

Claims

1. A method for detecting HPV-induced high-grade precancerous lesions and HPV-induced invasive cancers and nonHPV-induced gynaecological and anogenital cancers, said method comprising detection of a methylation classifier consisting of LHX8, ASCL1 and ST6GALNAC5 sequences in a cell whereby such hypermethylation
5 indicates the presence of HPV-induced precursor lesions with invasive potential, HPV-induced invasive cancers and nonHPV-induced gynaecological and anogenital cancers.
2. Method according to claim 1, wherein said HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma is a high-grade premalignant cervical lesion or invasive cervical cancer.
- 10 3. Method according to claim 1 or 2, wherein said HPV-induced invasive cancer is a high-risk HPV-induced invasive cancer.
4. Method according to claim 1, wherein said nonHPV-induced gynaecological cancers is an endometrial or ovarian cancer.
5. Method for detecting vulva cancer and vulvar HPV-induced precursor
15 lesions with invasive potential, said method comprising detection of a methylation classifier consisting of ASCL1 and one or more of ZIC1, ZNF-582 and LHX8 sequences in a cell whereby such hypermethylation indicates the presence of vulva cancer and vulvar HPV-induced precursor lesions with invasive potential.
6. Method for detecting anal cancer and anal HPV-induced precursor lesions
20 with invasive potential, said method comprising detection of a methylation classifier consisting of ASCL1 and one or more of ZIC1 and ZNF-582 sequences in a cell whereby such hypermethylation indicates the presence of anal cancer and anal HPV-induced precursor lesions with invasive potential.
7. Method for the detection of ovarian cancer and ovarian precursor lesions,
25 said method comprising detection of a methylation classifier consisting of ASCL1, and one or more of LHX8 and ZIC1 sequences in a cell whereby such hypermethylation indicates the presence of ovarian cancer and ovarian precursor lesions.
8. Method according to any one of the preceding claims, wherein said hypermethylation is detected in the CpG rich sequences as indicated in Figures 1, 2, 3,
30 7 and 8.
9. A method of detecting HPV-induced high-grade precancerous lesion and HPV-induced invasive cancers and nonHPV-induced gynaecological or anogenital

- cancer according to any of claims 1-4, or detection of vulva cancer and vulvar HPV-induced precursor lesions with invasive potential according to claim 5 and/or of detecting anal cancer and anal HPV-induced precursor lesions with invasive potential according to claim 6, and/or a method for the detection of ovarian cancer and ovarian precursor lesions, wherein said hypermethylation is an increased methylation of the methylation classifier LHX8, ASCL1, ZNF-582, ZIC1 and/or ST6GALNAC5 CpG rich promoter and/or gene sequences in a test cell as compared to a comparable normal cell.
10. Method according to any of claims 1-9, wherein the reagent is a methylation sensitive restriction endonuclease, chosen from the group consisting of BssHII, MspI, NotI and HpaII.
11. Method according to any of claims 1-9 wherein the method involves nanotechnology, preferably lab on a chip technology.
12. Method according to claim any of claims 1-9, 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
13. Method according to claim 12, wherein the reagent is a nucleic acid probe or primer that binds to the nucleic acid as indicated in Figure 1 or 2.
14. Method according to claim 13, wherein said nucleic acid probe or primer has a detectable label.
15. Method according to claim 12, 13 or 14, 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 ASCL1 as set forth in Figure 1 or to the sequence LHX8 as set forth in Figure 2 or to the sequence ST6GALNAC5 as set forth in Figure 3; or to the sequence ZIC1 as set forth in Figure 8 or to the sequence ZNF-582 as set forth in Figure 7;
 - b) a polynucleotide having at least 70% identity to one of the polynucleotides of a);
 - c) a polynucleotide complementary to one of the polynucleotides of a); and
 - d) a polynucleotide comprising at least 15 bases of one of the polynucleotides of a) or b).
16. Method according to any of claims 1 -13, wherein the methylation of the methylation classifier comprising of the LHX8, ASCL1, ZNF-582, ZIC1 and/or ST6GALNAC5 gene is determined

17. Use of the methylation classifier LHX8, ASCL1, and ST6GALNAC5 as a molecular diagnostic marker for the detection of HPV-induced high-grade precancerous lesion or HPV-induced invasive carcinoma or nonHPV-induced gynaecological or anogenital cancer, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion, carcinoma or cancer.
18. Use of the methylation classifier ASCL1, and one of ZIC1, ZNF-582 and LHX8 as a molecular diagnostic marker for the detection of vulva cancer or vulvar HPV-induced high-grade precancerous lesion, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or cancer.
19. Use of the methylation classifier ASCL1, and one of ZIC1 and ZNF-582 as a molecular diagnostic marker for the detection of anal cancer or anal HPV-induced high-grade precancerous lesion, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or cancer.
20. Use of the methylation classifier ASCL1, and one of LHX8 and ZIC1 as a molecular diagnostic marker for the detection of ovarian cancer, preferably wherein the methylation of said marker is predictive for the occurrence of said cancer.
21. Kit of parts for use in a method of detecting HPV-induced high- grade precancerous lesion or HPV-induced invasive carcinoma or nonHPV-induced gynaecological or anogenital cancer, said kit comprising
 - means for the detection of LHX8, ASCL1 and ST6GALNAC5 methylation wherein said means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of Figure 1 and the LHX8 nucleotide sequence of Figure 2 and the ST6GALNAC5 nucleotide sequence of Figure 3;
 - means for the detection of ASCL1 and ST6GALNAC5 methylation wherein said means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of Figure 1 and the ST6GALNAC5 nucleotide sequence of Figure 3; or
 - means for the detection of ASCL1 and LHX8 methylation wherein said means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of Figure 1 and the LHX8 nucleotide sequence of Figure 2; or
 - means for the detection of ASCL1 and at least one of ZIC1, ZNF-582 and LHX8 wherein said means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of Figure 1 and either of the ZNF-582 nucleotide sequence of Figure 7, the ZIC1 nucleotide sequence of Figure 8 and/or the LHX8 nucleotide sequence of Figure 2;

preferably further comprising;

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