.org/0000-0001-8588-6506>

REFERENCES

- Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papilloma virus following a national bivalent human papilloma virus vaccination programme in Scotland: a 7-year cross-sectional study. *Lancet Infect Dis.* 2017;17:1293-1302.
- Vänskä S, Luostarinen T, Baussano I, et al. Vaccination with moderate coverage eradicates oncogenic HPV if a gender-neutral strategy is applied. *J Infect Dis.* 2020;222:948-956.
- Gray P, Kann H, Pimenoff VN, et al. HPV seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: a cross-sectional cohort analysis following a cluster-randomised trial. *PLoS Med.* 2021;18:e1003588.
- Palmer TJ, Wallace L, Pollock KGJ, et al. Prevalence of cervical disease at age 20 after immunisation with bivalent HPV vaccine at age 12-13 in Scotland: retrospective population study. *BMJ.* 2019;365:11161.
- Rosenblum HG, Lewis RM, Gargano JW, Querec TD, Unger ER, Markowitz LE. Declines in prevalence of human papillomavirus vaccine-type infection among females after introduction of vaccine – United States, 2003-2018. *MMWR Morb Mortal Wkly Rep.* 2021;70:415-420.
- Willis AD. Rarefaction, alpha diversity, and statistics. *Front Microbiol.* 2019;10:10.
- Gray P, Luostarinen T, Vänskä S, et al. Occurrence of human papillomavirus type-replacement by sexual risk-taking behaviour group: post hoc analysis of a community randomized trial up to nine years after vaccination (IV). *Int J Cancer.* 2019;145:785-796.
- Louvanto K, Eriksson M, Elfström M, et al. Effectiveness of screening in human papillomavirus vaccinated women. *Int J Cancer.* 2020;147:440-447.
- Pimenoff VN, Tous S, Benavente Y, et al. Distinct geographic clustering of oncogenic human papillomaviruses multiple infections in cervical cancers: results from a world wide cross-sectional study. *Int J Cancer.* 2019;144:2478-2488.
- Pimenoff VN. *Challenges to Cervical Screening from Changing HPV Ecology*; Abstract. UK: EUROGIN Conference; 2022.
- Pimenoff VN, Tous S, Benevente Y, et al. Distinct geographic clustering of oncogenic human papillomaviruses multiple infections in cervical cancers: Results from a worldwide cross-sectional study. *Int J Cancer* 2019;144:2478-2488.
- 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-S68.
- Sultana F, Winch K, Saville M, Brotherton J. Is the positive predictive value of high-grade cytology in predicting high-grade cervical disease falling due to HPV vaccination. *Int J Cancer.* 2019;144:2964-2971.
- Lei J, Ploner A, Lehtinen M, et al. Impact of HPV vaccination on cervical screening performance: a population-based cohort study. *Br J Cancer.* 2020;123:155-160.
- Bowden SJ, Kalliala I, Veroniki AA, et al. The use of human papillomavirus DNA methylation in cervical intraepithelial neoplasia: a systematic review and meta-analysis. *EBio Med.* 2019;50:246-259.
- Vink FJ, Lissenberg-Witte BI, Meijer CJLM, et al. FAM19A4/miR124-2 methylation analysis as a triage test for HPV-positive women: cross-sectional and longitudinal data from a Dutch screening cohort. *Clin Microb Infect.* 2021;25:e1-e125. doi:10.1016/j.cmi.2020.03.018

17. Banila C, Lorincz AT, Scibior-Bentkowska D, et al. Clinical performance of methylation as a biomarker for cervical carcinoma in situ and cancer diagnosis: a worldwide study. *Int J Cancer*. 2022;150:290-302.
18. Widschwendter M, Jones A, Evans I, et al. Epigenome-based cancer risk prediction: rationale, opportunities and challenges. *Nat Rev Clin Oncol*. 2018;15:292-309.
19. Pimenoff VN, Mendes de Oliveira C, Bravo IG. Transmission between archaic and modern human ancestors during the evolution of the oncogenic HPV16. *Mol Biol Evol*. 2017;34:4-19.
20. Pimenoff VN, Houldcroft CJ, Rifkin R, Underdown S. The role of aDNA in understanding the co-evolutionary patterns of human sexually transmitted infections. *Genes*. 2018;25:E317.
21. Lehtinen M, Gray P, Louvanto K, Vänskä S. In 30 years gender-neutral vaccination eradicates oncogenic human papillomavirus (HPV) types while screening eliminates HPV-associated cancers. *Exp Rev Vaccines*. 2022;21:735-738. doi:10.1080/14760584.2022.2064279
22. Lehtinen M, Baussano I, Apter D, et al. Characteristics of a cluster-randomized phase IV HPV vaccination effectiveness trial. *Vaccine*. 2015;33:1284-1290.
23. Gray P, Kann H, Faust H, et al. Long-term of HPV type-replacement among young pregnant Finnish females before and after a community randomised HPV vaccination trial with moderate coverage. *Int J Cancer*. 2020;147:3511-3522.
24. Lehtinen M, Luostarinen T, Vänskö S, et al. Gender-neutral vaccination 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;143:2299-2310.
25. Orlando PA, Gatenby RA, Giuliano AR, Brown JS. Evolutionary ecology of human papillomavirus: trade-offs, coexistence, and origins of high-risk and low-risk types. *J Infect Dis*. 2012;205:272-279.
26. Soubeyrand B, Greenberg M, Tibayrenc M, et al. Vaccination: an evolutionary engine for pathogens? Conference report. *Infect Genet Evol*. 2014;27:137-141.
27. Brotherton JML, Malloy M, Budd AC, Saville M, Drennan K, Gertig DM. Effectiveness of less than three doses of quadrivalent human papillomavirus vaccine against cervical intraepithelial neoplasia when administered using a standard dose spacing schedule: observational cohort of young women in Australia. *Papillomavirus Res*. 2015;1:59-73.
28. Thamsborg LH, Napolitano G, Larsen LG, Lynge E. Impact of HPV vaccination on outcome of cervical cytology screening in Denmark - a register-based cohort study. *Int J Cancer*. 2018;143:1662-1670.
29. Soldan K, Elliss-Brookes, L, Sasieni P. The impact of HPV vaccination program on CIN3 and cervical cancer incidence in England; 2022. www.HPVWorld.com. Accessed April 12, 2022.
30. Palmer TJ, McFadden M, Pollock KGJ, et al. HPV immunisation and cervical screening - confirmation of changed performance of cytology as a screening test in immunised women: a retrospective population-based cohort study. *Br J Cancer*. 2016;114:582-589.
31. Mirabello L, Sun C, Ghosh A, et al. Methylation of human papillomavirus type 16 genome and risk of cervical precancer in a Costa-Rican population. *J Natl Cancer Inst*. 2012;104:556-565.
32. Kelly H, Benavente Y, Pavon MA, De Sanjose S, Mayaud P, Lorincz AT. Performance of DNA methylation assays for detection of high-grade cervical intraepithelial neoplasia (CIN2+): a systematic review and meta-analysis. *Br J Cancer*. 2019;121:954-965.
33. Brentnall AR, Vasiljevic N, Scibior-Bentkowska D, et al. HPV33 DNA methylation measurement improves cervical pre-cancer risk estimation of an HPV16, HPV18, HPV31 and EPB41L3 methylation classifier. *Cancer Biomark*. 2015;15:669-675.
34. Zeng R, Liu Y, Jiang Z-J, et al. EPB41L3 is a potential tumor suppressor gene and prognostic indicator in esophageal squamous cell carcinoma. *Int J Cancer*. 2018;143:1443-1454.
35. Clarke MA, Luhn P, Cage JC, et al. Discovery and validation of candidate host DNA methylation markers for detection of cervical precancer and cancer. *Int J Cancer*. 2017;141:701-711.
36. Nedjai B, Reuter C, Ahmad A, et al. Molecular progression to cervical precancer, epigenetic switch or sequential model. *Int J Cancer*. 2018;143:1720-1730.
37. Giuliano AR, Nedjai B, Lorincz AT, et al. Methylation of HPV 16 and EPB41L3 in oral gargles: associations with oropharyngeal cancer detection and tumor characteristics. *Int J Cancer*. 2020;146:1018-1030.
38. Tran YK, Böglér O, Gorse KM, Wieland I, Green MR, Newsham IF. A novel member of the NF2/ERM/4.1 superfamily with growth suppressing properties in lung cancer. *Cancer Res*. 1999;59:35-43.
39. Wang H, Xu M, Cui X, et al. Aberrant expression of the candidate tumor suppressor gene DAL-1 due to hypermethylation in gastric cancer. *Sci Rep*. 2016;6:21755. doi:10.1038/srep217
40. Cook DA, Krajden M, Brentnall AR, et al. Evaluation of a validated methylation triage signature for human papillomavirus positive women in the HPV FOCAL cervical cancer screening trial. *Int J Cancer*. 2019;144:2587-2594.
41. Hernandez-Lopez R, Lorincz AT, Torres-Ibarra L, et al. FRIDA study group methylation estimates the risk of precancer in HPV-infected women with discrepant results between cytology and HPV16/18 genotyping. *Clin Epigenet*. 2019;11:140. doi:10.1086/s13148-019-0743-9
42. Ramirez AT, Sanchez GI, Nedjai B, et al. Effective methylation triage of HPV positive women with abnormal cytology in a middle-income country. *Int J Cancer*. 2021;148:1383-1389.
43. Adcock R, Nedjai B, Lorincz AT, et al. DNA methylation testing with S5 for triage of high-risk HPV positive women. *Int J Cancer*. 2022;151:993-1004. doi:10.1002/ijc.34050
44. Louvanto K, Aro K, Nedjai B, et al. Methylation in predicting progression of untreated high-grade cervical intraepithelial neoplasia. *Clin Infect Dis*. 2020;70:2582-2590.
45. Hanahan D. Hallmarks of cancer. New dimensions. *Cancer Disc*. 2022;12(1):31-46. doi:10.1158/2159-8290.CD-21-1059
46. Bierkens M, Hesselink AT, Meijer CJ, et al. CADM1 and MAL promoter methylation levels in hrHPV-positive cervical scrapes increase proportional to degree and duration of underlying cervical disease. *Int J Cancer*. 2013;133:1293-1299.
47. De Strooper LMA, Meijer CJLM, Berkhof J, et al. Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. *Cancer Prev Res*. 2014;12:1251-1257.
48. Vink FJ, Meijer CJLM, Clifford G, et al. FAM19A4/miR124-2 methylation in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Int J Cancer*. 2020;147:1215-1222.
49. Verlaat W, Van Leuwen RW, Novianti PW, et al. Host-cell DNA methylation patterns during high-risk HPV-induced carcinogenesis reveal a heterogeneous nature of cervical pre-cancer. *Epigenetics*. 2018;13:769-778.
50. Steenbergen RDM, Snijders PJF, Heideman DAM, Meijer CJLM. Clinical implications of (epi) genetic changes in HPV-induced cervical precancerous lesion. *Nat Rev Cancer*. 2014;14:395-405.
51. Vink FJ, Dick S, Heideman DAM, et al. Classification of high-grade cervical intraepithelial neoplasia by p16ink4a, Ki-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management. *Int J Cancer*. 2021;149:707-716.
52. Vink FJ, Meijer CJLM, Hesselink AT, et al. FAM19A4/miR124-2 methylation testing and HPV16/18 genotyping in HPV-positive women under the age of 30 years. *Clin Infect Dis*. 2022;ciac433. doi:10.1093/cid/ciac
53. Kremer WW, Dick S, Heideman DAM, et al. Clinical regression of high-grade cervical intraepithelial neoplasia is associated with

- absence of *FAM19A4/miR124-2* DNA methylation (CONCERVE Study). *J Clin Oncol*. 2022;40. doi:[10.1200/JCO.21.02433](https://doi.org/10.1200/JCO.21.02433)
54. Leeman A, Ebisch RM, Kasius A, et al. Defining hrHPV genotypes in cervical intraepithelial neoplasia by laser capture microdissection supports reflex triage of self-samples using HPV16/18 and *FAM19A4/miR124-2* methylation. *Gynecol Oncol*. 2018;151:311-318.
 55. Bonde J, Floore A, Ejegod D, et al. Methylation markers *FAM19A4* and *miR124-2* as triage strategy for primary human papillomavirus screen positive women. *Int J Cancer*. 2021;148:396-405.
 56. De Strooper LMA, Berkhof H, Steenbergen RDM, et al. Cervical cancer risk in HPV-positive women after a negative *FAM19A4/miR124-2* methylation test: a post hoc analysis in the POBASCAM trial with 14 year follow-up. *Int J Cancer*. 2018;143:1541-1548.
 57. Dick S, Kremer WW, De Strooper LMA, et al. Long-term CIN3+ risk of HPV positive women after triage with *FAM19A4/ miR124-2* methylation analysis. *Gynecol Oncol*. 2019;154:368-373.
 58. Dick S, Vink FJ, Heideman DAM, Lissenberg-Witte BI, Meijer CJLM, Berkhof J. Risk-stratification of HPV-positive women with low-grade cytology by *FAM19A4/miR124-2* methylation & HPV genotyping. *Br J Cancer*. 2022;126:259-266.
 59. Lei J, Ploner A, Elfström KM, et al. HPV vaccination and the risk of invasive cervical cancer. *N Engl J Med*. 2020a;383:1340-1348.
 60. Lehtinen M, Lagheden C, Luostarinen T, et al. Human papillomavirus vaccine efficacy against invasive HPV-positive cancers: population-based follow-up of a cluster-randomized trial. *BMJ Open*. 2021;11:e050669. doi:[10.1136/bmjopen-2021-050669](https://doi.org/10.1136/bmjopen-2021-050669)
 61. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer world wide. *J Pathol*. 1999;189:12-19.
 62. Kapeu A, Youngman L, Jellum E, et al. Smoking is an independent risk factor of cervical cancer. *Am J Epidemiol*. 2009;169:480-488.
 63. Lehtinen M, Ault K, Lyytikäinen E, et al. Chlamydia trachomatis is an independent risk factor of CIN. *Sex Transm Infect*. 2011;87:372-376.
 64. Iversen L, Sivasubramaniam S, Lee AJ, Fielding S, Hannaford PC. Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. *Am J Obstet Gynecol*. 2017;216(580):e1-580 e9.
 65. Hoover RN, Adam E, Bond B, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. *N Engl J Med*. 2011;365:1304-1314.
 66. Bray F, Ferlay J, Soerjomataram I. Global cancer statistics 2018. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;69:394-424.
 67. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. *Sex Transm Dis*. 2014;41:660-664.
 68. Widschwendter A, Gatringer C, Ivarsson L, et al. Analysis of aberrant DNA methylation and human papillomavirus DNA in cervicovaginal specimens to detect invasive cervical cancer and its precursors. *Clin Cancer Res*. 2004;10:3396-3400.
 69. Fiegl H, Gatringer C, Widschwendter A, et al. Methylated DNA collected by tampons: a new tool to detect endometrial cancer. *Cancer Epidemiol Biomarkers Prev*. 2004;13:882-888.
 70. Teschendorff AE, Gao Y, Jones A, et al. DNA methylation outliers in normal breast tissue identify field defects that are enriched in cancer. *Nat Commun*. 2016;7:10478.
 71. Bartlett TE, Chindera K, McDermott J, et al. Epigenetic reprogramming of fallopian tube fimbriae in BRCA mutation carriers defines early ovarian cancer evolution. *Nat Commun*. 2016;7:11620.
 72. Teschendorff AE, Jones A, Fiegl H, et al. Epigenetic variability in cells of normal cytology is associated with the risk of future morphological transformation. *Genome Med*. 2012;4:24.
 73. Teschendorff AE, Jones A, Widschwendter M. Stochastic epigenetic outliers can define field defects in cancer. *BMC Bioinform*. 2016b;17:178.
 74. Barrett JE, Jones A, Evans I, et al. The DNA methylome of cervical cells can predict the presence of ovarian cancer. *Nat Commun*. 2022;13:448.
 75. Barrett JE, Herzog C, Jones A, et al. The WID-BC-index identifies women with primary poor prognostic breast cancer based on DNA methylation in cervical samples. *Nat Commun*. 2022b;13:449.
 76. Dubeau L. The cell of origin of ovarian epithelial tumours. *Lancet Oncol*. 2008;9:1191-1197.
 77. Barrett JE, Herzog C, Kim YN, et al. Susceptibility to hormone-mediated cancer is reflected by different tick rates of the epithelial and general epigenetic clock. *Genome Biol*. 2022;23(53):1-16. doi:[10.1186/s13059-022-02603-3](https://doi.org/10.1186/s13059-022-02603-3)

How to cite this article: Lehtinen M, Pimenoff VN, Nedjai B, et al. Assessing the risk of cervical neoplasia in the post-HPV vaccination era. *Int J Cancer*. 2023;152(6):1060-1068. doi:[10.1002/ijc.34286](https://doi.org/10.1002/ijc.34286)