

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

The inventors now have found a method for detecting HPV-induced high-grade precancerous lesions and HPV-induced invasive cancers. Hence 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. In one aspect, there is provided a method for improved detection of HPV-induced high-grade precancerous lesions and
20 HPV-induced invasive cancers, said method comprising detection of hypermethylation in the FAM19A4 gene and hypermethylation of hsa-miR-124-2 in a cell whereby such hypermethylation indicates the presence of HPV-induced precursor lesions with invasive potential and/or HPV-induced invasive cancers. Preferably, in such a method said HPV-induced high-grade
25 precancerous lesion or HPV-induced invasive carcinoma 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 disclosure the hypermethylation is detected in the CpG rich sequences as indicated in Figures 1 and 2.

The disclosure 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.

- 5 In a preferred embodiment of the disclosure the 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, 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 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 is the determination of the methylation of both the PRDM14 and the FAM19A4
20 gene. Also preferred is a combined detection of methylation of FAM19A4 and hsa-miR-124-2.

There is provided the use of PRDM14 and/or FAM19A4 as a molecular diagnostic marker for the detection of HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma. In another aspect,
25 there is provided the use of combination of FAM19A4 and hsa-miR124-2 as a molecular diagnostic marker for improving the in vitro detection of HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or carcinoma. Preferably, in such a use the
30 methylation of said marker is predictive for the occurrence of said lesion or carcinoma.

There is provided 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

-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.

Also disclosed is a kit of parts for use in a method of improved detection of HPV-induced high- grade precancerous lesion or HPV-induced invasive carcinoma, said kit comprising

10 -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

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the *PRDM14* 5' regulatory region, coding sequence, CpG rich part of first intronic sequence and transcribed 3' non-coding
20 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 sequence, CpG rich part of first intronic sequence and transcribed 3' non-
25 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 cervical cancer cells can be upregulated upon treatment with a DNA
30 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

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-
5 transfected cells, compared to the empty vector cells, 24, 48 and 72 hours post-transfection. (B) Flow cytometry analysis of cells double labelled with

Claims

1. An in vitro method for improved detection of HPV-induced high-grade precancerous lesions and HPV-induced invasive cancers, said method comprising
5 detection of hypermethylation in the FAM19A4 gene and hypermethylation of hsa-miR124-2 in a cell whereby such hypermethylation indicates the presence of HPV-induced precursor lesions with invasive potential and/or HPV-induced invasive cancers.
2. Method according to claim 1, wherein said HPV-induced invasive cancer
10 is a high-risk HPV-induced invasive cervical cancer.
3. Method according to any of the preceding claims, wherein FAM19A4 hypermethylation indicates the presence of an advanced CIN3 lesion.
4. Method according to any of the preceding claims wherein said HPV-induced high-grade precancerous lesion or HPV-induced invasive cancer is a high-
15 grade premalignant cervical lesion or invasive cervical cancer.
5. Method according to any one of the preceding claims, wherein said hypermethylation is detected in the CpG rich sequences as indicated in Figure 2.
6. A method of improved in vitro detection of HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma according to any of claims
20 1-6, wherein said hypermethylation is an increased methylation of FAM19A4 CpG rich promoter and /or genomic sequences and hypermethylation of hsa-miR124-2 in the test cell as compared to the comparable normal cell.
7. Method according to any of claims 1-6, wherein the hypermethylation is detected using a methylation sensitive restriction endonuclease, chosen from the
25 group consisting of BssHII, MspI, NotI and HpaII.

8. Method according to any of claims 1-6, wherein a methylation specific PCR is performed, which is based on bisulfite modification of DNA, followed by specific PCR reactions that target CpG rich sequences, preferably wherein a nucleic acid probe or primer is used that binds to the nucleic acid sequence FAM19A4 as set forth in Figure 2.

9. Method according to claim 8, wherein said nucleic acid probe or primer has a detectable label.

10. Method according to claim 8 or 9, wherein 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 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

d) a polynucleotide comprising at least 15 bases of a nucleotide of a) or b).

11. Use of the combination of FAM19A4 and hsa-miR124-2 as a molecular diagnostic marker for improving the in vitro detection of HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or carcinoma.

12. Use according to claim 10, wherein FAM19A4 and hsa-miR124-2 are used as a molecular diagnostic marker for the detection of an advanced CIN3 lesion.

13. Kit of parts for use in a method of improved detection of HPV-induced high- grade precancerous lesion or HPV-induced invasive carcinoma, said kit comprising

-means for the detection of FAM19A4 methylation and

5 hypermethylation of hsa-miR124-2 wherein said means comprise probes and/or primers specific for the the FAM19A4 nucleotide sequence of Figure 2 and means for the detection of hsamiR124-2 methylation

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