

5 RNAs (sncRNAs) isolated from urinary exosomes in combination with the Sentinel™ PCa, Sentinel™ CS and Sentinel™ HG tests. The Sentinel™ PCa, Sentinel™ CS and Sentinel™ HG tests are based on algorithmic analyses and comparisons of snRNA sequences catalogued from a large target population having no evidence of prostate cancer (NEPC) or having prostate cancer (GG1-GG5) for the Sentinel™ PCa Test; having low grade cancer (GG1) versus intermediate and high grade cancer (GG2-GG5) for the Sentinel™ CS Test; and having low and favorable intermediate grade cancer (GG1+GG2) versus unfavorable intermediate and high grade (GG3-GG5) cancer for the Sentinel™ HG Test . The three Sentinel™ Tests, that can be performed on a single urine sample, are used to sequentially determine whether a patient has prostate cancer or not, and whether patients with prostate cancer have low- or favorable intermediate-grade disease that can be monitored on active surveillance protocols or high-grade disease that needs immediate treatment.

### SUMMARY

15 **[00012]** In one example, the disclosure provides a method of screening a subject for prostate cancer comprising: (i) obtaining a biological sample from the subject, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein the collection of sncRNAs comprises SEQ ID NOs: 1-280, (iii) correlating the aggregate expression profile of SEQ ID NOs: 1-280 from the subject by comparing the aggregate expression level of SEQ ID NOs: 1-280 in a training data set from a target population having no evidence of prostate cancer (NEPC) or having prostate cancer; and (iv) classifying the subject as NEPC or has prostate cancer based on the results in (iii). This procedure is embodied in the Sentinel™ PCa test.

20 **[00012a]** In one aspect, the present disclosure refers to a method for screening a subject at risk for prostate cancer, the method comprising: (i) detecting the aggregate expression profiles of a signature collection of small non-coding RNAs (sncRNAs) from a biological sample obtained from the subject, wherein detecting aggregate expression profiles of the collection of signature sncRNAs comprises hybridizing probes specific for each of the cDNA derived from sncRNAs obtained from the biological sample, wherein the hybridizing probe comprises SEQ ID NO: 1; (ii) correlating the aggregate expression profiles of SEQ ID NO: 1 from the subject by comparing the aggregate expression profiles of SEQ ID NO: 1 in a training data set from a target population having no prostate cancer or prostate cancer; and (iii) determining the likelihood of a subject at risk for prostate cancer based on the results obtained from (ii) above.

25 **[00012b]** In one aspect, the present disclosure refers to a method for diagnosing a subject for prostate cancer, the method comprising: (i) detecting the aggregate expression profiles of a signature collection of small non-coding RNAs (sncRNAs) from a biological sample obtained from the subject, wherein detecting aggregate expression profiles of the collection of signature sncRNAs comprises hybridizing probes specific for each of the cDNA derived from sncRNAs obtained from the biological sample, wherein the hybridizing probes comprise SEQ. ID. NO: 1; (ii) correlating the expression of SEQ ID NO: 1 from the subject by comparing the expression of SEQ ID NO: 1 in a training data set from a target population having no prostate cancer or having prostate cancer; and (iii) determining the likelihood of a subject at risk for prostate based on the results obtained from (ii) above.

30 **[00013]** In yet another example, the disclosure provides a method of determining whether a patient diagnosed as having cancer has low-grade (GG1) or intermediate or high grade disease (GG2-GG5) or not, comprising: (i) obtaining a biological sample from a subject, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein the collection of sncRNAs comprises SEQ ID NOs: 281-560, (iii) correlating the aggregate expression profile of SEQ ID NOs: 281-560 from the subject by comparing the aggregate expression profile of SEQ ID NOs: 281-560 in a training data set from a

target population known to have low risk, low grade (GG1) or intermediate and high grade, intermediate and high risk prostate cancer (GG2-GG5) and (iv) classifying the subject as GG1 or GG2-GG5 based on the results obtained from (iii). This procedure is embodied in the Sentinel™ CS test.

5     **[00014]** In yet another example, the disclosure provides a method of determining whether a patient diagnosed as having cancer has high-grade (GG3-GG5) or not (low or intermediate grade disease (GG1 + GG2), comprising: (i) obtaining a biological sample from a subject, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein the collection of sncRNAs comprises SEQ ID NOs: 561-840, (iii)  
10    correlating the aggregate expression profile of SEQ ID NOs: 561-840 from the subject by comparing the aggregate expression profile of SEQ ID NOs: 561-840 in a training data set from a target population known to have high grade, high risk prostate cancer (GG3-GG5) or low- or intermediate-risk cancer (GG1+GG2) and (iv) classifying the subject as GG3-GG5 or GG1+GG2 based on the results obtained from (iii). This procedure is embodied in the Sentinel™ HG test.

15    **[00015]** In yet another example, the disclosure provides a method for treating a prostate cancer comprising: (i) obtaining a biological sample from a subject, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein the collection of sncRNAs comprises SEQ ID NOs: 281-840, (iii) correlating the aggregate expression profile of SEQ ID NOs: 1-840 from the subject by comparing the aggregate  
20    expression profile of SEQ ID NOs: 281-840 in a training data set from a target populations having NEPC, GG1, GG2, GG3, GG4 or GG5 prostate cancer, (iv) classifying the subject as having low-intermediate-grade, prostate cancer (GG1-GG2) or high-grade prostate cancer (GG3-GG5) based on the results obtained from (iii), and (v) treating the subject classified as having high-risk prostate cancer by administering one or more chemotherapeutic agents, hormones, immunotherapeutic,  
25    radiation, cryotherapy, surgery or a combination thereof.

**[00016]** In a further example, the disclosure provides a method for determining the likelihood of survival, disease recurrence or response to treatment for a subject with prostate cancer comprising: (i) obtaining a biological sample from a patient, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein

the collection of sncRNAs comprises SEQ ID NOs: 1-840, (iii) comparing the aggregate expression profile of SEQ ID NOs: 1-840 after treatment with that prior to treatment, (iv) correlating the aggregate expression profile of SEQ ID Nos: 1-840 from the subject by comparing the aggregate expression profile of SEQ ID Nos: 1-840 in a training data set from a target  
5 populations having no evidence of prostate cancer (NEPC) or having prostate cancer and the aggregate expression profile of SEQ ID Nos: 1-840 in a training data set from a target populations having Grade Group 1, 2, 3 or 4-5; and (v) determining the likelihood of survival, disease recurrence or response to treatment in a subject treated for prostate cancer.

**[00017]** In one example, the disclosure provides a method for predicting future prostate cancer in  
10 a subject comprising: (i) obtaining a biological sample from a patient, (ii) detecting the aggregate expression profile of a signature collection of small non-coding RNAs (sncRNAs) from the biological sample wherein the collection of sncRNAs comprises SEQ ID NOs: 1-280, (iii) correlating the aggregate expression profile of SEQ ID Nos: 1-280 from the subject by comparing the aggregate expression profile of SEQ ID Nos: 1-280 in a training data set from a target  
15 population from a target populations having Grade Group 1, 2, 3 or 4-5; (iv) determining the likelihood of a subject at risk of having Grade Group 2 -5 prostate cancer based on the results obtained from (iii), and (iv) treating the subject predicted with a high risk of developing aggressive prostate cancer by administering one or more chemotherapeutic agents, hormones, immunotherapeutic, radiation, cryotherapy, surgery or a combination thereof.

**[00017a]** In one aspect, the present disclosure refers to a system for determining if a subject has  
20 cancer or no cancer and classifying the subject with cancer as having (i) indolent (low grade, GG1), (ii) intermediate or high grade (GG2-GG5), (iii) low/intermediate risk (GG1-G2) or (iv) aggressive (high grade, GG3-GG5) prostate cancer comprising:

(i) a first processor configured to:

- 25 (a) interrogate sncRNA sequences of SEQ ID NO: 1 on first Open Array platform for informative sequences;
- (b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 1 and interaction between the sequences included in a first Classification algorithm;
- 30 (c) compare the Sentinel Score in (b) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have prostate cancer or no prostate cancer; and
- (d) determine whether the subject has prostate cancer or no prostate cancer;
- 35 wherein  
the subject determined to have prostate cancer is classified as having indolent (low grade, GG1) or intermediate or high grade (GG2-GG5) prostate cancer;

- (ii) a second processor configured to:
  - (a) interrogate sncRNA sequences of SEQ ID NO: 281 on a second Open Array platform for informative sequences;
  - (b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 281 and interaction between the sequences included in a second Classification algorithm;
  - (c) compare the Sentinel Score in (f) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have indolent (low grade, GG1) or high grade (GG2-GG5) prostate cancer; and
  - (d) determine whether the subject has indolent (low grade, GG1) or high grade (GG2-GG5) prostate cancer;
    - wherein
    - the subject determined to have high grade (GG2-GG5) prostate cancer is further classified as having low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5) prostate cancer;
- (iii) a third processor configured to
  - (a) interrogate sncRNAs sequences of SEQ ID NO: 561 on a third Open Array platform for informative sequences;
  - (b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 561- and interaction between the sequences included in a third Classification algorithm;
  - (c) compare the Sentinel Score in (j) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5) prostate cancer; and
  - (d) determine whether the subject has low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5).

**[00018]** In another example, the disclosure provides a system for determining whether a patient has no cancer or has cancer and classifying the subject with cancer as (i) indolent (low grade, GG1), (ii) intermediate or high grade (GG2-GG5), (iii) low/intermediate risk (GG1-GG2) or (iv) aggressive (high grade, GG3-GG5) prostate cancer comprising at least three processors configured to (a) interrogate sncRNA sequences for informative sequences, (b) determine and compare a Sentinel Score to determine if the subject has prostate cancer or no prostate cancer and to classify the prostate cancer stage group.

### **CLAIMS:**

1. A method for screening a subject at risk for prostate cancer, the method comprising:
  - (i) detecting the aggregate expression profiles of a signature collection of small non-coding RNAs (sncRNAs) from a biological sample obtained from the subject,  
5 wherein detecting aggregate expression profiles of the collection of signature sncRNAs comprises hybridizing probes specific for each of the cDNA derived from sncRNAs obtained from the biological sample,  
wherein the hybridizing probe comprises SEQ ID NO: 1;
  - (ii) correlating the aggregate expression profiles of SEQ ID NO: 1 from the subject by  
10 comparing the aggregate expression profiles of SEQ ID NO: 1 in a training data set from a target population having no prostate cancer or prostate cancer; and
  - (iii) determining the likelihood of a subject at risk for prostate cancer based on the results obtained from (ii) above.
2. The method of claim 1, wherein the expression of sncRNAs in the subject determined to  
15 be at risk for prostate cancer is re-analysed and compared to the aggregate expression profiles of SEQ ID NO: 281 in a training data set from a target population with indolent (low grade, GG1) or intermediate or high grade, (GG2-GG5) prostate cancer to further classify the subject as having indolent (low grade, GG1) or intermediate or high grade, (GG2-GG5) prostate cancer.
3. The method of claim 2, wherein the expression of sncRNAs in the subject determined to  
20 have aggressive (intermediate or high grade, GG2-GG5) prostate cancer is re-analysed and compared to the aggregate expression profiles of SEQ ID NO: 561 in a training set from a target population with low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5) prostate cancer to further classify the subject as having low/intermediate grade (GG2-GG5) or aggressive (high grade, GG3-GG5) prostate cancer.
- 25 4. The method of claim 3, wherein the subject classified as having aggressive (intermediate or high grade, GG2-GG5) prostate cancer is to be treated with one or more of radical

prostatectomy, brachytherapy of the prostate, radiotherapy of the prostate, neoadjuvant hormone therapy and adjuvant hormone therapy.

5. The method of claim 1, wherein the biological sample is cell free urine.

6. The method of claim 1, wherein the biological sample is a urinary exosome.

5 7. The method of claim 1, wherein the biological sample is a sncRNA extracted from the urinary exosome.

8. The method of claim 1, wherein the sncRNA comprises miRNA, C/D box snoRNA, H/ACA box snoRNA, scaRNA, piRNA, and lncRNA.

9. The method of claim 1, wherein the sncRNA comprises a miRNA and a snoRNA.

10 10. The method of claim 1, wherein the method for screening a subject at risk for prostate cancer comprises performing an Sentinel™ PCa (PCa) Test, wherein the PCa test comprises the steps of:

(i) interrogating sncRNAs sequences of SEQ ID NO: 1 on an Open Array platform for informative sequences;

15 (ii) determining a Sentinel Score that examines the aggregate expression profiles of all the sequences and interaction between the sequences included in a first Classification algorithm;

(iii) comparing the Sentinel Score in (ii) for the informative sequences to a Sentinel Score obtained for training data sets from patients known to have prostate cancer or no prostate cancer; and  
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(iv) determining whether the subject has prostate cancer or no prostate cancer.

11. The method of claim 10, wherein the method for screening a subject having prostate cancer is classified as having indolent or low grade (GG-1) or intermediate or high grade (GG-2 – GG-5) prostate cancer comprises performing a Sentinel™ Clinical Significance (CS) test, wherein the CS  
25 test comprises the steps of:

- (i) interrogating sncRNAs of SEQ. ID. NO: 281 in a second round on an Open Array platform for a different set of informative sequences;
- (ii) determining a Sentinel Score that examines the aggregate expression profiles of all the sequences and interactions between the sequences included in a second Classification algorithm;
- (iii) comparing the Sentinel Score in (ii) for the informative sequences to a Sentinel Score obtained for training data sets from patients GG1 prostate cancer and GG2-GG-5 prostate cancer; and
- (iv) determining whether the subject has GG1 prostate cancer or GG2-GG-5 prostate cancer.

12. The method of claim 11, wherein the method for screening a subject having prostate cancer is classified as having indolent or low grade (GG1) or intermediate or high grade (GG-2 – GG-5) prostate cancer further comprises performing a Sentinel™ High Grade (HG –test, wherein the CS test comprises the steps of:

- (i) interrogating sncRNAs of SEQ. ID. NO: 561 in a third round on an Open Array platform for a different set of informative sequences;
- (ii) determining a Sentinel Score that examines the aggregate expression profile of all the sequences and interactions between the sequences included in a third Classification algorithm;
- (iii) comparing the Sentinel Score in (ii) for the informative sequences to a Sentinel Score obtained for training data sets from patients low/intermediate grade (GG1-GG2) prostate cancer and high grade (GG3-GG-5) prostate cancer; and
- (iv) determining whether the subject has low/intermediate grade (GG1-GG2) prostate cancer or high grade (GG3-GG-5) prostate cancer.

13. The method of claim 1, wherein the step of detecting comprises a micro-array method, a reverse transcription polymerase chain reaction, a polymerase chain reaction, a nucleic acid hybridization, or a combination thereof.

14. The method of claim 1, wherein the aggregate expression profile of a signature collection of sncRNAs in a subject at risk for prostate cancer is a combination of higher or lower aggregate expression profile of the signature collection of sncRNAs (SEQ ID NO: 1) than the aggregate expression profile of the signature collection of sncRNAs (SEQ ID NO: 1) in a subject with no prostate cancer.

15. The method of claim 1, wherein the aggregate expression profile of a signature collection of sncRNAs in a subject with intermediate/high grade (GG2-GG5) prostate cancer is a combination of higher or lower aggregate expression profile of the signature collection of sncRNAs I (SEQ ID NO: 281) than the aggregate expression profile of the signature collection of sncRNAs (SEQ ID NO: 281) in a subject with indolent or low grade (GG1) prostate cancer.

16. A method for diagnosing a subject for prostate cancer, the method comprising:

- (i) detecting the aggregate expression profiles of a signature collection of small non-coding RNAs (sncRNAs) from a biological sample obtained from the subject, wherein detecting aggregate expression profiles of the collection of signature sncRNAs comprises hybridizing probes specific for each of the cDNA derived from sncRNAs obtained from the biological sample, wherein the hybridizing probes comprise SEQ. ID. NO: 1;
- (ii) correlating the expression of SEQ ID NO: 1 from the subject by comparing the expression of SEQ ID NO: 1 in a training data set from a target population having no prostate cancer or having prostate cancer; and
- (iii) determining the likelihood of a subject at risk for prostate based on the results obtained from (ii) above.

17. The method of claim 16, wherein the subject determined to be at risk for prostate cancer is re-analysed and compared to the expression of SEQ ID NO: 281 in a training data set from a target population with indolent or aggressive prostate cancer to further classify the subject as having either indolent (low grade, GG1) or -intermediate or high grade (GG2-GG5) prostate cancer.
- 5 18. The method of claim 17, wherein the subject determined to be at risk for intermediate or high grade (GG2-GG5) prostate cancer is re-analysed and compared to the expression of SEQ ID NO: 561 in a training data set from a target population with low/intermediate grade (GG1-GG2) prostate cancer to further classify the subject as having either indolent (low/intermediate grade, GG1-GG2) or aggressive or high grade (GG3-GG5) prostate cancer.
- 10 19. The method of claims 16 and 17, where the subject classified as having intermediate or high grade or aggressive prostate cancer is to be treated with one or more of radical prostatectomy, brachytherapy of the prostate, radiotherapy of the prostate, neoadjuvant hormone therapy and adjuvant hormone therapy.
20. The method of claim 16, wherein the biological sample is cell-free urine.
- 15 21. The method of claim 16, wherein the biological sample is a urinary exosome.
22. The method of claim 16, wherein the biological sample is a sncRNA extracted from the urinary exosome.
23. The method of claim 16, the sncRNA comprises miRNA, C/D box snoRNA, H/ACA box snoRNA, scaRNA, piRNA, and lncRNA.
- 20 24. The method of claim 16, wherein the sncRNA is miRNA, and snoRNA.
25. The method of claim 16, further comprising analyzing whether the aggregate expression profile of the collection of signature sncRNAs is higher or lower than the expression level of the collection of signature sncRNAs in an indolent prostate biological sample.
26. The method of claim 16, wherein the method for diagnosing a subject at risk for prostate cancer comprises performing an Sentinel™ Prostate Cancer (PCa) Test.
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27. The method of claim 17, wherein the method for diagnosing a subject as having indolent/low grade (GG-1) or intermediate/high risk (GG2-GG5) prostate cancer comprises performing a Sentinel™ Clinical Significance (CS) test.

28. The method of claim 18, wherein the method for diagnosing a subject as having  
5 low/intermediate grade (GG-1-GG2) or high risk (GG3-GG5) prostate cancer comprises performing a Sentinel™ High Grade (HG) test.

29. The method of claim 16, wherein the step of diagnosing comprises a micro-array method, a reverse transcription polymerase chain reaction, a polymerase chain reaction, a nucleic acid hybridization, or a combination thereof.

10 30. A system for determining if a subject has cancer or no cancer and classifying the subject with cancer as having (i) indolent (low grade, GG1), (ii) intermediate or high grade (GG2-GG5), (iii) low/intermediate risk (GG1-G2) or (iv) aggressive (high grade, GG3-GG5) prostate cancer comprising:

(i) a first processor configured to:

15 (a) interrogate sncRNA sequences of SEQ ID NO: 1 on first Open Array platform for informative sequences;

(b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 1 and interaction between the sequences included in a first Classification algorithm;

20 (c) compare the Sentinel Score in (b) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have prostate cancer or no prostate cancer; and

(d) determine whether the subject has prostate cancer or no prostate cancer;

wherein

the subject determined to have prostate cancer is classified as having indolent (low grade, GG1) or intermediate or high grade (GG2-GG5) prostate cancer;

(ii) a second processor configured to:

5 (a) interrogate sncRNA sequences of SEQ ID NO: 281 on a second Open Array platform for informative sequences;

(b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 281 and interaction between the sequences included in a second Classification algorithm;

10 (c) compare the Sentinel Score in (f) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have indolent (low grade, GG1) or high grade (GG2-GG5) prostate cancer; and

(d) determine whether the subject has indolent (low grade, GG1) or high grade (GG2-GG5) prostate cancer;

15 wherein

the subject determined to have high grade (GG2-GG5) prostate cancer is further classified as having low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5) prostate cancer;

(iii) a third processor configured to

20 (a) interrogate sncRNAs sequences of SEQ ID NO: 561 on a third Open Array platform for informative sequences;

(b) determine a Sentinel Score that examine the aggregate expression profile of all the sequences of SEQ ID NO: 561- and interaction between the sequences included in a third Classification algorithm;

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- (c) compare the Sentinel Score in (j) for the informative sequences to a Sentinel Score obtained in training data sets from patients known to have low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5) prostate cancer; and
  - (d) determine whether the subject has low/intermediate risk (GG1-GG2) or aggressive (high grade, GG3-GG5).