

still 10% have or acquire $\geq$ CIN 3. Moreover, cytology is not an option for self-sampled cervico-vaginal specimens that can be taken at home, since these are not representative for the cytological status of the cervix. Another mode is to use HPV16/18 genotyping. This however leaves women with non-
 5 HPV16/18 types who are, although to a lesser extent, also at risk of $\geq$ CIN 2/3 and $\geq$ adenocarcinoma in situ. Therefore, there is a need for supplementary or alternative triage tools to stratify hrHPV positive women into those with and without $\geq$ CIN 2/3 and $\geq$ adenocarcinoma in situ.

10 ~~SUMMARY OF THE INVENTION~~

The inventors now have found a method for detecting HPV-induced high-grade precancerous lesions and HPV-induced invasive cancers. Hence in one aspect, there is provided a method for detecting HPV-induced high-grade precancerous lesions and HPV-induced invasive cancers, wherein said
 15 method comprises the detection of hypermethylation in the PRDM14 and/or FAM19A4 gene in a cell whereby such hypermethylation indicates the presence of HPV-induced precursor lesions with invasive potential and HPV-induced invasive cancers. Preferably, in such a method said HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma
 20 is a high-grade premalignant cervical lesion or invasive cervical cancer, more preferably a high-risk HPV-induced invasive cancer.

In a preferred embodiment of the ~~invention-disclosure~~ the hypermethylation is detected in the CpG rich sequences as indicated in Figures 1 and 2.

25 The ~~disclosure invention~~ also relates to a method as defined above wherein said hypermethylation is an increased methylation of PRDM14 and/or FAM19A4 CpG rich promoter and /or genomic sequences in the test cell as compared to the comparable normal cell.

In a preferred embodiment of the ~~disclosure invention~~ the
 30 detection of (hyper)methylation is performed by using a methylation sensitive restriction endonuclease, chosen from the group consisting of BssHII, MspI, NotI and HpaII. In an alternative preferred embodiment of the ~~disclosure invention~~, the detection of (hyper)methylation is performed via a methylation specific PCR,

which is based on bisulfite modification of DNA, followed by specific PCR reactions that target CpG rich sequences. Preferably in such a method a nucleic acid probe or primer is used that binds to the nucleic acid as indicated in Figure 1 or 2, and more preferably said nucleic acid probe or
 5 primer has a detectable label.

In another embodiment of the disclosure ~~invention~~ the nucleic acid probe has a nucleotide sequence selected from the group consisting of:

- a) a polynucleotide sequence capable of hybridizing under stringent conditions to the sequence PRDM14 as set forth in Figure 1 or to
 10 the sequence FAM19A4 as set forth in Figure 2;
- b) a polynucleotide having at least 70% identity to the polynucleotide of a);
- c) a polynucleotide complementary to the polynucleotide of a); and
- 15 d) a polynucleotide comprising at least 15 bases of a nucleotide of a) or b).

Further preferred in the present method of the disclosure ~~invention~~ is the determination of the methylation of both the PRDM14 and
 20 the FAM19A4 gene. Also preferred is a combined detection of methylation of FAM19A4 and hsa-miR-124-2.

In another aspect, there is provided ~~Also part of the invention is~~ the use of PRDM14 and/or FAM19A4 as a molecular diagnostic marker for the detection of HPV-induced high-grade precancerous lesion or HPV-
 25 induced invasive carcinoma. Preferably, in such a use the methylation of said marker is predictive for the occurrence of said lesion or carcinoma.

In another aspect, there is provided ~~The invention also comprises a~~ kit of parts for use in a method of detecting HPV-induced high- grade precancerous lesion or HPV-induced invasive carcinoma, said kit comprising
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-means for the detection of PRDM14 and/or FAM19A4 methylation wherein said means comprise probes and/or primers specific for the PRDM14 nucleotide sequence of Figure 1 and/or the FAM19A4 nucleotide sequence of Figure 2 and

5 -means for the detection of HPV infection, wherein said means comprise probes and primers specific for HPV.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the *PRDM14* 5' regulatory region, coding sequence, 10 CpG rich part of first intronic sequence and transcribed 3' non-coding sequences. Transcription start is indicated in bold, coding sequence is in upper case. A promoter sequence of 1000 bp and intron 1, both containing CpG rich sequences, are in lower case.

Figure 2 shows the *FAM19A4* 5' regulatory region, coding 15 sequence, CpG rich part of first intronic sequence and transcribed 3' non-coding sequences. Transcription start is indicated in bold, coding sequence is in upper case. A promoter sequence of 1000 bp and intron 1, both containing CpG rich sequences, are in lower case.

Figure 3 shows that the mRNA expression of PRDM14 in SiHa 20 cervical cancer cells can be upregulated upon treatment with a DNA methylation inhibitor (DAC). Treatment of SiHa cervical cancer cells was performed with the methylation inhibitor 2'-deoxy-5'-azacytidine (5'-aza or DAC) at 0.2 μ M and 5 μ M concentration. Other treatments were PBS and ethanol (EtOH). All treatments were in duplicate. Controls are human 25 reference RNA and a -RT control (without reverse transcriptase added to the cDNA reaction mixture).

Figure 4. Effects of ectopic PRDM14 expression in SiHa, CaSki and 93VU147T cells. (A) The percentage of GFP⁺ cells declines in the PRDM14-transfected cells, compared to the empty vector cells, 24, 48 and 72 hours 30 post-transfection. (B) Flow cytometry analysis of cells double labelled with