THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Eurogin Roadmap 2017: triage strategies for the management of HPV-positive women in cervical screening programmes

OPEN ACCESS

Eurogin Roadmap 2017: triage strategies for the management of HPV-positive women in cervical screening programmes

Cuschieri K[1*], Ronco G[2], Lorincz A[3], Smith L[4]Ogilvie G[5], Mirabello L[6], Carozzi F[7], Cubie H[8], Wentzensen N[6], Snijders P[9], Arbyn M[10] Monsonego J[11], Franceschi S[12]

1: Scottish HPV Reference Laboratory, Department of Laboratory Medicine, NHS Lothian, Royal Infirmary of Edinburgh, 51 Little France Crescent, EH16 4SA, Kate.Cuschieri@nhslothian.scot.nhs.uk, Tel: 00 44 131 242 6039, Fax: 00 44 131 242 6008. *corresponding author

2: Centre for Cancer Prevention (CPO). AOU Città della Salute e della Scienza.via Cavour 39, 10123 Torino,Italy.

3: Wolfson Institute of Preventive Medicine, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ.

4: University of British Columbia and BC Women's Hospital and Health Centre, 4500 Oak Street, Vancouver, BC, Canada V6H 3N1.

5: Faculty of Medicine, University of British Columbia, Canada

6: Division of Cancer Epidemiology and Genetics, National Cancer Institute, US.

7: Cancer Prevention Regional Laboratory, ISPO, Cancer Prevention and Research Institute, Florence, Italy

8: Global Health Academy, University of Edinburgh, Teviot Quad, Edinburgh EH8 9PG.

9. Department of Pathology, VU University Medical Center (VUmc), Amsterdam, The Netherlands

10: Unit of Cancer Epidemiology, Belgian Cancer Centre, Scientific Institute of Public Health, Brussels, Belgium

11: Institut du Col, Paris, France

12: International Agency for Research on Cancer, Lyon, France

Short Title: Triage strategies for High-risk HPV positive women

Key words: HPV, triage, screening, roadmap

Novelty and impact: Second line or "triage" tests that can risk–stratify HR-HPV positive women are needed urgently for cervical screening. Through the vehicle of the "EUROGIN roadmap", we present the current state of the art in triage options, in addition to describing emerging technologies and challenges to implementation. Given that several countries have, or are soon to implement, HPV based screening nationally, such a review is timely.

Abstract

Cervical cancer screening will rely, increasingly, on HPV testing as a primary screen. The requirement for triage tests which can delineate clinically significant infection is thus prescient. In this EUROGIN 2017 roadmap, justification behind the most evidenced triages is outlined, as are challenges for implementation.

Cytology is the triage with the most follow-up data; the existence of an HR-HPV positive, cytology negative group presents a challenge and re-testing intervals for this group (and choice of re-test) require careful consideration. Furthermore, cytology relies on subjective skills and while adjunctive dual-staining with p16/Ki67 can mitigate inter-operator/site disparities, clinician-taken samples are required. Comparatively, genotyping and methylation markers are objective and are applicable to self-taken samples, offering logistical advantages including in low and middle income settings. However, genotyping may have diminishing returns in immunised populations and type(s) included must balance absolute risk for disease to avoid low specificity. While viral and cellular methylation markers show promise, more prospective data are needed in addition to refinements in automation.

Looking forward, systems that detect multiple targets concurrently such as next generation sequencing platforms will inform the development of triage tools. Multi-step triage strategies may be be beneficial provided they do not create complex, unmanageable pathways. Inevitably, the balance of risk to cost(s) will be key in decision making, although defining an acceptable risk will likely differ between settings. Finally, given the significant changes to cervical screening and the variety of triage strategies, appropriate education of both health care providers and the public is essential.

Introduction

HPV testing for cervical cancer screening is now a reality with several countries, either adopting this modality at the programme level or introducing it through the execution of regional pilot studies (1). Support for this approach to screening is also endorsed by various professional societies and organisations which have global influence and reach (2-5). While there is a wide and growing consensus that HPV molecular testing is the most accurate and cost-effective method of primary

screening, there is a comparative lack of consensus regarding the optimal means of risk stratification or "triage" of primary HPV infections. This is evidenced by the heterogeneity of triage algorithms either proposed or applied across various settings [(6) and Table 1]. Given that most HPV infections follow a benign course, effective triage is crucial to ensure the screening participant is not subject to the burden of unnecessary follow-up and that resources are used efficiently. This point is further emphasised by the fact that compared to cytology based primary screening, HPV testing will generate significantly more screen "positives" in the initial round of screening (7).

As a growing number of countries prepare for HPV primary screening, the triage issue becomes particularly timely. This urgency was reflected at the EUROGIN 2016 meeting, where several of the scientific papers focussed on the application of triage strategies. Consequently, a group of experts were tasked with using the vehicle of the now established "Eurogin roadmap" (the eleventh Roadmap release since 2007) to outline evidence, benefits and challenges surrounding current triage strategies and to summarise some of the key technical developments in both sampling and diagnostics that may bear influence.

Cytology

Arguably, the strategy with the longest available follow up data is cytology. Cytological triage of HPV-positive women has been evaluated within three European randomised controlled trials, initially designed to compare HPV testing (alone or with cytology concurrently) to cytology screening over two screening rounds (8-10).

Notwithstanding minor protocol differences across these trials, women with abnormal cytology were referred to colposcopy while the remainder were recalled to repeat an HPV test; alone or with cytology, after 6-18 months and referred to colposcopy in the case of persistent positivity (type specific in one study). All studies showed a significantly reduced occurrence of CIN3+ in the HPV arm at round 2. In addition, a pooled analysis of 4 studies with data on two screening rounds, including the three described, (and one without triage), found significantly less cancer in the HPV arm with no evidence of heterogeneity between studies. Conversely, the biopsy rate was similar between arms in the studies with cytological triage but higher in the trial without it (11). Thus, cytological triage with HPV repeat-testing in cytology-negative women can be considered validated. In the aforementioned trials, cytology interpretation was blind to HPV result. In a Finnish trial (performed over one screening round) cytology was performed on HPV-positive women, with knowledge of HPV status. Interestingly, the detection of CIN was higher in the HPV arm even when considering only referrals due to reflex

abnormal cytology (12). This suggests that knowledge of HPV positivity may affect the performance of cytology. This observation is consistent with a sub-study of the NTCC trial where HPV positive Paps were dispatched for external cytology review to a laboratory blinded to information other than HPV status. The cross-sectional relative sensitivity for CIN2+ of HPV informed vs blind cytology was 1.58 (95% CI = 1.22 to 2.01) (13). An important consideration, however, is that as cytology relies on subjective morphological assessment, between-site variation in interpretation and performance can be expected (14). In 2012, in ten Italian routine, local programmes based on HPV testing, the proportion of HPV positive women judged with ≥ASC-US varied from 20.0% to 56.9%. (14). This said, it should be noted that, as a consequence of the screening protocol applied (HPV+/cytologically normal women at baseline were referred to colposcopy if still HPV+ at 12 months), this variation had a relative small impact on the colposcopy referral rate, at baseline or at 12 months (only a 4.2% increase for a 10% increase in immediate referral) (15) However, the total number of colposcopies is also dependent on the follow-up guidelines of women with a negative colposcopy.

 More generally the above data show that when judging the performance of any triage approach, the entire process should be taken into account i.e. not just the immediate triage test. Improvements to immediate triage tests can be fully exploited only if the interval to re-testing is considered carefully. Finally, a practical consideration of cytology-triage is that it requires a highly-skilled workforce and significant investment in ongoing quality assurance to perform optimally. This is possible and has precedent in high-income countries with the relevant infrastructure, yet even in this context, the increasing move to HPV primary screening will bring about sizeable reductions in the overall cytology workload. Adequate recruitment, training and retention of cytology staff in an era of HPV primary screening may prove challenging and efforts to address this are required. A novel approach to automated cytology that achieves performance similar to expert manual evaluation may serve as mitigation to these challenges (16).

HPV Genotyping

Current HPV tests include 13-14 HPV genotypes that vary substantially in their association with cervical cancer and pre-cancer (Table 2). The variation in risk has prompted development of genotyping strategies for triage purposes. In cervical cancer, by far the most important type is HPV16, followed by HPV18. However, there is some regional variation of type prevalence that could affect which types

are included in screening and triage assays (17). While HPV16 is also dominant in CIN3, HPV31, 33, 52, and 58 are more common than HPV18 (Table 2). There are several important considerations that affect HPV genotyping-based triage: 1. Detection of a specific HPV genotype cannot differentiate between a transient infection and a prevalent precancer. 2. HPV genotype detection predicts risk of precancer over many years. Thus, women may be at increased risk of future precancer, but they may not have any detectable lesions. 3. Deciding which types to include in a triage genotyping assay must balance the prevalence of the type in disease and in the healthy population, measured by the absolute risk of disease related to genotype. These points demonstrate that unavoidably, there is some unnecessary colposcopy referral with genotyping. Table 3 shows the absolute risk of CIN3+ and CIN2+ for genotypes in a large population of HPV-positive women. Importantly, the ranking of types may differ depending on chosen endpoint; CIN3+ is generally preferable since many CIN2s regress spontaneously (18). Several commercial HPV assays, some of which have been FDA-approved and or clinically validated via other means offer partial genotyping, typically measuring at least HPV16 and HPV18 (19). Current US guidelines recommend immediate referral of HPV16/18 positive women with normal cytology to colposcopy in a HPV-cytology co-testing strategy (20). A recently FDA-approved strategy, endorsed by interim expert consensus, recommends immediate referral of HPV16/18-positive women, with cytology triage of women positive for other HR types (2). Furthermore, recent data from the US show that extended genotyping (up to nine types or combinations) combined with cytology can provide refined risk-stratification through the identification of type-specific persistence (21).

p16 +/- Ki67

p16INK4a (or p16) is a cellular protein which highlights disruption of the retinoblastoma (RB)/E2F pathway related to activity of the HPV oncogene E7. The diagnostic application is through inmunocytochemistry (or histochemistry), initially as a single marker and now as a dual stain with Ki67 (a proliferation marker which confers additional specificity) [22-25]. While p16 staining still requires a level of morphological interpretation, it can reduce pattern-complexity by allowing focus on a small subset of p16-stained cells. In contrast, for the dual stain, the criterion for positivity is a single cell with a simultaneous brown cytoplasm (p16) and a red nucleus (Ki67). The longitudinal accuracy of p16 immunostaining (without Ki67) as a triage of HPV positive women was studied within the NTCC trial (26). In women 35–60 years, the risk of CIN3+ at 3 years was 4.7% among HPV+/p16+ women compared to 0.8% in HPV+/p16- women. Furthermore, 83.7% of women who had a CIN3+ at follow-up were p16+ at baseline. The authors concluded that HPV+/p16+ positive women warrant immediate colposcopy whereas HPV+/p16- women could defer follow-up for at least 2 years. More

recently, three studies have reported on dual-stained cytology as a triage of HPV-positive women, within primary screening cohorts (27-29); all of which have indicated that dual-staining may enhance the sensitivity of cytology. The largest (n=7727) was nested into the Athena trial (29) and showed that for CIN3+ detection, sensitivity of dual-stained cytology vs Pap cytology was significant higher (74.9% vs. 51.9%), as was NPV and PPV; whereas specificity was equivalent. Immediate colposcopy referral of all HPV16/18+ women combined with dual-stained cytology of women positive for non-16/18 genotypes provided the highest sensitivity for CIN3+ (86.8%). Additionally, a recent Scottish study showed higher sensitivity but lower specificity of dual stained, compared to conventionally stained, cytology (30). It has been argued that the dual stain can reduce the requirement for "expert" cytology through simplifying interpretation and may improve inter-operator variability compared to traditional cytology (31). However, a recent study showed important differences in inter-observer reproducibility according to a laboratory's experience highlighting the need for robust training and quality assurance (32). In summary p16/ki67 dual stain is a credible tool for risk stratification of HPV-positive women and compares favourably to cytology (33). Head to head comparisons now need to consider the cost effectiveness of this strategy compared with other stand-alone and combination options.

Cellular and Viral Methylation Assays for Triage of HPV Infection

Methylation has a fundamental role in the development and outcome of malignancies and can be measured accurately and easily by automated methods. Approximately ten human genes have consistently elevated methylation in cervical precancers and hypermethylation in most cancers, hence the appetite to focus on methylation targets for triage purposes (34,35). Of prominence are *CADM1, EPB41L3, FAM19A4, MAL, miR-124, PAX1 and SOX1*. Methylation of certain HPV genes including L1 are also associated with precancer and invasive disease, especially for types HPV16, HPV18, HPV31, HPV33 and HPV45 (36,37). Table 4 shows the performance of human and HPV gene classifiers as triage tests for cervical precancers (38). *MAL and CADM1* have been investigated extensively in hrHPV+ women; in one study of a screening population with a precancer and cancer endpoint (collectively CIN2+) these genes gave a sensitivity of 84% (95%CI 72-93), specificity of 52% (48-57), and AUC of 0.72 (39). In the same set of women the sensitivity and specificity of cytology were 66% (50-79%) and 79% (74-83%) respectively (40). More-recent studies on screening and colposcopy populations using a variety of human gene targets showed sensitivity ranging from 69 to 74% with specificity (for CIN2+) ranging from 66% to 76% (38). A more comprehensive approach is to test for methylation of HPV *and* human genes. For example, a combination of *DAPK1 and* HPV on samples from a US colposcopy population gave a sensitivity of 80% and a specificity of 89% for CIN2+. Another study on a combination

of *EPB41L3* and HPV in a UK colposcopy population gave a sensitivity of 90%, specificity of 49%, AUC=0.82 (38). Given that performance in a colposcopy population does not necessarily translate to performance in a screening population the *EPB41L3*-HPV gene combination was validated in a separate study of hrHPV positive women from a screening population in the UK and gave a sensitivity of 74% (59-85%), specificity of 65% (60-70%), and AUC=0.78 (Table 4) (41). In comparison HPV16 and HPV18 genotyping on the same samples had a significantly poorer performance as a triage (P<0.0001). Methylation testing is still in the early stages but is showing good promise as an accurate molecular classifier. Technical improvements will likely improve clinical performance and can be expected in the next 5 years. Furthermore, even if methylation testing can deliver equivalent (rather than improved) performance compared to robust cytology, there are still positive aspects to this method including objectivity, consistency and applicability to automation and self-taken samples.

Special considerations – triage of self-collected specimens

Offering self-sampling of cervico-vaginal material for HPV testing (HPV self-sampling) is an effective tool to increase screening coverage (42). Moreover HPV testing on self-samples is as accurate as on clinician-taken samples if target-amplification assays are used (43). However, as cytology is not reliably applicable to self-taken samples, (43,44) offering cytology as a triage of women positive on their self-sample would necessitate a clinic visit. This confers a significant risk of loss to follow-up yet the issue would be circumvented if the triage test could be applied directly to the residual self-sample. In contrast to microscopy-based assays, molecular tests do not require the preservation of intact cells and may be used directly. The detection of (hyper) methylation of host cell genes by quantitative methylation-specific PCR (qMSP) on both self-collected vaginal lavages and brushes is feasible and shows promise as a triage (45-49).

A recent RCT where qMSP for host genes *MAL* and *miR124-2* was compared to cytology (on an additional smear) as a triage of HPV positive self-samples, demonstrated that the qMSP was non inferior to cytology for the detection of CIN2+ (50). While the clinical performance of the qMSP did not exceed that of cytology, the logistic advantages/efficiencies of being able to apply the screening and triage assay to the same sample are clear. There is also evidence to suggest that certain methylation markers have the advantage of giving a very high reassurance for absence of cervical cancer or advanced CIN2/3 lesions with a high short-term progression risk in test negative women (50,51). Finally, as with the other markers considered in this roadmap, it is perhaps prudent not to consider methylation biomarkers as only applicable in isolation. A study in which the performance of the *FAM19A4* and *miR124-2* methylation biomarker was assessed (51) showed that the addition of

HPV16/18 genotyping results increased the sensitivity for CIN2+ significantly. The combination approach of 16/18 genotyping with methylation has parallels with combining cytology with HPV16/18 genotyping, with the former offering a pathway to full molecular screening and triage.

The role of next generation sequencing and viral genomics

Advances in next-generation sequencing (NGS) technologies have enabled the sequencing of HPV whole-genomes in a high-throughput, cost-efficient manner. The large-scale study of HPV genome variability will advance a deeper understanding of HPV biology and mechanisms of HPV carcinogenicity, which may help to improve the design of triage tools in the future. NGS has already generated important information about carcinogenesis and natural history of HPV (52-54). For example, it has demonstrated that HPV 16 sub-lineages confer differential risks for disease as well as different tropisms for morphological lesion-type [53, 55-70]. Among 3,215 HPV16-positive women in the U.S., the HPV16 A4, D2, and D3 sub-lineages conferred significantly increased risks for glandular lesions compared to the more common A1/A2 sub-lineages (53). An international study of invasive cancers also found an enrichment of specific lineages in adenocarcinomas (67). A triage test incorporating detection of a variants specific for adenocarcinoma could be used to enhance the detection of glandular lesions. At the level below a sub-lineage there is an HPV isolate which is a genome differing by ≥2 nucleotides from all others. NGS has shown that thousands of unique HPV16 isolates exist (54).

Further, transient HPV16 infections have been shown to have a higher number of single nucleotide polymorphisms compared to transforming infections (54). Notably, the strict genetic conservation of E7 was associated with a greater risk of HPV16 driven carcinogenesis (54). The level of genetic variation in specific regions of the viral genome is important and could inform the design of future triage tools. Finally, given that NGS is capable of rapid sequencing of both host and viral genes the technology lends itself to identifying several potential biomarkers of significance to infection and precancer concurrently such as HPV genotyping detection, variant classification and detection of somatic mutations.

Low and middle income countries (LMIC)

Cervical screening in LMIC is clearly associated with very different challenges from screening in high income countries (HIC). WHO Guidelines recommend visual inspection with acetic acid (VIA) for

population based screening or HPV testing if it can be afforded (3). However, most current HPV tests are designed for HIC and a new generation of tests which address the challenges in LMIC are needed (Text-box 1). Unless these issues are addressed, both primary HPV testing and associated triage could remain largely HIC issues. Options for triage in LMIC are associated with particular challenges. The lack of cytologists, pathologists and associated quality assurance usually means cytology-based protocols cannot be contemplated. Effective triage in a single "screen and treat" visit would overcome the challenges of follow-up and restricted opportunities for intervention in LMIC, but requires a simple, quick, affordable and objective biomarker test performed on the same sample as the primary screen.

Neither limited genotyping nor current biomarkers would satisfy these conditions. Work in rural Malawi has shown that trained staff who maintain a regular workload and adequate continued professional development can use VIA directly to differentiate treatable lesions and suspicious / advanced cancers (71,72) but that prior knowledge of HPV results can aid judgment and inform clinical management (73). Sending all HPV positive women for immediate treatment will result in over treatment, whereas restricting VIA to HPV positives may well entail a significant loss in sensitivity yet could address problems of capacity and retention of competent VIA providers. Inevitably, the trade-off between resources available and programme outcomes needs to be assessed country by country and this is an area of great interest as outlined in recent reviews (74,75). Many LMIC have not implemented HPV primary screening due to the cost and challenges identified, whereas roll-out of a VIA service, while potentially more practicable is challenged by the need for ongoing quality assurance and monitoring. Use of less than 3 doses of HPV vaccine together with GAVI pricing have greatly advanced the potential for LMIC to consider national programmes (76). A combination of vaccination of adolescent girls and VIA for adult women may provide a better approach than HPV primary screening and an appropriate, as yet unidentified triage test although more evidence is needed to support this. To this end, projects built around the HPV FASTER recommendation [(of extending routine vaccination programmes to women of up to 30 years of age (and to the 45-50-year age groups in certain settings)], paired with at least one HPV-screening test at age 30 years will provide important data (77).

Triage for immunised women

The impact that HPV immunisation programmes have conferred on both infection and disease is now clear at the population level in several countries. (78) The evidence of herd immunity and the potential utility of even one dose of vaccine adds to these encouraging observations, for the countries

9

that can afford immunisation programmes (79,80). However, immunisation does present certain challenges for screening. The predictive value of any screening test will be influenced by the level of disease in the population (81). Furthermore, the fraction of HR-HPV infections in vaccinated populations attributable to 16/18 will be significantly lower; in a recent study of females immunised aged 12-13, over 90% of residual HR-HPV infections at age of first screen were not HPV 16 or 18 (82). As a wealth of evidence demonstrates that non 16/18 HR-HPV types confer a lower risk for CIN2+, the PPV of HR-HPV screening may reduce so appropriate triages are even more relevant in vaccinated populations (1). Limited genotyping in this population is likely to have diminishing returns given the scarcity of 16/18 positive infections and it is arguable that non-viral targets may be more appropriate or assays that offer genotyping beyond HPV 16/18. There have been few assessments of cytology triage specifically in immunised women (with or without adjunctive staining). Evidence of deterioration in the predictive value of cytology as a primary screen in immunised women has been documented, although this does not translate into poor performance as a triage of HR-HPV positive samples (83). It is also worth noting that the extent of triage in immunised populations will reduce given evidence from modelling studies that indicate 10-year screening starting aged 30 is optimal (84,85). Conclusions from modelling endeavours, while extremely helpful, clearly incorporate various assumptions and differing levels of vaccine-uptake, type of vaccine and dimensions of programme (including the detail of catch up immunisation) will exert influence. This makes a comprehensive, "one size fits all" solution difficult (1). In line with this, one of the recommendations from a recent Italian conference on screening for vaccinated women was that modifications to the status quo should only be imposed on women vaccinated routinely rather than as part of a catch up programme (86). Furthermore, offering optimal screening and triage to increasingly mixed populations of immunised and unimmunised women, without creating impractical algorithms, and conflicting public-facing message(s) is a key challenge facing the community.

Appropriate educational initiatives

The transition from cytology to HPV-based screening presents unique challenges for patients and providers. With HPV screening, receipt of positive findings may result in a shift in the clinical discussion from an oncologic to a communicable disease approach. Despite the fact that HPV is the most prevalent STI worldwide (87), there is a lack of awareness about HPV among the general population (88,89). Women may feel anxiety, or shame when informed they are HPV positive (90). Clinicians often feel ill-equipped to manage the questions of HPV positive women (91). These concerns could ultimately affect acceptance of a superior screening technology if implementation is not carefully

planned, irrespective of what the subsequent triage may be. Key messaging should be developed to address the concerns around an HPV positive result: De-stigmatize HPV by emphasizing the high prevalence; Highlight HPV positivity does not indicate a woman has or will develop cervical cancer; Ensure women are aware HPV may have been present for years, and does not reflect partner infidelity or promiscuity; Move the focus of HPV testing from "STI identification" to an enhanced test for cervical cancer prevention. A key indicator of a woman's intention to receive HPV testing is endorsement by her care provider (92); therefore, clinicians play a significant role alleviating distress by being prepared for the concerns and questions women will have. Cytology has been the primary cervical screening tool for decades. As a result, programs must invest appropriate time and resources to plan for HPV-based testing irrespective of the triage used. Without such investment, lack of engagement from both patients and providers could jeopardize successful acceptance, resulting in decreased attendance for screening or increased health system costs due to non-compliance with extended screening interval guidelines.

Conclusions

The choice of appropriate triage strategy for HPV positive women is one of the key issues facing the cervical-screening community at present. As outlined above, there are various options associated with varying levels of evidence that either exploit considerable, existing expertise in morphological assessment or take advantage of recent developments in molecular technologies. Currently, there is no single approach which offers a binary solution of referral to colposcopy for the positives and routine recall for the negatives, with the HPV positive-triage negative group (whether by cytology, typing, or methylation markers) representing a challenge. Technical refinement of molecular approaches and/or the appropriate combination of more than one option, either concurrently or in a step-wise fashion may deliver benefits provided the complexity and cost are not prohibitive. Defining an acceptable level of risk is also important to help calibrate triage tools to appropriate performance standards at the population level. For European settings, it has been proposed that triage positive women should have (minimum) 20% risk of CIN2+ to indicate colposcopy, whereas triage negative women should have a risk of <2% of CIN2+ to indicate routine recall (93). These settings are lower in the US (94). Longitudinal data from national HPV primary screening programmes, where alternative triage options have been used will continue to be important and should ideally be stratified by vaccination status. Finally, should there be a move to an entirely molecular option for cervical screening, possibly in combination with self-sampling, communications around this paradigm shift must be managed carefully to ensure informed informed engagement and sustainable uptake.

Acknowledgements

Country	Screening policy†	1st triage	2nd triage	Reference
Australia/ New Zea- land	HPV alone	HPV16/18+: colposcopy hrHPV other: LBC: • if HSIL+: colposcopy • if <=LSIL: 2nd triage	At 12 M: hrHPV testing: • If +: colposcopy, • If –: routine screening	http://wiki.cancer.org.au/australiawiki/images/a/ad/National_Cervical_Screening_Program_guidelines_long-form_PDF.pdf
England (pilot study) *	HPV alone	Current **pilot** (6 sites) algorithm: cytology, if ASC-US+: colposcopy, if NILM: 2nd triage. HPV16/18 typing also assessed in component of pilot sites where persistent 16/18 positivity at 1st and 2nd triage = colposcopy	For pilot studies: At 12 M: hrHPV testing: • If +: colposcopy, • If –: routine screening TBD for roll out	https://www.gov.uk/government/publications/human-papillomavirus-hpv-primary-screening-protocol
Germany **	cotesting	If ASC-H, AIS, HSIL: colposcopy, if NILM-LSIL: 2nd triage.	At 6-12 M: cotesting • If + (any test): colposcopy • If –: routine screening	P. Hillemans, Department of Gynaecology and Obstetrics, Hannover Medical School; https://www.g-ba.de/institution/presse/pressemitteilungen/641/

Ireland ***	HPV alone	Cytology, if ASC-US+: colposcopy, if NILM: 2nd triage. Inclusion of limited typing likely but not yet defined.	To be determined.	Health Technology Assessment (HTA) of human papillomavirus testing as the primary screening method for prevention of cervical cancer. Health Information and Quality Authority. May 2017 https://www.hiqa.ie/sites/default/files/2017-05/HPV%20HTA%20technical%20report-%2026052017_updated.pdf
Italy ****	HPV alone	Reflex cytology, if ASC-US+: colposcopy, if NILM: 2nd triage.	At 12 M: hrHPV testing: • If +: colposcopy, • If –: routine screening	http://www.gisci.it/documenti/convegni/firenze2014/workshop/carozzi_gisci_20140611.pdf
Netherlands	HPV alone	Cytology, if ASC-US+: colposcopy, if NILM: 2nd triage.	At 6M: cytology • If ASC-US_. Referral to colposcopy • If NILM: routine screening	http://www.britishcytology.org.uk/resources/Primary_HPV_screening_The_Dutch_experience.pdf
Norway *****	HPV alone	Reflex cytology if ASC-US+: colposcopy. If reflex cytology=NILM 2nd triage.	At 12 M: hrHPV testing • If +: colposcopy, • If –: routine screening	Ameli Trope & Mari Nygard, Norwegian Cancer Registry
Scotland	HPV alone	Cytology, if ASC-US+: colposcopy., if NILM: 2nd triage.	At 12 M: hrHPV testing: • If +: colposcopy, • If –: routine screening	Tracey Curtis, National Services Division and Timothy Palmer, Univeristy of Edinburgh
Sweden ******	HPV alone	Cytology, if ASC-US+: colposcopy	At 36M: cytology • If ASC-US_. Referral to colposcopy If NILM: routine screening	http://www.socialstyrelsen.se/riktlinjer/nationellascreeningprogram/livmoderhalscancer-screeningme

Turkey	HPV alone	HPV genotyping and evaluation of CP which is taken at same time as specimen for HPV screening (only used when hrHPV+). If cytology=ASC-US+: or if HPV16/18 regardless of cytology results: colposcopy. If HPV positive for other than HPV16/18 and smear is NILM: 2nd triage.	At 3-6M: hrHPV testing. If hrHPV-: routine screening. if hrHPV+: genotyping and evaluation of CP with same algorithm as 1ary triage. If cyto=NILM and other hrHPV: 3rd triage 3-6M later.	Turkey: M. Gultekin, Cancer Control Department, Public Health Institute, Ministry of Health, Ankara. http://kanser.gov.tr/Dosya/Kitaplar/turkce/Turkiye_Kanser_Kontrol_Program_ing.pdf
USA	Cotest	Immediate referral of HPV-positive ASC-US, any LSIL+ HPV+/NILM 2nd triage.	Repeat HPV/cytology. If either HPV+ or ASC-US+, referral to colposcopy If co-test negative, routine screening.	Saslow et al (2012), Ref. 20
	HPV alone	HPV16/18+: colposcopy hrHPV other: reflex LBC, if >=ASC-US: colposcopy; if reflex LBC=NILM: 2nd triage.	at 12M: repeat cotest. If either HPV+ or ASC-US+, referral to colposcopy If co-test negative, routine screening.	Huh et al (2015), Ref. 2

Table 1: Active or proposed triage policies for HPV-based screening

ASCUS: Atypical Squamous Cells of Undetermined Significance

CP: conventional Pap smear

LSIL: Low grade squamous intraepithelial lesion

NILM: Negative for intraepithelial lesion or malignancy

† For a description of the details of the screening policies see Wentzensen et al (1)

*Final decision on 1st & 2nd triage options for national roll out in England to be determined.

** Triage policy still needs national approval in Germany.

*** 2nd triage test is not yet determined for Ireland.

**** - Cytology for women 25-33 years. HPV alone for women 34-64 years

***** - Cytology for women 25-33 years. HPV alone for women 34-69 years.

******Cytology is offered to women 23-29 with HPV only interval=3y, age 30–49, with one cotest at age 41; HPV only, interval=7y, age 50–64. Non-participating continue to be invited yearly up to age of 70.

HPV type	Invasive cancer (n=40,679)	CIN3 (n=11,618)
HPV16	64.7	54.5
HPV18	16.5	4.9
HPV58	5.5	10.8
HPV33	5.1	11.0
HPV45	4.3	1.7
HPV52	3.7	10.9
HPV31	3.5	10.7
HPV39	1.5	1.5
HPV59	1.3	0.8
HPV35	1.2	3.1
HPV56	0.9	1.5
HPV51	0.8	3.5
HPV68	0.6	1.1
Multiple	11.9	15.8

Table 2: Prevalence of carcinogenic HPV types as a proportion of HPV-positive samples in cervical cancers and CIN3 worldwide [genotype-specific positivity includes that contributed by multiple HPV infections. [Adapted from Guan IJC 2012 (16)].

Type or type combination	CIN3+ absolute risk	CIN2+ absolute risk
16	22.5	35.6
18	11.7	20.7
31	8.5	20.7
33/58	8.5	18.8
45	6.1	11.1
52	5.8	16.2
51	2.7	8.9
39/68/35	2.1	8.1
59/56/66	1.5	5.6

Table 3: Absolute risk of CIN3+ and CIN2+ for genotypes and genotype combinations in HPV-positive women from Kaiser Permanente Northern California.

Genes	Sensitivity %	Specificity %	PPV %
C13ORF18JAM3 ANKRD18CP	74	76	NA
FAM19A4	70	66	55
EPB41L3 HPV16/18/31/33	74	65	28
EPB41L3 HPV16/18/31/33	90	49	51
CADM1 MAL miR-124	89	50	52
PAX1	44	95	83
PCDHA4 PCDHA13	75	80	73
DLX1 ITGA4 RXFP3 SOX17 ZNF671	77	86	NA
CADM1 MAL	62	78	49
JAM3 EPB41L3 TERT	55 60 62	90 57 62	NA NA NA
FAM19A4	69	70	42
MAL Mir-124	72	49	29
CADM1 MAL miR-124	48	81	NA
JAM3 TERT EPB41L3 C13ORF18	65	77	NA
CADM1 MAL	84	52	25

Table 4. Performance characteristics of selected DNA methylation studies in exfoliated cervical cells for CIN2+.The combination tests typically used any gene marker as positive, but see reference 37 for specific details.

- Small collection volumes are cheaper and make storage, transport, and disposal of plastics and fluids easier; systems designed a priori for cytology are wasteful
- Readily available simple blood collection tubes could be used for sample collection
- Self-collected vaginal specimens are more acceptable than clinician-collected specimens to women and especially in cultures where gynaecological examination requires a husband's permission
- Tampon-like collection devices are easy to use, favoured by women in certain settings and may be able to be transported without fluid
- HPV tests should be cheap, simple to do, and reproducible by all levels of healthcare providers with short turnaround time (< 2 hours).
- Point of care testing by clinic staff would enable 'screen and treat' programmes where women could complete the screening process in a single visit

- Text Box 1 - Elements to consider in the development of new generation of HPV tests appropriate for use in LMIC

References

1. Wentzensen N, Arbyn M, Berkhof J, Bower M, Canfell K, Einstein M, Farley C, Monsonego J, Franceschi S. Eurogin 2016 Roadmap: how HPV knowledge is changing screening practice. Int J Cancer. 2017; 140:2192-2200.

2. Huh WK, Ault KA, Chelmow D, Davey DD, Goulart RA, Garcia FA, Kinney WK, Massad LS, Mayeaux EJ, Saslow D, Schiffman M, Wentzensen N, Lawson HW, Einstein MH. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol. 2015; 136 :178-82.

3. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. WHO 2013. http://apps.who.int/iris/bitstream/10665/94830/1/9789241548694_eng.pdf

4. https://www.gov.uk/guidance/cervical-screening-programme-overview

5. Anttila A, Arbyn A, De Vuyst H, Dillner J, Dillner L, Franceschi S, Patnick J, Ronco G, Segnan N, Suonio E, Törnberg S & von Karsa L eds. European guidelines for quality assurance in cervical cancer screening. Second edition, Supplements. Office for Official Publications of the European Union, Luxembourg, 2015

6. Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. J Clin Virol. 2016; 76 Suppl 1:S49-S55.

7. Isidean SD, Mayrand MH, Ramanakumar AV, Rodrigues I, Ferenczy A, Ratnam S, Coutlée F, Franco EL; CCCaST Study Group. Comparison of Triage Strategies for HPV-Positive Women: Canadian Cervical Cancer Screening Trial Results. Cancer Epidemiol Biomarkers Prev. 2017;26 : 923-929.

8. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K Rådberg T, Strander B, Johansson B, Forslund O, Hansson BG, Rylander E, Dillner J . Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007; 357: 1589-1597.

9. Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NW, Heideman DA, Kenter GG, Cuzick J, Snijders PJ, Meijer CJ. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol. 2012;13:78–88

10. 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;10:672–82.

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

12. Kotaniemi-Talonen L, Nieminen P, Anttila A, Hakama M. Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting.Br J Cancer 2005;93:862-7.

13. Bergeron C, Giorgi-Rossi P, Cas F, Schiboni ML, Ghiringhello B, Dalla Palma P , Minucci D, Rosso S, Zorzi M, Naldoni C, Segnan N ,Confortini M, Ronco G. Informed cytology for triaging HPV positive women: sub-study nested in the NTCC randomized controlled trial. J.Natl Cancer Inst. 2015 107 (12): dju423 doi: 10.1093/jnci/dju423.

14. Sørbye SW, Suhrke P, Revå BW, Berland J, Maurseth RJ, Al-Shibli K. Accuracy of cervical cytology: comparison of diagnoses of 100 Pap smears read by four pathologists at three hospitals in Norway. BMC Clin Pathol. 2017;17:18.

15. Ronco G, Zappa M, Franceschi S, Tunesi S, Caprioglio A, Confortini M, Del Mistro A, Carozzi F, Segnan N, Zorzi M, Giorgi-Rossi P; Italian HPV Survey Working Group. Impact of variations in triage cytology interpretation on human papillomavirus-based cervical screening and implications for screening algorithms. Eur J Cancer. 2016;68:148-155

16 Schiffman M, Yu K, Zuna R, Terence Dunn S, Zhang H, Walker J, Gold M, Hyun N, Rydzak G, Katki HA, Wentzensen N. Proof-of-principle study of a novel cervical screening and triage strategy: Computer-analyzed cytology to decide which HPV-positive women are likely to have ≥CIN2. Int J Cancer. 2017; 140:718-725.

17 Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, Clifford GM. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012;131:2349-59.

18 Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. Obstet Gynecol. 2009;113:18-25.

19 Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ, Poljak M. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect. 2015;21:817-26.

20 Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, Garcia FA, Moriarty AT, Waxman AG, Wilbur DC, Wentzensen N, Downs LS Jr, Spitzer M, Moscicki AB, Franco EL, Stoler MH, Schiffman M, Castle PE, Myers ER; American Cancer Society; American Society for Colposcopy and Cervical Pathology; American Society for Clinical Pathology. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. Am J Clin Pathol. 2012;137:516-42.

21 Schiffman M, Burk RD, Boyle S, Raine-Bennett T, Katki HA, Gage JC, Wentzensen N, Kornegay JR, Aldrich C, Tam T, Erlich H, Apple R, Befano B, Castle PE. A study of genotyping for management of human papillomavirus-positive, cytology-negative cervical screening results. J Clin Microbiol. 2015;53:52-9.

22 Von Knebel Doeberitz M. New markers for cervical dysplasia to visualise the genomic chaos created by aberrant oncogenic papil- lomavirus infections. Eur J Cancer. 2002;38:2229–2242.

23 Tsoumpou I, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P, Malamou-Mitsi V, Paraskevaidis E p16INK4a immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009;35 :210–220.

24 Bergeron C, Ikenberg H, Sideri M, Denton K, Bogers J, Schmidt D, Alameda F, Keller T, Rehm S, Ridder R; PALMS Study Group. . Prospective evaluation of p16/Ki-67 dual-stained cytology for managing women with abnormal Papanicolaou cytology: PALMS study results. Cancer Cytopathol. 2015;123:373–381.

25 Kruse A-J, Baak JPA, Helliesen T,Kjellevold KH, Bol MG, Janssen EA . Evaluation of MIB-1-positive cell clusters as a diagnostic marker for cervical intraepithelial neoplasia. Am J Surg Pathol. 2002;26:1501–1507

26 Carozzi F, Gillio-Tos A, Confortini M, Del Mistro A, Sani C, De Marco L, Girlando S, Rosso S, Naldoni C, Dalla Palma P, Zorzi M, Giorgi-Rossi P, Segnan N, Cuzick J, Ronco G; NTCC working group. Risk of high-grade cervical intraepithelial neoplasia during follow-up in HPV-positive women according to baseline p16-INK4A results: a prospective analysis of a nested substudy of the NTCC randomised controlled trial, Lancet Oncol. 2013;14: 168–176.

27 Wentzensen N, Fetterman B, Castle PE, Schiffman M, Wood SN, Stiemerling E, Tokugawa D, Bodelon C, Poitras N, Lorey T, Kinney W. p16/Ki-67 dual stain cytology for detection of cervical precancer in HPV-positive women. J Natl Cancer Inst. 2015;107:djv257.

28   Gustinucci D, Rossi PG,Cesarini E, Broccolini M, Bulletti S, Carlani A, D'angelo V, D'amico MR, Di Dato E, Galeazzi P, Malaspina M, Martinelli N, Spita N, Tintori B, Giaimo MD, Passamonti B Use of cytology, E6/E7 mRNA, and p16 [INK4a] –Ki-67 to define the management of human papillomavirus (HPV)–positive women in cervical cancer screening. Am J Clin Pathol. 2016;145:35–45.

29 Wright TC Jr, Behrens CM, Ranger-Moore J, Rehm S, Sharma A, Stoler MH, Ridder R. Triaging HPV-positive women with p16/Ki-67 dual-stained cytology: Results from a sub-study nested

into the ATHENA trial. Gynecologic Oncology 2017;144: 51–56

30 Stanczuk GA, Baxter GJ, Currie H, Forson W, Lawrence JR, Cuschieri K, Wilson A, Patterson L, Govan L, Black J, Palmer T, Arbyn M. Defining Optimal Triage Strategies for hrHPV Screen-Positive Women-An Evaluation of HPV 16/18 Genotyping, Cytology, and p16/Ki-67 Cytoimmunochemistry. Cancer Epidemiol Biomarkers Prev2017;26: 1629-1635.

31 Wentzensen N, Fetterman B, Tokugawa D,Schiffman M, Castle PE, Wood SN, Stiemerling E, Poitras N, Lorey T, Kinney W . Interobserver reproducibility and accuracy of p16/Ki-67 dual-stain cytology in cervical cancer screening. Cancer Cytopathol. 2014;122:914– 920.,

32 Benevolo M, Allia E, Gustinucci D, Rollo F, Bulletti S, Cesarini E, Passamonti B, Giovagnoli MR, Carico E, Carozzi FM, Mongia A, Fantacci G, Confortini M, Rubino T, Fodero C, Prandi S, Marchi N, Farruggio A, Coccia A, Macrì L, Ghiringhello B, Ronco G, Bragantini E, Polla E, Maccallini V, Negri G, Giorgi Rossi P; New Technologies for Cervical Cancer Screening 2 (NTCC2) Working Group. Interobserver reproducibility of cytologic p16INK4a /Ki-67 dual immunostaining in human papillomavirus-positive women. Cancer. 2017;125:212-220.

33 Murphy N, Heffron CC, King B, Ganuguapati UG, Ring M, McGuinness E, Sheils O, O'Leary JJ. p16INK4A positivity in benign, premalignant and malignant cervical glandular lesions: a potential diagnostic problem. Virchows Arch. 2004;445:610-615

34 Wentzensen N, Sherman ME, Schiffman M, Wang SS. Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. Gynecol Oncol. 2009;112:293-9.

35 Clarke MA, Luhn P, Gage JC, Bodelon C, Dunn ST, Walker J, Zuna R, Hewitt S, Killian JK, Yan L, Miller A, Schiffman M, Wentzensen N. Discovery and validation of candidate host DNA methylation markers for detection of cervical precancer and cancer. Int J Cancer. 2017; 141:701-710.

36 Mirabello L, Sun C, Ghosh A, Rodriguez AC, Schiffman M, Wentzensen N, Hildesheim A, Herrero R, Wacholder S, Lorincz A, Burk RD. Methylation of human papillomavirus type 16 genome and risk of cervical precancer in a Costa Rican population. J Natl Cancer Inst. 2012 ;104:556-65.

37 Wentzensen N, Sun C, Ghosh A, Kinney W, Mirabello L, Wacholder S, Shaber R, LaMere B, Clarke M, Lorincz AT, Castle PE, Schiffman M, Burk RD. Methylation of HPV18, HPV31, and HPV45 genomes and cervical intraepithelial neoplasia grade 3. J Natl Cancer Inst. 2012; 104:1738-49.

38 Lorincz A. The virtues and weaknesses of DNA methylation as a test for cervical cancer prevention. Acta Cytologica. 2016; 60:501-512

39 Hesselink AT, Heideman DAM, Steenbergen RDM, Coupe VMH, Overmeer RM, Rijkaart D, Berkhof J, Meijer CJLM, Snijders PJF. Combined promoter methylation analysis of *CADM1* and *MAL*: an objective triage tool for high-risk human papillomavirus DNA-positive women. Clin Cancer Res 2011;17:2459-465.

40 Kalantari M, Osann K, Calleja-Macias IE, Kim S, Yan B, Jordan S, Chase DM, Tewari KS, Bernard HU, Methylation of human papillomavirus 16, 18, 31, and 45 L2 and L1 genes and the cellular DAPK gene: Considerations for use as biomarkers of the progression of cervical neoplasia. Virology 2014;448:314–321.

41 Lorincz AT, Brentnall AR, Scibior-Bentkowska D, Reuter C, Banwait R, Cadman L, Austin J, Cuzick J, Vasiljević N. Validation of a DNA methylation HPV triage classifier in a screening sample.Int J Cancer 2016;138:2745-2751.

42 Verdoodt F, Jentschke M, Hillemanns P, Racey CS, Snijders PJ, Arbyn M. Reaching women who do not participate in the regular cervical cancer screeningprogramme by offering self-sampling kits: a systematic review and meta-analysisof randomised trials. Eur J Cancer. 2015;51:2375-85.

43 Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao FH, Hillemanns P, Anttila A. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol. 2014;15:172-83.

44 Snijders PJ, Verhoef VM, Arbyn M, Ogilvie G, Minozzi S, Banzi R, van Kemenade FJ, Heideman DA, Meijer CJ. High-risk HPV testing on self-sampled versus clinician-collected specimens: a review on the clinical accuracy and impact on population attendance in cervical cancer screening. Int J Cancer. 2013;132:2223-36.

45 Garcia F, Barker B, Santos C, Brown EM, Nuño T, Giuliano A, Davis J. Cross-sectional study of patient- and physician-collected cervical cytology and human papillomavirus. Obstet Gynecol. 2003;102:266-72.

46 Brink AA, Meijer CJ, Wiegerinck MA, Nieboer TE, Kruitwagen RF, van Kemenade F, Fransen Daalmeijer N, Hesselink AT, Berkhof J, Snijders PJ. High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods, with comparison of a novel self-sampling device to a conventional endocervical brush. J Clin Microbiol. 2006;44:2518-23.

47 Eijsink JJ, Yang N, Lendvai A, Klip HG, Volders HH, Buikema HJ, van Hemel BM, Voll M, Coelingh Bennink HJ, Schuuring E, Wisman GB, van der Zee AG. Detection of cervical neoplasia by DNA methylation analysis in cervico-vaginal lavages, a feasibility study. Gynecol Oncol. 2011;120:280-283.

48 Boers A, Bosgraaf RP, van Leeuwen RW, Schuuring E, Heideman DA, Massuger LF, Verhoef VM, Bulten J, Melchers WJ, van der Zee AG, Bekkers RL, Wisman GB. DNA methylation analysis in self-sampled brush material as a triage test in hrHPV-positive women. Br J Cancer. 2014;111:1095-101.

49 Chang CC, Huang RL, Liao YP, Su PH, Hsu YW, Wang HC, Tien CY, Yu MH, Lin YW, Lai HC. Concordance analysis of methylation biomarkers detection in self-collected and physician-collected samples in cervical neoplasm. BMC Cancer. 2015;15:418.

50 Verhoef VM, Bosgraaf RP, van Kemenade FJ, Rozendaal L, Heideman DA, Hesselink AT, Bekkers RL, Steenbergen RD, Massuger LF, Melchers WJ, Bulten J, Overbeek LI, Berkhof J, Snijders PJ, Meijer CJ. Triage by methylation-marker testing versus cytology in women who test HPV-positive on self-collected cervicovaginal specimens PROHTECT-3): a randomised controlled non-inferiority trial. Lancet Oncol. 2014;15:315-22.

51 De Strooper LM, Verhoef VM, Berkhof J, Hesselink AT, de Bruin HM, van Kemenade FJ, Bosgraaf RP, Bekkers RL, Massuger LF, Melchers WJ, Steenbergen RD, Snijders PJ, Meijer CJ, Heideman DA. Validation of the FAM19A4/mir124-2 DNA methylation test for both lavage- and brush-based self-samples to detect cervical (pre)cancer in HPV-positive women. Gynecol Oncol. 2016;141:341-7.

52 Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14:395-405

53 Cullen M, Boland JF, Schiffman M, Zhang X, Wentzensen N, Yang Q, Chen Z, Yu K, Mitchell J, Roberson D, Bass S, Burdette L, Machado M, Ravichandran S, Luke B, Machiela MJ, Andersen M, Osentoski M, Laptewicz M, Wacholder S, Feldman A, Raine-Bennett T, Lorey T, Castle PE, Yeager M, Burk RD, Mirabello L. Deep sequencing of HPV16 genomes: A new high-throughput tool for exploring the carcinogenicity and natural history of HPV16 infection. Papillomavirus Research 2015; 1: 3-11.

54 Mirabello L, Yeager M, Cullen M, Boland JF, Chen Z, Wentzensen N, Zhang X, Yu K, Yang Q, Mitchell J, Roberson D, Bass S, Xiao Y, Burdett L, Raine-Bennett T, Lorey T, Castle PE, Burk RD, Schiffman M. HPV16 Sublineage Associations With Histology-Specific Cancer Risk Using HPV Whole-Genome Sequences in 3200 Women. J Natl Cancer Inst 2016; 108: djw100.

55 Mirabello L, Yeager M, Yu K, Clifford G, Xiao Y, Zhu.Bin, Cullen M, Boland JF, Wentzensen N, Nelson CW, Raine-Bennett T, Chen Z, Bass S, Song L, Yang Q, Steinberg M, Burdett L, Dean M, Roberson D, Mitchell J, Lorey T, Franceschi S, Castle PE, Walker J, Zuna R, Kreimer AR, C BD, Hildesheim A, Gonzalez P, Porras C, Burk RD, Schiffman M. HPV16 E7 genetic conservation is critical to carcinogenesis. Cell. 2017;170:1164-1174.

56 Hildesheim A, Schiffman M, Bromley C, Wacholder S, Herrero R, Rodriguez AC, Bratti MC, Sherman ME, Scarpidis U, Lin Q-Q, Terai M, Bromley RL, Buetow K, Apple RJ, Burk RD. Human Papillomavirus Type 16 Variants and Risk of Cervical Cancer. Journal of the National Cancer Institute 2001; 93: 315-8.

57 Pientong C, Wongwarissara P, Ekalaksananan T, Swangphon P, Kleebkaow P, Kongyingyoes B, Siriaunkgul S, Tungsinmunkong K, Suthipintawong C. Association of human papillomavirus type 16 long control region mutation and cervical cancer. Virology Journal 2013; 10: 30 doi: 10.1186/1743-422X-10-30

58 Xi LF, Koutsky LA, Hildesheim A, Galloway DA, Wheeler CM, Winer RL, Ho J, Kiviat NB. Risk for High-Grade Cervical Intraepithelial Neoplasia Associated with Variants of Human Papillomavirus Types 16 and 18. Cancer Epidemiology Biomarkers & Prevention 2007; 16: 4-10.

59 Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, Hildesheim A, Desalle R, Befano B, Yu K, Safaeian M, Sherman ME, Morales J, Guillen D, Alfaro M, Hutchinson M, Solomon D, Castle PE, Burk RD. A Population-Based Prospective Study of Carcinogenic Human Papillomavirus Variant Lineages, Viral Persistence, and Cervical Neoplasia. Cancer Research 2010; 70: 3159-69.

60 Cornet I, Gheit T, Iannacone MR, Vignat J, Sylla BS, Del Mistro A, Franceschi S, Tommasino M, Clifford GM. HPV16 genetic variation and the development of cervical cancer worldwide. Br J Cancer 2013; 108: 240-4.

61 Gheit T, Cornet I, Clifford GM, Iftner T, Munk C, Tommasino M, Kjaer SK. Risks for Persistence and Progression by Human Papillomavirus Type 16 Variant Lineages Among a Population-Based Sample of Danish Women. Cancer Epidemiology Biomarkers & Prevention 2011; 20: 1315-21.

62 Zehbe I, Voglino G, Delius H, Wilander E, Tommasino M. Risk of cervical cancer and geographical variations of human papillomavirus 16 E6 polymorphisms. The Lancet 1998; 352: 1441-2.

63 Zuna RE, Moore WE, Shanesmith RP, Dunn ST, Wang SS, Schiffman M, Blakey GL, Teel T. Association of HPV16 E6 variants with diagnostic severity in cervical cytology samples of 354 women in a US population. International Journal of Cancer 2009; 125: 2609-13.

64 Sichero L, Ferreira S, Trottier H, Duarte-Franco E, Ferenczy A, Franco EL, Villa LL. High grade cervical lesions are caused preferentially by non-European variants of HPVs 16 and 18. International Journal of Cancer 2007; 120: 1763-8.

65 Berumen J, Ordoñez RM, Lazcano E, Salmeron J, Galvan SC, Estrada RA, Yunes E, Garcia-Carranca A, Gonzalez-Lira G, Madrigal-de la Campa A. Asian-American Variants of Human Papillomavirus 16 and Risk for Cervical Cancer: a Case–Control Study. Journal of the National Cancer Institute 2001; 93: 1325-30.

66 Freitas LB, Chen Z, Muqui EF, Boldrini NAT, Miranda AE, Spano LC, Burk RD. Human Papillomavirus 16 Non-European Variants Are Preferentially Associated with High-Grade Cervical Lesions. PLoS ONE 2014; 9: e100746.

67 Burk RD, Terai M, Gravitt PE, Brinton LA, Kurman RJ, Barnes WA, Greenberg MD, Hadjimichael OC, Fu L, McGowan L, Mortel R, Schwartz PE, Hildesheim A. Distribution of Human Papillomavirus Types 16 and 18 Variants in Squamous Cell Carcinomas and Adenocarcinomas of the Cervix. Cancer Research 2003; 63: 7215-20.

68 Quint KD, de Koning MNC, van Doorn L-J, Quint WGV, Pirog EC. HPV genotyping and HPV16 variant analysis in glandular and squamous neoplastic lesions of the uterine cervix. Gynecologic Oncology 2010; 117: 297-301.

69 Rabelo-Santos SH, Villa LL, Derchain SF, Ferreira S, Sarian LOZ, Ângelo-Andrade LAL, Do Amaral Westin MC, Zeferino LC. Variants of human papillomavirus types 16 and 18: Histological findings in women referred for atypical glandular cells or adenocarcinoma in situ in cervical smear. International Journal of Gynecological Pathology 2006; 25: 393-7.

70 Nicolás-Párraga S, Alemany L, de Sanjosé S, Bosch FX, Bravo IG, on behalf of the RHT, groups HVs. Differential HPV16 variant distribution in squamous cell carcinoma, adenocarcinoma and adenosquamous cell carcinoma. International Journal of Cancer 2017; 140: 2092-100.

71 Campbell C, Kafwafwa S, Brown H, Walker G, Madetsa B, Deeny M, Kabota B, Morton D, Ter Haar R, Grant L, Cubie HA. Use of thermo-coagulation as an alternative treatment modality in a 'screen and treat' programme of cervical screening in rural Malawi. Int J Cancer 2016; 139: 908-915.

72 Cubie HA; Morton D, Kawonga E, Mautanga M, Mwenitete I, Teakle N, Ngwira B, Walker H, Walker G, Kafwafwa S, Kabota B, Ter Haar R, Campbell C. Using the cartridge-based GeneXpert® HPV assay to assess HPV prevalence in women attending cervical screening in rural Malawi. J Clin Virol. 2017; 87: 1-4.

73 Beatrice Kabota, Nkhoma CCSP Co-ordinator; personal communication

74 Campos NG, Tsu V, Jeronimo J, Myundura M, Kim JJ. Evidence-based policy choices for efficient and equitable cervical cancer screening programs in low-resource settings. Cancer Med. 2017;6:2008-2014.

75 Santesso N, Mustafa RA, Schuneman HJ, Arbyn M, Blumenthal PD, Cain J, Chirenje M, Denny L, De Vuyst H, Eckert LO, Forhan SE, Franco EL, Gage JC, Garcia F, Herrero R, Jeronimo J, Lu ER, Luciani S, Quek SC, Sankaranarayanan R, Tsu V, Broutet N; Guideline Support Group . World Health Organisation Guidelines for treatment of cervical intraepithelial neoplasia 2-3 and screen-and-treat strategies to prevent cervical cancer. Int J Gynaecol Obstet. 2016; 132:252-258.

76 Kreimer AR, Rodriguez AC, Hildesheim A, Herrero R, Porras C, Schiffman M, González P,Solomon D, Jiménez S, Schiller JT, Lowy DR, Quint W, Sherman ME, Schussler J, Wacholder S; CVT Vaccine Group. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst. 2011;103:1444-51.

77 Bosch FX, Robles C, Díaz M, Arbyn M, Baussano I, Clavel C, Ronco G, Dillner J, Lehtinen M, Petry KU, Poljak M, Kjaer SK, Meijer CJ, Garland SM, Salmerón J,Castellsagué X, Bruni L, de Sanjosé S, Cuzick J. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. Nat Rev Clin Oncol. 2016;13:119-32.

78 Drolet M, Bénard É, 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 P, Markowitz L, Mboup A, 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;15:565-80.

79 Cameron RL, Kavanagh K, Pan J, Love J, Cuschieri K, Robertson C, Ahmed S, Palmer T, Pollock KG. Human Papillomavirus Prevalence and Herd Immunity after Introduction of Vaccination Program, Scotland, 2009-2013. Emerg Infect Dis. 2016;22:56-64.

80 Kreimer AR, Struyf F, Del Rosario-Raymundo MR, Hildesheim A, Skinner SR, Wacholder S, Garland SM, Herrero R, David MP, Wheeler CM; Costa Rica Vaccine Trial Study Group Authors, González P, Jiménez S, Lowy DR, Pinto LA, Porras C, Rodriguez AC, Safaeian M, Schiffman M, Schiller JT, Schussler J, Sherman ME; PATRICIA Study Group Authors, Bosch FX, Castellsague X, Chatterjee A, Chow SN, Descamps D, Diaz-Mitoma F, Dubin G, Germar MJ, Harper DM, Lewis DJ, Limson G, Naud P, Peters K, Poppe WA, Ramjattan B, Romanowski B, Salmeron J, Schwarz TF, Teixeira JC, Tjalma WA; HPV PATRICIA Principal Investigators/Co-Principal Investigator Collaborators; GSK Vaccines Clinical Study Support Group. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica Vaccine and PATRICIA Trials. Lancet Oncol. 2015;16:775-86.

81 Ogilvie G, Nakisige C, Huh WK, Mehrotra R, Franco EL, Jeronimo J. Optimizing secondary prevention of cervical cancer: Recent advances and future challenges. Int J Gynaecol Obstet. 2017;138 Suppl 1:15-19.

82 Kavanagh K, Pollock KG, Cuschieri K, Palmer T, Cameron RL, Watt C, Bhatia R, Moore C, Cubie H, Cruickshank M, Robertson C. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. Lancet Infect Dis. 2017;17:1293-1302.

83 Palmer TJ, McFadden M, Pollock KG, Kavanagh K, Cuschieri K, Cruickshank M, Cotton S, Nicoll S, Robertson C. 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 Mar;114:582-9.

84 Burger EA, Pedersen K, Sy S, Kristiansen IS, Kim JJ. Choosing wisely: a model-based analysis evaluating the trade-offs in cancer benefit and diagnostic referrals among alternative HPV testing strategies in Norway. Br J Cancer. 2017 Sep;117:783-790.

85   Kim JJ, Burger EA, Sy S, Campos NG. Optimal Cervical Cancer Screening in Women Vaccinated Against Human Papillomavirus. J Natl Cancer Inst. 2016;109: djw216.

86 Giorgi Rossi P, Carozzi F, Federici A, Ronco G, Zappa M, Franceschi S; Italian Screening in HPV vaccinated girls Consensus Conference group. Cervical cancer screening in women vaccinated against human papillomavirus infection: Recommendations from a consensus conference. Prev Med. 2017;98:21-30.

87 H. Trottier, E.L. Franco. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24 Suppl 1:S1-15.:

88 Klug SJ, Hukelmann M, Blettner M. Knowledge about infection with human papillomavirus: a systematic review. Prev Med. 2008; 46:87-98.

89 Giles M, Garland S. A study of women's knowledge regarding human papillomavirus infection, cervical cancer and human papillomavirus vaccines. Aust N Z J Obstet Gynaecol. 2006;46:311-5.

90 Hendry M, Pasterfield D, Lewis R, Clements A, Damery S, Neal RD,Adke R,Weller D, Campbell C, Patnick J, Sasieni P, Hurt C, Wilson S, Wilkinson C . Are women ready for the new cervical screening protocol in England? A systematic review and qualitative synthesis of views about human papillomavirus testing. Br J Cancer. 2012;107:243-54.

91 Smith L, van Niekerk D, Coldman A, Krajden M, Franco E, Ogilvie S. Recommendations for Implementing Human Papillomavirus-Based Cervical Cancer Screening: Lessons Learned from the HPV FOCAL Trial. J Obstet Gynaecol Can. 2016;38:723-6.

92 Ogilvie GS, Smith LW, van Niekerk DJ,Khurshed F, Krajden M, Saraiya M, Goel V, Rimer BK, Greene SB, Hobbs S, Coldman AJ, Franco EL. Women's intentions to receive cervical cancer screening with primary human papillomavirus testing. Int J Cancer. 2013; Dec 15;133:2934-43.

93 Arbyn M, Xu L, Verdoodt F,Cuzick J, Szarewski A, Belinson JL, Wentzensen N, Gage JC, Khan MJ . Genotyping for HPV16 and HPV18 in women with minor cervical lesions: a systematic review and meta-analysis. Ann Intern Med. 2017; 166: 118-12

94 Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, Cheung LC, Raine-Bennett T, Gage JC, Kinney WK. Benchmarking CIN 3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S28-35.