

presence of HPV-induced precursor lesions with invasive potential, HPV-induced
invasive cancers and/or nonHPV-induced gynaecological and anogenital cancers.
Preferably, in such a method said HPV-induced high-grade precancerous lesion or
HPV-induced invasive carcinoma is a high-grade premalignant cervical lesion or
5 invasive cervical cancer, more preferably a high-risk HPV-induced invasive cancer.
Preferably, said nonHPV-induced gynaecological cancer is a endometrial cancer.
Preferably said nonHPV-induced anogenital cancer is a vulvar or penile cancer.

In one aspect, the present disclosure refers to a method for detecting HPV-
induced high-grade precancerous lesions and HPV-induced invasive cancers and
10 nonHPV-induced gynaecological and anogenital cancers, said method comprising
detection of a methylation classifier consisting of LHX8, ASCL1 and ST6GALNAC5
sequences in a sample comprising anogenital, gynaecological and/or oropharyngeal
cells and/or a urine sample, whereby hypermethylation indicates the presence of
HPV-induced precursor lesions with invasive potential, HPV-induced invasive cancers
15 and nonHPV-induced gynaecological and anogenital cancers.

Also comprised in another example in the present disclosure is a method for
detecting vulva cancer and vulvar HPV-induced precursor 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
20 hypermethylation indicates the presence of vulva cancer and vulvar HPV-induced
precursor lesions with invasive potential. In one aspect, the present disclosure refers
to a method for detecting vulva cancer and vulvar HPV-induced precursor lesions with
invasive potential, said method comprising detection of a methylation classifier
consisting of ASCL1 and LHX8 and optionally one or more of ZIC1 and ZNF-582
25 sequences in a sample comprising vulvar cells and/or a urine sample, whereby
hypermethylation indicates the presence of vulva cancer and vulvar HPV-induced
precursor lesions with invasive potential.

Further comprised in another example in the disclosure is a method for
detecting anal cancer and anal HPV-induced precursor lesions with invasive potential,
30 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.

Also comprised in another example of the disclosure is a method for the detection of ovarian cancer and ovarian precursor lesions, 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. In one aspect, the present disclosure refers to a method for the detection of ovarian cancer and ovarian precursor lesions, said method comprising detection of a methylation classifier consisting of ASCL1 and LHX8 and optionally ZIC1 sequences in a sample comprising ovarian cells and/or a urine sample, whereby hypermethylation indicates the presence of ovarian cancer and ovarian precursor lesions.

In a preferred example of the disclosure the hypermethylation is detected in the sequences as indicated in Figures 1, 2, 3, 7 and 8. In a further preferred example said hypermethylation is an increased methylation of the methylation classifier LHX8, ASCL1, ZNF-582, ZIC1 and ST6GALNAC5 CpG rich promoter and/or gene sequences, such as regulatory sequences, in a test cell as compared to a comparable normal cell.

In another aspect, the present disclosure refers to a method of detecting HPV-induced high-grade precancerous lesion and HPV-induced invasive cancers and nonHPV-induced gynaecological or anogenital cancer as disclosed herein, or detection of vulva cancer and vulvar HPV-induced precursor lesions with invasive potential as disclosed herein and/or a method for the detection of ovarian cancer and ovarian precursor lesions as disclosed herein, 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.

In a preferred example 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 another preferred example of the disclosure the detection of (hyper)methylation is performed using nanotechnology.

In an alternative preferred example 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 the reagent is a nucleic acid probe or primer
 5 that binds to the nucleic acid as indicated in Figure 1, 2 or 3, and more preferably said nucleic acid probe or primer has a detectable label.

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

10 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;

15 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).

Further preferred in the present method of the disclosure the methylation of the gene sequence of LHX8, ASCL1, ZNF-582, ZIC1 and ST6GALNAC5 or the regulatory sequence of these genes is determined.

20 Also part of the disclosure is the use of LHX8, ASCL1, ZNF-582, ZIC1 and ST6GALNAC5 as a molecular diagnostic marker for the detection of HPV-induced invasive carcinoma, nonHPV-induced gynaecological or anogenital carcinoma, and their high-grade precancerous lesion. Further comprised in another example of the disclosure is the use of the methylation classifier LHX8, ASCL1, and ST6GALNAC5 as
 25 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. In one aspect, the present disclosure refers to the use of hypermethylation of the methylation classifier
 30 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 in a sample comprising anogenital, gynaecological and/or oropharyngeal cells and/or a urine sample, preferably wherein the methylation of said marker is predictive for the occurrence of
 35 said lesion, carcinoma or cancer. Also comprised in another example of the disclosure is the 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. In one aspect, the present
 40 disclosure refers to use of hypermethylation of the methylation classifier ASCL1 and LHX8, and optionally one of ZIC1 and ZNF-582 as a molecular diagnostic marker for the detection of vulva cancer or vulvar HPV-induced high-grade precancerous lesion in a sample comprising vulvar cells and/or a urine sample, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or cancer.
 45 Further comprised in another example of the disclosure is the 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. Next, comprised in another example of the disclosure is the use of the methylation classifier ASCL1, and one of LHX8 and ZIC1 as
 5 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. In one aspect, the present disclosure refers to use of hypermethylation of the methylation classifier ASCL1, and LHX8 and optionally ZIC1 as a molecular diagnostic marker for the detection of ovarian cancer in a sample comprising ovarian cells and/or a urine
 10 sample, preferably wherein the methylation of said marker is predictive for the occurrence of said cancer.

The disclosure also comprises a kit of parts for use in a method of detecting HPV-induced invasive carcinoma, nonHPV-induced gynaecological or anogenital carcinoma and their high- grade precancerous lesion said kit comprising

15 -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;

20 -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 ASLCL1 and LHX8 methylation wherein said means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of
 25 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
 30 sequence of Figure 2;

The kit preferably further comprises -means for the detection of HPV infection, wherein said means comprise probes and primers specific for HPV.

In another example, the present disclosure refers to a 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

5 - 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; or

 -means for the detection of ASCL1 and LHX8 methylation wherein said
10 means comprise probes and/or primers specific for the ASCL1 nucleotide sequence of Figure 1 and the LHX8 nucleotide sequence of Figure 2; and

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

BRIEF DESCRIPTION OF THE DRAWINGS

15 Figure 1 shows the ASLC1 promoter region and CpG rich sequence exon 1; the coding sequence is given in upper case.

 Figure 2 shows the LHX8 5' regulatory region and coding sequence (exon 1). The coding sequence is in upper case.

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 sample comprising anogenital,
5 gynaecological and/or oropharyngeal cells and/or a urine sample, whereby hypermethylation 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
10 precancerous lesions and HPV-induced invasive cancers are HPV-induced cervical, anal, rectal, penile, vulvar or vaginal invasive cancers and lesions.
3. Method according to claim 1, wherein said HPV-induced high-grade precancerous lesion or HPV-induced invasive cancers is a high-grade premalignant cervical lesion or invasive cervical cancer.
- 15 4. Method according to claim 1, 2 or 3, wherein said HPV-induced invasive cancer is a high-risk HPV-induced invasive cancer.
5. Method according to claim 1, wherein said nonHPV-induced gynaecological cancers is an endometrial or ovarian cancer.
6. Method for detecting vulva cancer and vulvar HPV-induced precursor
20 lesions with invasive potential, said method comprising detection of a methylation classifier consisting of ASCL1 and LHX8 and optionally one or more of ZIC1 and ZNF-582 sequences in a sample comprising vulvar cells and/or a urine sample, whereby hypermethylation indicates the presence of vulva cancer and vulvar HPV-induced precursor lesions with invasive potential.
- 25 7. Method for the detection of ovarian cancer and ovarian precursor lesions, said method comprising detection of a methylation classifier consisting of ASCL1 and LHX8 and optionally ZIC1 sequences in a sample comprising ovarian cells and/or a urine sample, whereby hypermethylation indicates the presence of ovarian cancer and ovarian precursor lesions.
- 30 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, 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 a method
5 for the detection of ovarian cancer and ovarian precursor lesions according to claim 6, 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, further comprising use of a
10 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 any of claims 1-9, wherein a methylation specific PCR
15 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, further comprising use of 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
20 has a detectable label.
15. Method according to claim 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
25 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 complementary to one of the polynucleotides of a).
16. Method according to any of claims 1 -15, wherein the methylation of the
30 methylation classifier comprising of the LHX8 and ASCL1, and optionally ZNF-582, ZIC1 and/or ST6GALNAC5 gene is determined.
17. Method according any of claims 1-16, wherein said sample comprises a cervical scrape, a vaginal scrape, a cervico-vaginal lavage and/or a cervico-vaginal swab or urine.

18. Use of hypermethylation 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 in a sample comprising anogenital,
5 gynaecological and/or oropharyngeal cells and/or a urine sample, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion, carcinoma or cancer.
19. Use of hypermethylation of the methylation classifier ASCL1 and LHX8, and optionally one of ZIC1 and ZNF-582 as a molecular diagnostic marker for the
10 detection of vulva cancer or vulvar HPV-induced high-grade precancerous lesion in a sample comprising vulvar cells and/or a urine sample, preferably wherein the methylation of said marker is predictive for the occurrence of said lesion or cancer.
20. Use of hypermethylation of the methylation classifier ASCL1, and LHX8 and optionally ZIC1 as a molecular diagnostic marker for the detection of ovarian
15 cancer in sample comprising ovarian cells and/or a urine sample, preferably wherein the methylation of said marker is predictive for the occurrence of said cancer.