Novel biomarkers for cervical screening and surveillance of women treated for cervical precancer

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4. Aim and outline of this thesis

1. Cervical cancer and its premalignant lesions

1.1 Epidemiology

Cervical cancer is a worldwide public health problem that claims the lives of more than 270,000 women every year. It is the fourth most common cancer among women worldwide, with approximately 528,000 newly diagnosed cases in 2012.[1]

More than 85% of cervical cancer deaths occur in women living in low- and middle-income countries with incidence rates highest in sub-Saharan Africa (age-standardized rate (ASR) > 30 per 100,000 woman-years), South Central Asia (ASR 284.6 per 100,000) and Melanesia (ASR 23.9 per 100,000).[1,2,3,4] In high-income countries early diagnosis and treatment of cervical precancerous lesions by implementation of screening has led to a significant reduction in the burden of disease.[5-7]

In The Netherlands, cervical cancer comprises approximately 1.9% of all newly diagnosed malignancies.[8] In 2015, the number of new cancer cases was 715 and the number of death cases was 198. The 5-year overall survival probability in women with cervical cancer was 66% in year 2004-2012.

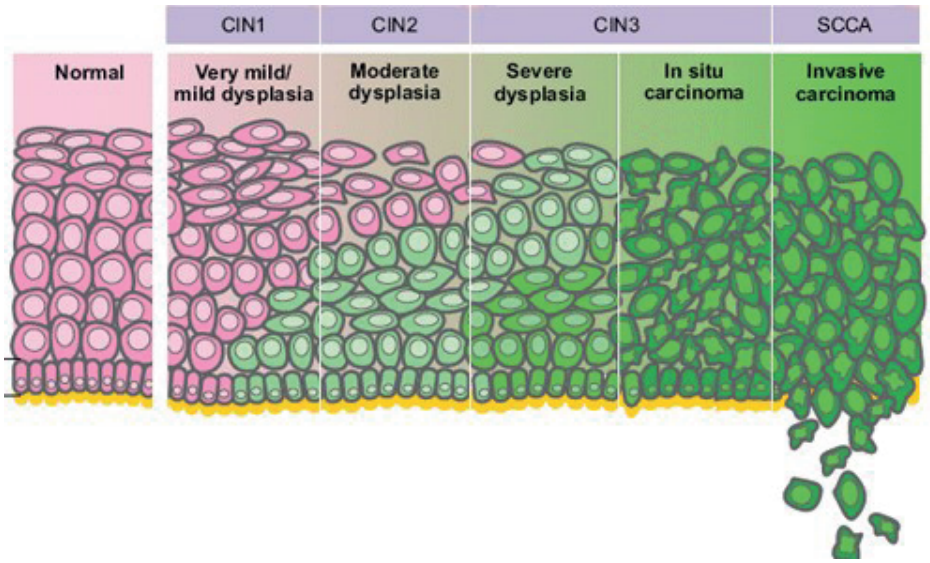
1.2 Cervical cancer and its precursor lesions

Cancer of the cervix uteri originates in the cervix, the lower-most portion of the uterus. Cylindrical in shape, the cervix consists of ectocervix (the outer part) covered with squamous epithelium and the endocervix (the inner part) covered with glandular columnar cells. The squamocolumnar junction (SCJ) is located at the site where the columnar epithelium and the squamous epithelium meet. Its location varies throughout life as a result of metaplastic changes in the columnar epithelium of the cervix. Before puberty the SCJ is usually located at the external os; in parous women it may be on the ectocervix; after the menopause the SCJ is usually within the endocervical canal. The region between the original and new SCJ is called the transformation zone.[9]

Histologically, cervical cancers can be classified into different subtypes. The majority (80%) comprise squamous cell carcinomas (SCC) and a smaller fraction (15-20%) comprise adenocarcinomas (ACs) and adenosquamous carcinomas (5%). Invasive cervical cancers are preceded by a long phase of preinvasive disease (precursor lesions). In case of SCC, these histologically recognizable precursor lesions, cervical intraepithelial neoplasia (CIN) are graded from 1 to 3 (Figure 1). CIN1 shows dysplasia in less than one third of the epithelium, CIN2 in two thirds of the epithelium and CIN3 in more than two thirds of the epithelium. During the progression from CIN1 to CIN3 also an increase in the degree of atypia in the individual cells is seen. Invasive SCC is diagnosed when the basal membrane has been invaded.

CIN lesions can regress, persist or progress. CIN1 has the highest regression rate whereas CIN3, the lowest.[10,11] Vice versa, CIN3 has the highest progression rate to

Figure 1.1 Schematic representation of histological classification during cervical cancer development. [Adapted from: Funk et al. 2007][17]



CIN, cervical intraepithelial neoplasia.

cervical cancer of approximately 30-50% in 30 years.[12,13] Based on their relative risk of progression to cancer, CIN1 are called low-grade lesions, whereas CIN2 and CIN3 are referred to as high-grade lesions.[14,15] Progression of a high-grade CIN lesion to cancer takes 15-30 years.[12,16] However, based on morphology, no distinction can be made between high-grade lesions with a low or high risk of progression to cancer. Therefore, all women with a high-grade lesion are recommended for a large loop excision of the transformation zone (LLETZ), in order to prevent the progression to SCC. Consequently this results in a considerable amount of overtreatment.

2. Human Papillomavirus and cervical carcinogenesis

A persistent infection with high-risk human papillomavirus (HPV) is assumed to be the necessary etiological factor in the development of cervical cancer.[18-21]

Human papillomaviruses are small, double-stranded DNA viruses and belong to the Papillomaviridae family. The viral genome of HPV is about 8kb in size and contains eight genes.[22] These are grouped into early genes (E1, E2, E4, E5, E6, E7), expression of which is necessary for viral replication, and late genes (L1 and L2), encoding the capsid proteins.[23,24]

To date more than 140 HPV types have been identified.[25] A new HPV type is defined when more than 10% of the viral DNA sequence of E6, E7 and L1 differs from any other known HPV type.[26] HPV can be categorized into cutaneous types (infecting the epithelial cells of the skin) and mucosal types (infecting the mucosal lining of mouth, throat, respiratory tract, anogenital epithelium). HPVs can be further subdivided into high- and low-risk types. The majority of HPV types are low-risk (lr), or non-oncogenic types. They infect the cutaneous epithelium and cause benign wart-like lesions. High-risk (hr), or oncogenic HPV types act as carcinogens in the development of cervical cancer and a subset of other anogenital and head-and-neck cancers. Currently, twelve HPV types are defined by the World Health Organisation (WHO) as being carcinogenic , i.e. HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (IARC class1) and one type is classified as probable carcinogenic, i.e. HPV68 (IARC class 2A). Several other types are considered as possible carcinogenic, i.e. HPV26, 53, 66, 67, 70 73, and 82 (IARC class2B).[27] The HPV types differ in their oncogenic potential and prevalence rates, with HPV16 accounting for approximately 50% of cervical cancers worldwide, followed by HPV18 (~16%), and HPV33 (~4%), accounting for approximately 16% and 4% of cervical cancers, respectively.[28]

2.1 Prevalence of HPV

The prevalence of HPV in the female population depends on geographic area and age. In a meta-analysis, the global prevalence in the female population of any HPV infection was estimated to be 12%, with the highest rates in sub-Saharan Africa (24%), Eastern Europe (21%) and Latin America (16%).[29] The lifetime risk of acquiring a genital HPV infection is estimated at 80%.[3,30]

Since HPV transmission in the genital tract is mainly through sexual contact[31], its prevalence is highest shortly after starting sexual intercourse.[32] Among women in the Netherlands, prevalence is highest between the age of 18 and 24 years (approximately 20%) and decreases with increasing age to less than 3% in women over 45 years of age.[30,33] The prevalence is approximately 5% in women (30-60 years of age) who participate in cervical screening.[30]

Although HPV infections are very common, only a minority (1-3%) of infected women will ultimately develop cervical cancer. When infected with HPV, about 80% of women cure the infections without developing CIN lesions. About 20% of the HPV infections are associated with CIN lesions and most of them (mainly CIN1 and part of the CIN2 lesions) are so-called 'productive' infections with transient behavior. They will be cleared by the hosts' immune system within 1-2 years after exposure.[19, 32, 34-37] In ~20% of these productive infections , the HPV infection remains persistent and may become a "transforming" infection, characterized by deregulated viral gene expression. Addition-

ally, HPV infections have been suggested to directly result in a transforming infection. A transforming infection is the first step into the development of cervical cancer.

2.2 HPV-mediated cervical carcinogenesis

Productive infections

HPV preferentially infect the basal cells of the transformation zone, but may also infect the basal cells of the ecto- or endocervix. Upon cellular differentiation the viral DNA is replicated and virus will be produced in the upper layers of the epithelium. Since HPVs encode only 8 proteins, they must employ host cell factors to regulate viral transcription and replication. In differentiated, non-dividing cells, the replication machinery is normally inactive, but it will be reactivated by the viral onco-proteins, E6 and E7 to allow viral replication. In this so-called productive infection, new virus particles are formed and released from terminally differentiated cells. These infections correspond with mild to moderate cellular abnormalities that are histomorphologically comparable with CIN1/2.

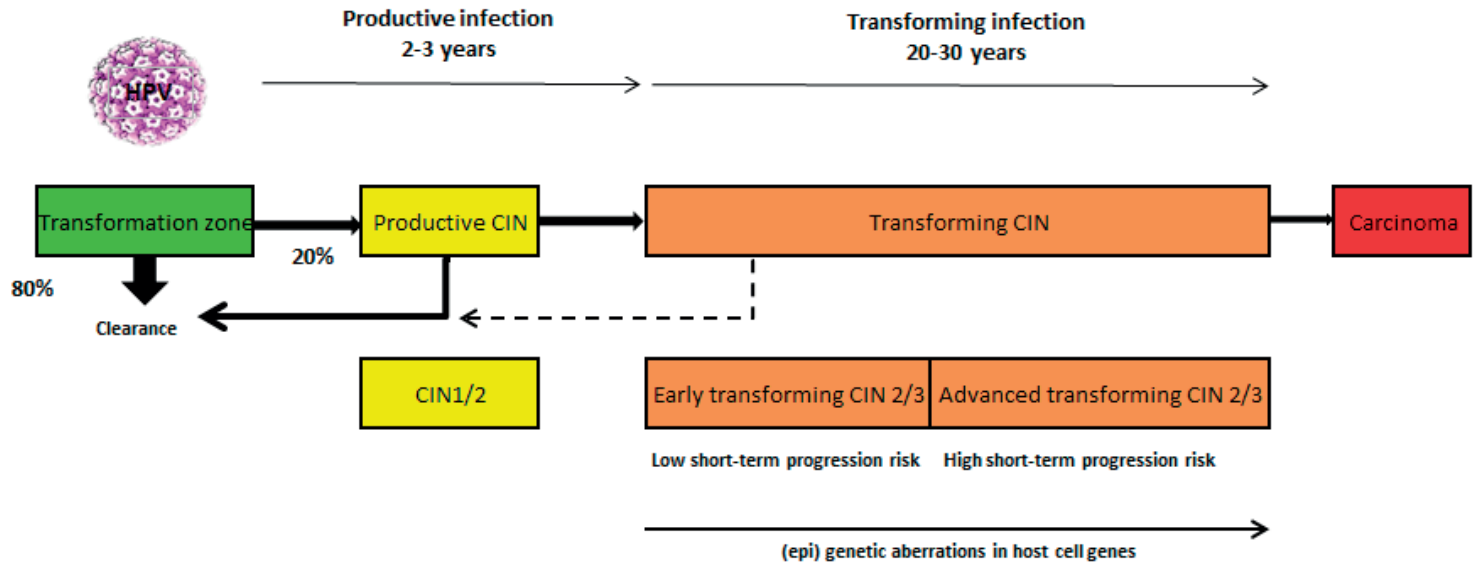
Transforming infections

Cervical cancer development is a result of a so-called transforming infection in which E6 and E7 are deregulated and become expressed in dividing cells. These latter infections are associated with a subset of CIN2, CIN3 and cancer. This indicates that CIN2 represents a heterogeneous group of lesions caused by productive as well as transforming infections.[16]

Expression of the viral oncoproteins E6 and E7 gene in dividing cells results in a deregulation of the cell cycle. The HPV E6 protein targets the p53 tumor suppressor protein for rapid degradation. As a consequence, the normal activities of p53 which governs cell cycle arrest, apoptosis, and DNA repair are abrogated.[14, 16, 34] HPV E7 binds to the retinoblastoma tumor suppressor (pRB). This binding disrupts the complex between pRB and the cellular transcription factor E2F, resulting in the liberation of E2F and subsequent S-phase entry and cell proliferation.[38, 39]

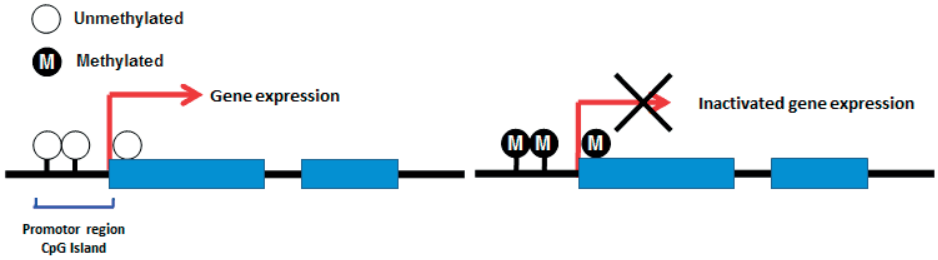
An alternative concept of cervical carcinogenesis has recently been described by Herfs et al. according to which infection of the SCJ-cells directly results in a transforming infection and the development of true precancerous lesions (CIN3). A distinct population of cuboidal cells of embryonic origin at the SCJ has been proposed as the cells of origin for cervical cancer and its high-grade precursor lesions.[40, 41] These so-called SCJ-cells uniquely express a number of genes that are also detectable in the far majority of invasive cervical cancers linked to HPV. This may suggest that precancerous lesions exhibiting the SCJ-specific signature are at increased risk of progression to cancer.

Figure 1.2 HPV-mediated cervical carcinogenesis.



Concept of cervical cancer development. *Adapted from Snijders J.Pathol 2006 and Steenbergen et al.2014*[14,16] HPV infection of the transformation zone can result in clearance, or in a productive infection (CIN1and a subset of CIN2), or in a transforming infection (remaining subset of CIN2 and CIN3), and finally cervical cancer. As a consequence of a persistent HPV infection, additional (epi) genetic changes are necessary for progression from a productive HPV infection to a transforming HPV infection associated with part of CIN2 and CIN3 , finally resulting in cervical cancer. Depending on the duration of lesion existence, as characterized by the duration of the HPV infections, and the number of chromosomal aberrations transforming CIN can be distinguished into early transforming CIN 2/3 with a low short-term progression risk to cancer, and advanced transforming CIN 2/3 with a high short-term progression risk (for more details see text).

Figure 1.3 Schematic representation of promotor methylation in a normal cell and in a cancer cell. DNA methylation is a chemical modification by a methyl group on cytosines at the CpG sequences of DNA. When a region that regulates gene expression (a promoter region) is methylated, the gene cannot be expressed. [Adapted from http://www.ncc.go.jp/en/nccri/divisions/14carc/14carc01_1.html]



Ultimately, as a consequence of the persistence of an HPV infection, additional genetic and epigenetic host cell alterations are acquired that drive progression towards cancer.[14, 16] These final steps may last 20-30 years (Figure 1.2).[121442] This long latency period indicate that CIN2/3 represent a heterogeneous group of lesions in terms of duration of lesion existence.[16]

Based on the duration of existence of the CIN lesion, as defined by the duration of the HPV infection and on the presence of chromosomal aberrations, transforming CIN can be distinguished in i) early transforming CIN2/3 with a low short-term progression risk to cancer, characterized by a HPV infection < 5 years and few chromosomal aberrations and ii) advanced transforming CIN 2/3 with a high short-term progression risk[16], characterized by an HPV infection> 5 years and numerous chromosomal aberrations.

2.2.1 DNA methylation

Epigenetic alterations are reversible changes in gene function without changes in DNA sequence, and include DNA-methylation of tumor suppressor genes. DNA methylation involves the covalent binding of a methyl group (CH3) at the carbon-5 position of cytosine located 5’ of a guanine to generate a 5’methylcytosine.

In normal tissue tumor suppressor genes with CpG islands in their promotor regions are generally unmethylated. Methylation of these CpG islands as seen in cancer cells can result in gene silencing and therefore contribute to cell transformation (Figure 3).[43-45] Interestingly, for a number of tumor suppressor genes, like cell adhesion molecule 1 (CADM1), T-lymphocyte maturation associated protein (MAL) and FAM19A4, the levels of promoter methylation have been found to increase proportionally to the degree of underlying cervical disease[46-48] and are extremely high in cervical cancer. Moreover, levels of CADM1, MAL and FAM19A4 promoter methylation are significantly increased in CIN3 lesions of women with a HPV infection that had persisted over a long time period (≥5 years) compared to those with an early CIN3 lesion resulting from a more

recently acquired HPV infection. CIN3 lesions with a long-term HPV infection have also a significantly higher number of chromosomal aberrations compared to CIN3 with a short-term HPV infection.[49] This has led to the concept that CIN2/CIN3 with a cancer-like methylation profile can be considered advanced CIN2/3 lesions with a high short term risk of cervical cancer in contrast to methylation negative CIN2/3.[16]

3. Prevention of cervical cancer

The prevention of cervical cancer includes primary, secondary and tertiary prevention. The aim of primary prevention is to prevent cervical cancer in healthy women without disease, for instance by prophylactic vaccination; secondary prevention is the detection of preclinical disease i.e CIN lesions or early cancer in women who have the disease (HPV infection) but are asymptomatic, for instance through screening and followed by treatment. Tertiary prevention consists of measures to reduce recurrence or progression of invasive disease.

3.1 Primary cervical cancer prevention: prophylactic vaccination

Primary prevention of cervical cancer is possible by prophylactic vaccination of HPV-naïve women (women without current infection and without prior exposure), thus prior to sexual debut. For prophylactic vaccines, the proteins coded by the L1 gene of a given HPV type spontaneously configure empty viral capsids or virus like particles (VLPs) when expressed in baculoviral or yeast expression systems. Early studies of HPV16 L1 VLP vaccines showed that they are well tolerated and generate high levels of antibodies against HPV16.[50] Currently, the HPV16/18 AS04-adjuvanted bivalent vaccine (Cervarix®, GSK)[51, 52 53], the HPV6/11/16/18 quadrivalent vaccine (Gardasil®, Merck)[54, 55], and a nine-valent vaccine (Gardasil 9®, Merck)[54, 55] are licensed. Although protection is type-specific, both the bivalent and the quadrivalent vaccine have shown cross-protective potential against certain oncogenic non-HPV16/18 types.

Because protection by prophylactic vaccines against cervical cancer cannot be demonstrated within 20 years, intermediate endpoints like protection against CIN2+ or a persistent HPV infection of 6 months or longer, have been accepted to show efficacy of the vaccines.[56] Vaccines given in a three or two dose scheme have shown long persistence of antibodies (>10 years) and high efficacy against CIN2.[57-60]

HPV vaccination will not stop the need for cervical cancer screening in the coming 20 years. First of all, currently vaccinated girls are protected against HPV types which, together, are responsible for the majority (77%; 70% by HPV 16/18, 7% by cross-protection)[61,62], but not all, cervical cancer cases. Secondly, vaccine uptake in the

Netherlands and many other countries is suboptimal. In the first year after introduction (2012) vaccine coverage in the Netherlands was 56% with an increase to 59% 2 years later.[63] The uptake of HPV vaccination is lower as expected and is probably influenced by the novelty of the vaccine, a vaccine targeting a sexually transmitted infection and negative media attention.[64] In comparison, the HPV vaccination coverage in Australia in 2015 was 77.4% and in England 89.4%.[65, 66] Concerning abovementioned reasons, cervical screening will stay an important secondary prevention strategy for women. Important research questions remain, like possible extension of the screening interval for HPV-vaccinated women, the optimal number of HPV-screening tests necessary in vaccinated women or possible expanding the indications for HPV vaccination.[67]

3.2 Secondary cervical cancer prevention: screening

Cervical cancer screening programmes are in place in almost all European countries as a secondary prevention method. In the Netherlands, cytological screening has been available since the mid-1970s to women (35-55 years of age)in the Nijmegen, Utrecht and Rotterdam area, consisting of a combination of opportunistic screening and local and regional invitational programmes.[68] Over time, a nationwide screening programme has been introduced, which was restructured in 1996. From then on till the end of 2016, women between 30 and 60 years of age have been screened at 5-year intervals with a Pap-smear. In 2017, a novel cervical screening programme will be implemented, using primary HPV testing and cytology triage at baseline and after 6 months.[69] Women are screened in five rounds at the ages of 30, 35, 40, 50 and 60. Since a negative HPV result gives a better protection against cervical cancer than a negative cytology result[70], screening intervals are extended to 10 years from the age of 40. However, women who

Table 1.1 Terminology in use for classifying cervical cytology. [Adapted from: Bulk et al 2006 and Bulkmans et al 2004][71 72]

CISOE-A	C0	S1, E1-2, O1-2		S2-3, O3, E3	S4, E4-5	S5, O4-5	S6, O6, E6	S7, E7	S8-9, O7-8, E9
Pap	Pap0	Pap1		Pap2	Pap3a1	Pap3a2	Pap3b	Pap4	Pap5
Description	Inadequate	Normal		Borderline	Mild	Moderate	Severe	Carcinoma in situ	Carcinoma
BETHESDA 2001	Unsatisfactory for evidence	Negative	Atrophy	ASC-H		HSIL			SCC
				ASC-US	LSIL				
				AGC	AGC favour neoplasic			AIS	AC

CISOE-A: C composition, I inflammation, S squamous epithelium, O other abnormalities and endometrium, and E endo-cervical columnar epithelium; ASC-H, atypical squamous cells cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; AGC, atypical glandular cells; LSIL, low grade squamous intra-epithelial lesion; HSIL, high grade squamous intra-epithelial lesion; AIS, endocervical adenocarcinoma in situ; SCC, squamous cell carcinoma; AC, adenocarcinoma

test HPV positive, and repeat cytology negative at the age of 40, 50 or 60, are re-tested after 5 years since their CIN2+ risk is too high to delay re-screening.

3.2.1 Cytology-based screening

Cervical lesions can be detected by cytomorphological examination of cervical squamous and columnar epithelial cells from the transformation zone. Morphological changes are graded based on the subjective interpretation of the degree of abnormality. There are several commonly used classifications. In the Netherlands, the CISOE-A classification (in Dutch KOPAC-B) is used which can be easily converted into the (internationally used) Bethesda system (table 1). CISOE-A is a descriptive extension added to the Pap classification. Five items are scored with a nine-tiered score: C for composition, I for Inflammation, S for Squamous epithelium, O for Other abnormalities and endometrium, and E for Endocervical columnar epithelium.[71]

A Pap1 score indicates normal cytology. Women with Pap1 comprise 96.5% of all screened women and these are recalled at the subsequent screening round in 5 years.[69] Management for women with Pap3a2 (moderate dyskaryosis), Pap3b (severe dyskayosis), Pap4 (suspicious of Carcinoma in Situ) and Pap5 (suspicious of invasive disease) is also straightforward. These women are directly referred for colposcopy.[72, 73]

However, women with a Pap2/3a1 (borderline or mild dyskaryosis, BMD) are not directly referred since only 10-20% will have or will develop CIN2/3.[74, 75, 76] These women are advised to return for a follow-up smear after 6 and 18 months and are referred for colposcopy in case of a persistent abnormality (≥BMD) at one of these moments. Besides triaging women with BMD by cytology, triaging with HPV is a good alternative.[35, 74,76-81] Several studies and meta-analyses have demonstrated that immediate HPV testing is highly effective in identifying women with underlying clinically relevant cervical disease compared to repeat cytology testing.[76, 82, 83] Kocken et al[83] demonstrated that women with baseline BMD and normal cytology after 6 months had a 5-year CIN3+ risk of 5%, while this risk was <0.1% in women with BMD and a negative HPV test at baseline. These women are recommended to return to routine screening[73, 79, 84-86] since this risk is similar to the risk of women who test negative for cytology after 6 and 18 months.[35, 74, 76, 83] Women with BMD at baseline that are HPV positive have a CIN3+ risk of almost 40% and are in need for immediate referral.[72, 73, 83, 84, 87]

For this reason, as of 2006 an additional HPV test in the six month follow-up visit of women with BMD has been recommended by the Netherlands Society of Pathology (NVVP).[88]

Although the cytological screening programme has led to a decrease in cervical cancer in developed countries, there are several limitations. The first limitation is the low sensitivity for detecting a precancerous lesions (at best 70%).[89, 90, 91] This low sensitivity of a single cytological smear is compensated by repeated screening, in The Netherlands

every five years. Moreover, cytological reading is subjective, geographically dependent and labor-intensive test.[92] It also has limited reproducibility, which leads to variable accuracy.[93, 94] In addition, while the incidence of squamous cell carcinomas (SCCs) has decreased, the incidence of adenocarcinomas (AdCAs) and adenocarcinoma in situ (ACIS) has not shown a marked decrease since the implementation of organized screening. This may partly be explained by the higher location of these lesions in the endocervical canal, which leads to absence or less abnormal cells in the cervical smear associated with the failure of cytology to detect adenocarcinoma precursor lesions.[7] Finally, the participation rate to the screening programme is not optimal (~65% per screening round[95, 96]). Since more than 50% of cervical carcinomas are diagnosed in women without a history of screening, it is important to put efforts into attracting non-attendees.[97]

3.2.2 HPV-based screening

Several randomized controlled trials[70,98-103] have shown that primary HPV screening or combination screening (HPV testing and cytology) leads to 30% increase in CIN2+ detection and 20% increase in CIN3+ detection in women >30 years of age compared to cytology alone. In the next round, the trials showed a 50% reduction in CIN3+ in baseline HPV-negative women, compared to women with normal cytology at baseline. In a pooled analysis of four of these trials comprising 176,464 screened women in total, the results were established for end-point cancer.[104]

Altogether, trial data strongly support initiation of HPV-based screening for women aged 30 years and older and an extension of the screening interval after a negative HPV test. In the new Dutch screening programme, an extension from 5 to 10 years in HPV-negative women, older than 40 years of age, has been incorporated. Moreover, cost-effectiveness studies have shown that the new HPV based screening programme with extended screening interval is more cost-effective compared to the cytology based screening programme.[69]

Although HPV testing increases the protection against cervical (pre)cancer compared to cytology, it has a 3-4% lower specificity than cytology[105] because a substantial number of transient HPV infections is detected. Since only persistent infections are associated with an increased risk of CIN2+[16,106], a positive HPV test needs an additional reflex or follow-up triage test[107] in order to control the number of over-referrals and over-treatment.

Rijkaart et al. indicated that a triage strategy for HPV-positive women may be considered acceptable if the negative predictive value (NPV) for end-point CIN3+ is at least 98%. A NPV of 98% means that the CIN3+ risk after a negative triage test within 4 years of follow-up is less than 2% which is an accepted risk for CIN3+ after baseline BMD cytology and normal cytology at 6 and 18 months (1.2%).[108, 109] Furthermore, the positive predictive value (PPV) of a triage strategy should be at least 10% but preferably

20%.[109] The identification of the optimal triage strategy for HPV-positive women is still subject of study. In the new HPV-based screening programme of The Netherlands, HPV-positive women are followed by cytology at baseline and after 6 months and referred for colposcopy if cytology is abnormal. This strategy is supported by several studies.[78, 110-114] It results in a feasible balance between the safety of a triage strategy (i.e. low CIN3+ risk) and the screening burden for patients and clinicians[108, 109, 115-117] (i.e. modest colposcopy referral rate).

However, although cytology is currently considered as an appropriate triage tool for HPV-positive women, there is still room for improvement and need for more objective non-morphological biomarkers. Cytology is subjective, geographically dependent and the result of cytology is influenced by prior knowledge of the HPV status.[118]

At present, several biomarkers are being studied as possible candidates for the triage of HPV-positive women; alternative algorithms to triage HPV-positive women for colposcopy are based on more objective morphological markers or molecular non-morphological biomarkers. In this thesis, we will focus on the utility of a more objective morphological marker; p16/Ki-67 dual-stained cytology.

p16 ^{INK4a} is a cell cycle-dependent kinase inhibitor, which becomes overexpressed in response to viral oncogene E7 expression. It is widely accepted that p16 ^{INK4a} is a sensitive and specific marker of dysplastic cells of the cervix and is a useful biomarker to support the diagnosis of CIN2/3. Since virus-transformed cells expressing high levels of p16^{INK4a} have also kept their full proliferative capacity, co-expression of the proliferation marker Ki-67 and p16 ^{INK4a} in a single cells serves as an unequivocal sign of transforming HPV infections and is therefore indicative for the presence of CIN2+.[119]

3.3 Post-treatment surveillance

3.3.1 Follow-up of women treated for CIN2/3

In addition to the use of HPV testing as a primary screening tool and as a triage test in women with BMD cytology, various studies have demonstrated the value of HPV testing in post-treatment surveillance.[105, 120-123]

The rate of post-treatment disease varies between countries from approximately 4 to 17%.[120, 124-126] It can be divided in residual (incomplete excision of the original CIN lesion with a persistent HPV infection) and recurrent, incident disease, resulting from a newly acquired infection with a different HPV type[127] or a re-infection with the same type. Although most post-treatment disease is diagnosed within 2 years of treatment[128-130], the risk of developing post-treatment disease remains elevated for over 10 years.[120, 131-133]

Current follow-up protocols are mainly based on serial cytology. Until recently, in the Netherlands, women were monitored for at least 2 years following treatment by cervical cytology at 6, 12 and 24 months post-treatment and, if necessary, yearly thereafter

until three consecutive smears are read as normal.[134] After three consecutive negative smears women return to the cervical screening programme.

However, the Pap test has some inherent flaws, particularly its limited sensitivity, which makes repeat testing necessary.[135] Additionally, many women do not complete follow-up; only 45-60% of treated women complete the entire follow-up scheme in the first two years after treatment.[136-138]

The post-treatment surveillance by serial cytology can be simplified. Two large studies have shown that women who have normal cytology and are negative for HPV at their follow-up screening appointment are at very low risk of residual disease .[120,121] Kitchener et al. assessed of the role of HPV testing as test of cure in the National Health Service (NHS) Cervical Screening Programme for England.[121] The incidence of histological abnormalities over 2 years among 917 women who were cytology negative and HPV negative (n=717) at 6 months after treatment for CIN2/3 was 0.5% (3 CIN2, 1 CIN3, 1 VAIN and 1 adenocarcinoma) and sufficiently low to recommend return to routine recall. Kocken et al.[120] reported a 5-year post-treatment CIN2+ risk of 3% (and 0.7% for CIN3+) among women with 3 consecutive negative Pap smears at 6, 12 and 24 months. Adding HPV testing to cytology testing (co-testing) at 6 months after treatment resulted in a similar 5-year CIN2+ risk of 3.0%. This risk could be further reduced to 1% in case co-testing was performed at 6 and 24 months. This latter risk is similar to that of women with normal cytology in population-based screening. Based on these studies, the NHS and the Dutch Society of Obstetrics and Gynaecology (NVOG) have both incorporated HPV testing in post-treatment surveillance.[139-140]

3.3.2 HPV vaccination for preventing post-treatment CIN2/3

Recently introduced prophylactic HPV vaccines (Gardasil and Cervarix) have shown to be effective in preventing HPV infection[141], but without any therapeutic efficacy against pre-existing HPV infections or pre-malignant lesions. Interestingly, Joura et al.[142] showed in a post-hoc study that vaccination with the quadrivalent HPV vaccine within two years after treatment resulted in a significantly reduced risk of 64.9% for CIN2+ lesions. Although this retrospective analysis was not designed or powered to evaluate the effects of vaccination after treatment for CIN2/3, results are promising. Also Kang et al. reported in a retrospective cohort study that women who underwent LLETZ and received HPV vaccination after treatment were less likely to develop post-treatment disease (median follow-up of 3.5 years), even among women positive for HPV 16 or 18 at baseline (vaccinated: 2.5% versus non-vaccinated 8.5%).[143] A likely explanation is that the viral load is sufficiently low after a LLETZ procedure to neutralize HPV by the antibody titers evoked by the prophylactic vaccines.

3.3.3 Further developments in post-treatment protocol

The differentiation between recurrent post-treatment disease and residual post-treatment disease is difficult. HPV testing in post-treatment surveillance results in a higher sensitivity compared to cytology testing. However, since the number of HPV-positive test results is higher than the number of CIN2+ detected, there is room for optimizing the follow-up by combined cytology and HPV testing. An interesting new development is the surveillance of treated women with HPV in combination with an objective biomarker instead of cytology. In this thesis, we have evaluated the utility of methylation markers in the follow-up of women treated for CIN2/3.

As mentioned in paragraph 2.2, recent studies on DNA methylation in HPV-positive women have shown that methylation markers are particularly effective in detecting cervical cancer and persistent, high grade CIN with a high short term risk of cancer in need of treatment (advanced CIN3). In this thesis, the value of CADM1 and MAL methylation analysis in monitoring women treated for CIN2/3 will be further explored.

4. Aim and outline of this thesis

Cervical cancer is the fourth most common female malignancy worldwide. Cervical cancer screening aims to prevent cervical cancer by detecting early pre-cancerous changes in the cervix. It has been proven that screening significantly reduces incidence and mortality of cervical cancer. It has also been shown that the cervical cancer screening programme is associated with an improved rate of cure of invasive cervical cancer. The challenge is to choose the screening strategy with the most optimal balance between the safety of a triage strategy and the screening burden for patients and clinicians.

The aim of the first part of this thesis is to examine whether cytology based cervical cancer screening can be improved by using p16/Ki-67 dual staining in cytological smears. In addition we evaluated in HPV-based screening programmes whether P16/ki67 dual staining is effective in the triage of HPV-positive women.

In the second part we focus on the follow-up management of women treated for CIN2/3.

PART 1: Screening

In current cytology based cervical cancer screening programme approximately 2.5% of participating women have a borderline or mild dyskayotic (BMD) test result. Triage testing is necessary since only 10-20% of these women harbor CIN2+. Recently, cross-sectional studies have shown a significantly higher specificity for p16/Ki-67 dual-stained cytology compared with HPV triage, without a substantial loss of sensitivity for high-grade cervical lesions. The aim of chapter 2 was to evaluate the use of p16/Ki-67dual-stained cytology as a triage test at baseline for identifying women with BMD,

and underlying CIN3+ and CIN2+ within a population-based screening setting with long-term follow-up (VUSA-Screen study). Results were compared with baseline HPV triage (**Chapter 2**).

Although many countries are planning to implement primary HPV screening, the proper triage algorithm for HPV-positive women is still under debate. In **Chapter 3** we evaluated the 5-year cervical (pre) cancer risk of women with different combinations of HPV and cytology test results. Special attention was paid to HPV-positive women with negative triage-tests since their residual risk determines the safety of dismissal from further follow-up. In line with this, we studied the effect of p16/Ki67 dual stained cytology on HPV-positive women (**Chapter 4**).

PART 2: Post-treatment considerations

It is well known that women treated for high-grade cervical lesions face an increased risk of post-treatment disease. It is of utmost importance to determine the most optimal surveillance strategy.

For this reason, we first systematically reviewed all literature published between 2003 and 2011 to determine whether HPV testing should be incorporated in post-treatment testing (**Chapter 5**). In addition, based on these data and data from other studies from the Netherlands, we proposed a new post-treatment surveillance protocol (**Chapter 6**). Furthermore, we studied the role of molecular methylation markers in post-treatment women. Post-treatment CIN comprises a heterogeneous group comprising persistent lesions resulting from residual (i.e. incompletely treated) disease with persistence of the same HPV genotype and incident (i.e. early onset) lesions. Because of different CIN2+ risks, post-treatment surveillance should differentiate residual (with a persistent HPV infection) from incident disease. We questioned whether these two types of lesions can be distinguished by means of methylation markers and whether methylation markers offer alternative options for patient management (**Chapter 7**).

The general discussion (**Chapter 8**) provides an overview of the arguments to implement HPV testing in primary and secondary cervical cancer screening. At last, clinical consequences and future perspectives will be discussed.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer.* 2010;127(12):2893-2917.
2. Arbyn M, Castellsague X, de Sanjose S, et al. Worldwide burden of cervical cancer in 2008. *Ann. Oncol.* 2011;22(12):2675-2686.
3. IARC. WHO/ICO information centre on HPV and Cervical Cancer (HPV information Centre). "Human Papillomavirus and Related Cancers in World." [Report]. Cited June 20th, 2016; Available from: http://www.google.nl/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0ahUKEwiVueaI3cbOAhWFBBoKHbIxDqUQFggkMAE&url=http%3A%2F%2Fwww.hpvcentre.net%2Fstatistics%2Freports%2FXWX.pdf&usg=AFQjCNGkoHxRAiG7omF6B11qoSbSTSvcyA
4. Forman D, de Martel C, Lacey CJ, et al. Global burden of human papillomavirus and related diseases. *Vaccine.* 2012;30 Suppl 5:F12-23.
5. Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet.* 2004;364(9430):249-256.
6. Bulk S, Visser O, Rozendaal L, Verheijen RH, Meijer CJ. Incidence and survival rate of women with cervical cancer in the Greater Amsterdam area. *Br. J. Cancer.* 2003;89(5):834-839.
7. de Kok IM, van der Aa MA, van Ballegooijen M, et al. Trends in cervical cancer in the Netherlands until 2007: has the bottom been reached? *Int. J. Cancer.* 2011;128(9):2174-2181.
8. International Agency for Research on Cancer (IARC). "Most frequent cancers in women". [webpage]. Cited May 26th, 2016. Available from: http://eco.iarc.fr/EUCAN/Country.aspx?ISOCountryCd=528.
9. Richart RM. A theory of cervical carcinogenesis. *Obstet. Gynecol. Surv.* 1969;24(7 Pt 2):874-879.
10. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int. J. Gynecol. Pathol.* 1993;12(2):186-192.
11. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. *Am. J. Obstet. Gynecol.* 1969;105(3):386-393.
12. Vink MA, Bogaards JA, van Kemenade FJ, de Melker HE, Meijer CJ, Berkhof J. Clinical progression of high-grade cervical intraepithelial neoplasia: estimating the time to preclinical cervical cancer from doubly censored national registry data. *Am. J. Epidemiol.* 2013;178(7):1161-1169.
13. McCredie MR, Sharples KJ, Paul C, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol.* 2008;9(5):425-434.
14. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J. Pathol.* 2006;208(2):152-164.
15. Blaustein's Pathology of the Female Genital Tract (Kurman, Blaustein's Pathology of the Female Genital Tract) 6th ed. 2011.
16. 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(6):395-405.
17. Gius D, Funk MC, Chuang EY, et al. Profiling microdissected epithelium and stroma to model genomic signatures for cervical carcinogenesis accommodating for covariates. *Cancer Res.* 2007;67(15):7113-7123.
18. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat. Rev. Cancer.* 2002;2(5):342-350.
19. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet.* 2007;370(9590):890-907.
20. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999;189(1):12-19.
21. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine.* 2006;24 Suppl 3:S3/1-10.
22. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology*. 2004;324(1):17-27.
23. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. *Clin. Sci. (Lond.).* 2006;110(5):525-541.
24. Bosch FX, Broker TR, Forman D, et al. Comprehensive control of human papillomavirus infections and related diseases. *Vaccine.* 2013;31 Suppl 7:H1-31.
25. Biological agents. Volume 100 B. A review of human carcinogens. *IARC Monogr. Eval. Carcinog. Risks Hum.* 2012;100(Pt B):1-441.
26. Torrisi A, Del Mistro A, Onnis GL, Merlin F, Bertorelle R, Minucci D. Colposcopy, cytology and HPV-DNA testing in HIV-positive and HIV-negative women. *Eur. J. Gynaecol. Oncol.* 2000;21(2):168-172.
27. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. *Vaccine.* 2012;30 Suppl 5:F55-70.
28. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine.* 2006;24 Suppl 3:S3/26-34.
29. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J. Infect. Dis.* 2010;202(12):1789-1799.
30. Coupe VM, Berkhof J, Bulkmans NW, Snijders PJ, Meijer CJ. Age-dependent prevalence of 14 high-risk HPV types in the Netherlands: implications for prophylactic vaccination and screening. *Br. J. Cancer.* 2008;98(3):646-651.
31. Burchell AN, Winer RL, de Sanjose S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine.* 2006;24 Suppl 3:S3/52-61.
32. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J. Clin. Virol.* 2005;32 Suppl 1:S16-24.
33. Wentzensen N, Schiffman M, Dunn ST, et al. Grading the severity of cervical neoplasia based on combined histopathology, cytopathology, and HPV genotype distribution among 1,700 women referred to colposcopy in Oklahoma. *Int. J. Cancer.* 2009;124(4):964-969.

34. Stanley M. Immune responses to human papillomavirus. *Vaccine.* 2006;24 Suppl 1:S16-22.
35. Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *Lancet.* 1999; 354(9172):20-25.
36. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *J. Infect. Dis.* 2007;195(11):1582-1589.
37. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N. Engl. J. Med.* 1998; 338(7):423-428.
38. Doorbar J. Papillomavirus life cycle organization and biomarker selection. *Dis. Markers.* 2007; 23(4):297-313.
39. von Knebel Doeberitz M. New markers for cervical dysplasia to visualise the genomic chaos created by aberrant oncogenic papillomavirus infections. *Eur. J. Cancer.* 2002;38(17):2229-2242.
40. Herfs M, Yamamoto Y, Laury A, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. *Proc. Natl. Acad. Sci. U. S. A.* 2012;109(26):10516-10521.
41. Herfs M, Vargas SO, Yamamoto Y, et al. A novel blueprint for 'top down' differentiation defines the cervical squamocolumnar junction during development, reproductive life, and neoplasia. *J. Pathol.* 2013;229(3):460-468.
42. Vink MA, van de Kassteele J, Wallinga J, Teunis PF, Bogaards JA. Estimating seroprevalence of human papillomavirus type 16 using a mixture model with smoothed age-dependent mixing proportions. *Epidemiology.* 2015;26(1):8-16.
43. Hesselink AT, Heideman DA, Steenbergen RD, et al. 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(8):2459-2465.
44. Overmeer RM, Louwers JA, Meijer CJ, et al. Combined CADM1 and MAL promoter methylation analysis to detect (pre-)malignant cervical lesions in high-risk HPV-positive women. *Int. J. Cancer.* 2011;129(9):2218-2225.
45. Wentzensen N, Zuna RE, Sherman ME, et al. Accuracy of cervical specimens obtained for biomarker studies in women with CIN3. *Gynecol. Oncol.* 2009;115(3):493-496.
46. Overmeer RM, Henken FE, Snijders PJ, et al. Association between dense CADM1 promoter methylation and reduced protein expression in high-grade CIN and cervical SCC. *J. Pathol.* 2008;215(4): 388-397.
47. Overmeer RM, Henken FE, Bierkens M, et al. Repression of MAL tumour suppressor activity by promoter methylation during cervical carcinogenesis. *J. Pathol.* 2009;219(3):327-336.
48. De Strooper LM, Meijer CJ, 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. (Phila.).* 2014;7(12):1251-1257.
49. Bierkens M, Wilting SM, van Wieringen WN, et al. HPV type-related chromosomal profiles in high-grade cervical intraepithelial neoplasia. *BMC Cancer.* 2012;12:36.
50. Harro CD, Pang YY, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J. Natl. Cancer Inst.* 2001;93(4):284-292.
51. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet.* 2006;367(9518): 1247-1255.
52. Paavonen J, Naud P, Salmeron J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet.* 2009;374(9686): 301-314.
53. Malagon T, Drolet M, Boily MC, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect. Dis.* 2012;12(10):781-789.
54. Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol.* 2005;6(5):271-278.
55. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N. Engl. J. Med.* 2007;356(19):1928-1943.
56. Primary End-points for Prophylactic HPV Vaccine Trials. Editors: IARC HPV Working Group. Source: Lyon (FR): International Agency for Research on Cancer; 2014.
57. Romanowski B, de Borba PC, Naud PS, et al. Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years. *Lancet.* 2009;374(9706): 1975-1985.
58. Wheeler CM, Skinner SR, Del Rosario-Raymundo MR, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. *Lancet Infect. Dis.* 2016.
59. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol.* 2012;13(1):89-99.
60. Schiller JT, Castellsague X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine.* 2012;30 Suppl 5:F123-138.
61. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* 2010;11(11): 1048-1056.
62. Mesher D, Soldan K, Howell-Jones R, et al. Reduction in HPV 16/18 prevalence in sexually active young women following the introduction of HPV

immunisation in England. *Vaccine.* 2013;32(1): 26-32.
63. RIVM. " Groeiend vertrouwen in HPV-inenting". [webpage]. Cited June 23th, 2016. Available from. http://www.rivm.nl/bibliotheek/rapporten/150202003.pdf.
64. de Melker H, Kenter G, van Rossum T, Conyn-van Spaendonck M. [Developments in HPV vaccination]. *Ned. Tijdschr. Geneeskd.* 2012;156(47): A5410.
65. Public Health England. HPV vaccination coverage report: 1 September 2014 to 31 August 2015. [report] Cited May 26th 2016. Available from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/487514/HPV_2014_15_ReportFinal181215_v1.1.pdf.
66. National HPV Vaccination Program Register "HPV Vaccination Coverage 2015". [webpage]Cited May 26th, 2016. Available from http://www.hpvregister.org.au/research/coverage-data/HPV-Vaccination-Coverage-2015.
67. Bosch FX, Robles C, Diaz M, et al. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. *Nat. Rev. Clin. Oncol.* 2016;13(2): 119-132.
68. van der Graaf Y, Peer PG, Zielhuis GA, Vooijs PG. Cervical cancer survival in Nijmegen region, The Netherlands, 1970-1985. *Gynecol. Oncol.* 1988; 30(1):51-56.
69. RIVM. Uitvoeringstoets 2013.Health Councel of the Netherlands. Population screening for cervical cancer. The Hague: Health Councel of the Netherlands, 2011; publication no.2011/07.2014.
70. Rijkaart DC, Berkhof J, Rozendaal L, et al. 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(1):78-88.
71. Bulk S, Van Kemenade FJ, Rozendaal L, Meijer CJ. The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardisation since 1996. *J. Clin. Pathol.* 2004;57(4): 388-393.
72. Bulkmans NW, Rozendaal L, Snijders PJ, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int. J. Cancer.* 2004;110(1):94-101.
73. Castle PE, Sideri M, Jeronimo J, Solomon D, Schiffman M. Risk assessment to guide the prevention of cervical cancer. *Am. J. Obstet. Gynecol.* 2007; 197(4):356.e351-356.
74. Zielinski DG, Snijders PJ, Rozendaal L, et al. High-risk HPV testing in women with borderline and mild dyskaryosis: long-term follow-up data and clinical relevance. *J. Pathol.* 2001;195(3):300-306.
75. Clavel C, Masure M, Bory JP, et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Br. J. Cancer.* 2001;84(12):1616-1623.
76. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine.* 2006;24 Suppl 3:S3/78-89.
77. Bais AG, Rebolj M, Snijders PJ, et al. Triage using HPV-testing in persistent borderline and mildly dyskaryotic smears: proposal for new guidelines. *Int. J. Cancer.* 2005;116(1):122-129.
78. Cuzick J, Szarewski A, Cubie H, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet.* 2003;362(9399):1871-1876.
79. Rijkaart DC, Berkhof J, van Kemenade FJ, et al. Comparison of HPV and cytology triage algorithms for women with borderline or mild dyskaryosis in population-based cervical screening (VUSA-screen study). *Int. J. Cancer.* 2010;126(9):2175-2181.
80. Bulk S, Bulkmans NW, Berkhof J, et al. Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months. *Int. J. Cancer.* 2007;121(2): 361-367.
81. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J. Natl. Cancer Inst.* 2003;95(1):46-52.
82. Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J. Natl. Cancer Inst.* 2004; 96(4):280-293.
83. Kocken M, Berkhof J, van Kemenade FJ, et al. Long-term CIN3+ risk in women with abnormal cytology; role of hrHPV testing. *Br. J. Cancer.* 2012; 106(5):817-825.
84. Safaeian M, Solomon D, Wacholder S, Schiffman M, Castle P. Risk of precancer and follow-up management strategies for women with human papillomavirus-negative atypical squamous cells of undetermined significance. *Obstet. Gynecol.* 2007; 109(6):1325-1331.
85. Levi AW, Harigopal M, Hui P, Schofield K, Chhieng DC. Use of high-risk human papillomavirus testing in patients with low-grade squamous intraepithelial lesions. *Cancer Cytopathol.* 2011;119(4):228-234.
86. Katki HA, Kinney WK, Fetterman B, et al. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. *Lancet Oncol.* 2011;12(7):663-672.
87. Manos MM, Kinney WK, Hurley LB, et al. Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. *JAMA.* 1999;281(17):1605-1610.
88. van Kemenade FJ, Wiersma T, Helmerhorst TJ. [New version of the pathology practice guideline for cervical cytology: sharpened criteria for adequacy; expanded use of new techniques]. *Ned. Tijdschr. Geneeskd.* 2007;151(23):1283-1286.
89. Bulkmans NW, Rozendaal L, Voorhorst FJ, Snijders PJ, Meijer CJ. Long-term protective effect of high-risk human papillomavirus testing in population-based cervical screening. *Br. J. Cancer.* 2005; 92(9):1800-1802.
90. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int. J. Cancer.* 2006;119(5):1095-1101.
91. Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. *Vaccine.* 2006;24 Suppl 3:S3/63-70.
92. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, et al. Cost-effectiveness of cervical-cancer screening in five developing countries. *N. Engl. J. Med.* 2005; 353(20):2158-2168.

93. Nanda K, McCrory DC, Myers ER, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. *Ann. Intern. Med.* 2000;132(10):810-819.
94. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. *Am. J. Epidemiol.* 1995;141(7): 680-689.
95. van Lier et al. RIVM. "Vaccinatiegraad Rijksvaccinatieprogramma Nederland Verslagjaar 2015". [Report]. Cited May 26th, 2016. Available from: http://www.rivm.nl/dsresource?objectid=rivmp:311706&type=org&disposition=inline.
96. Bos AB, Rebolj M, Habbema JD, van Ballegooijen M. Nonattendance is still the main limitation for the effectiveness of screening for cervical cancer in the Netherlands. *Int. J. Cancer.* 2006;119(10):2372-2375.
97. Gok M, Heideman DA, van Kemenade FJ, et al. Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: Characteristics of the responders. *Eur. J. Cancer.* 2012;48(12):1799-1808.
98. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol.* 2006;7(7):547-555.
99. Naucler P, Ryd W, Tornberg S, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N. Engl. J. Med.* 2007;357(16):1589-1597.
100. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. *J. Natl. Cancer Inst.* 2008; 100(7):492-501.
101. Leinonen M, Nieminen P, Kotaniemi-Talonen L, et al. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. *J. Natl. Cancer Inst.* 2009; 101(23):1612-1623.
102. Kitchener HC, Almonte M, Thomson C, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *Lancet Oncol.* 2009; 10(7):672-682.
103. Dijkstra MG, Snijders PJ, Arbyn M, Rijkaart DC, Berkhof J, Meijer CJ. Cervical cancer screening: on the way to a shift from cytology to full molecular screening. *Ann. Oncol.* 2014;25(5):927-935.
104. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet.* 2014; 383(9916):524-532.
105. Arbyn M, Ronco G, Anttila A, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. *Vaccine.* 2012; 30 Suppl 5:F88-99.
106. Cuzick J, Bergeron C, von Knebel Doeberitz M, et al. New technologies and procedures for cervical cancer screening. *Vaccine.* 2012;30 Suppl 5: F107-116.
107. Andrae B, Kemetli L, Sparen P, et al. Screening-preventable cervical cancer risks: evidence from a nationwide audit in Sweden. *J. Natl. Cancer Inst.* 2008;100(9):622-629.
108. Rijkaart DC, Berkhof J, van Kemenade FJ, et al. Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. *Int. J. Cancer.* 2012;130(3):602-610.
109. Dijkstra MG, van Niekerk D, Rijkaart DC, et al. Primary hrHPV DNA testing in cervical cancer screening: how to manage screen-positive women? A POBASCAM trial substudy. *Cancer Epidemiol. Biomarkers Prev.* 2014;23(1):55-63.
110. Rijkaart DC, Coupe VM, van Kemenade FJ, et al. Comparison of Hybrid capture 2 testing at different thresholds with cytology as primary cervical screening test. *Br. J. Cancer.* 2010;103(7): 939-946.
111. Ronco G, Segnan N, Giorgi-Rossi P, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J. Natl. Cancer Inst.* 2006;98(11):765-774.
112. Naucler P, Ryd W, Tornberg S, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J. Natl. Cancer Inst.* 2009;101(2):88-99.
113. Whitlock EP, Vesco KK, Eder M, Lin JS, Senger CA, Burda BU. Liquid-based cytology and human papillomavirus testing to screen for cervical cancer: a systematic review for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 2011;155(10):687-697, w214-685.
114. Ogilvie GS, Krajden M, van Niekerk DJ, et al. Primary cervical cancer screening with HPV testing compared with liquid-based cytology: results of round 1 of a randomised controlled trial -- the HPV FOCAL Study. *Br. J. Cancer.* 2012;107(12):1917-1924.
115. Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. *Br. J. Cancer.* 2003; 89(1):88-93.
116. Gok M, Heideman DA, van Kemenade FJ, et al. HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. *BMJ.* 2010;340:c1040.
117. Gok M, van Kemenade FJ, Heideman DA, et al. Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program. *Int. J. Cancer.* 2012;130(5):1128-1135.
118. Moriarty AT, Nayar R, Arnold T, et al. The Tahoe Study: bias in the interpretation of Papanicolaou test results when human papillomavirus status is known. *Arch. Pathol. Lab. Med.* 2014;138(9): 1182-1185.
119. McLaughlin-Drubin ME, Crum CP, Munger K. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. *Proc. Natl. Acad. Sci. U. S. A.* 2011;108(5):2130-2135.
120. Kocken M, Helmerhorst TJ, Berkhof J, et al. Risk of recurrent high-grade cervical intraepithelial neoplasia after successful treatment: a long-term multi-cohort study. *Lancet Oncol.* 2011;12(5): 441-450.
121. Kitchener HC, Walker PG, Nelson L, et al. HPV testing as an adjunct to cytology in the follow up of women treated for cervical intraepithelial neoplasia. *BJOG.* 2008;115(8):1001-1007.

122. Bulkmans NW, Berkhof J, Rozendaal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet.* 2007; 370(9601):1764-1772.
123. Nobbenhuis MA, Meijer CJ, van den Brule AJ, et al. Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. *Br. J. Cancer.* 2001;84(6):796-801.
124. Alvarez RD, Helm CW, Edwards RP, et al. Prospective randomized trial of LLETZ versus laser ablation in patients with cervical intraepithelial neoplasia. *Gynecol. Oncol.* 1994;52(2):175-179.
125. Bigrigg A, Haffenden DK, Sheehan AL, Codling BW, Read MD. Efficacy and safety of large-loop excision of the transformation zone. *Lancet.* 1994; 343(8888):32-34.
126. Nuovo J, Melnikow J, Willan AR, Chan BK. Treatment outcomes for squamous intraepithelial lesions. *Int. J. Gynaecol. Obstet.* 2000;68(1):25-33.
127. Bleeker MC, Meijer CJ, Berkhof J. Follow-up after treatment for cervical intraepithelial neoplasia. *BMJ.* 2012;345:e7186.
128. Melnikow J, McGahan C, Sawaya GF, Ehlen T, Coldman A. Cervical intraepithelial neoplasia outcomes after treatment: long-term follow-up from the British Columbia Cohort Study. *J. Natl. Cancer Inst.* 2009;101(10):721-728.
129. Paraskevaidis E, Arbyn M, Sotiriadis A, et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat. Rev.* 2004;30(2): 205-211.
130. Persad VL, Pierotic MA, Guijon FB. Management of cervical neoplasia: a 13-year experience with cryotherapy and laser. *J. Low. Genit. Tract Dis.* 2001; 5(4):199-203.
131. Kalliala I, Anttila A, Pukkala E, Nieminen P. Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study. *BMJ.* 2005;331(7526):1183-1185.
132. Soutter WP, de Barros Lopes A, Fletcher A, et al. Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia. *Lancet.* 1997; 349(9057):978-980.
133. Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int. J. Cancer.* 2006;118(8):2048-2055.
134. NVOG. National Guideline 'Cervical Intraepithelial Neoplasia.'2004. Cited January 20th, 2015; Available from: http://www.oncoline.nl/cervicale-intraepitheliale-neoplasie.
135. Bollen LJ, Tjong AHSP, van der Velden J, et al. Prediction of recurrent and residual cervical dysplasia by human papillomavirus detection among patients with abnormal cytology. *Gynecol. Oncol.* 1999;72(2):199-201.
136. Bais AG, Eijkemans MJ, Rebolj M, et al. Post-treatment CIN: randomised clinical trial using hrHPV testing for prediction of residual/recurrent disease. *Int. J. Cancer.* 2009;124(4):889-895.
137. Eijsink JJ, de Bock GH, Kuiper JL, et al. Routine follow-up intervals in patients with high-grade squamous intraepithelial lesions (HSIL) and free excision margins can safely be increased in the first two years after Large Loop Excision of the Transformation Zone (LLETZ). *Gynecol. Oncol.* 2009; 113(3):348-351.
138. Zielinski GD, Rozendaal L, Voorhorst FJ, et al. HPV testing can reduce the number of follow-up visits in women treated for cervical intraepithelial neoplasia grade 3. *Gynecol. Oncol.* 2003;91(1):67-73.
139. NVOG. National Guideline "CIN, AIS en VAIN". [webpage] 2016. Cited July 4th, 2016. Available from: http://www.oncoline.nl/cin-ais-en-vain.
140. NHS Cervical Screening Programme. "HPV Triage and Test of Cure Implementation Guide". [Report]. Cited May 26th, 2016. Available from: http://www.csp.nhs.uk/files/F000198_F000196_NHSCSP Good Practice Guide no 3 HPV implementation guidance.pdf.
141. Harper DM. Prevention of human papillomavirus infections and associated diseases by vaccination: a new hope for global public health. *Public health genomics.* 2009;12(5-6):319-330.
142. Joura EA, Garland SM, Paavonen J, et al. Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data. *BMJ.* 2012;344:e1401.
143. Kang WD, Choi HS, Kim SM. Is vaccination with quadrivalent HPV vaccine after loop electrosurgical excision procedure effective in preventing recurrence in patients with high-grade cervical intraepithelial neoplasia (CIN2-3)? *Gynecol. Oncol.* 2013; 130(2):264-268.