

As shown in Pasechnikov et al (2014) and Kim et al, current screening methodologies usually use either invasive (biopsy or endoscopy) or imaging-based methods (which often exposes to the patients to radioactivity and/or is costly).

- Pasechnikov V, Chukov S, Fedorov E, Kikuste I, Leja M. *Gastric cancer: prevention, screening and early diagnosis*. *World J Gastroenterol*. 2014;20(38):13842–13862. doi:10.3748/wjg.v20.i38.13842
- Kim GH, Liang PS, Bang SJ, Hwang JH. *Screening and surveillance for gastric cancer in the United States: Is it needed?* *Gastrointest Endosc*. 2016 Jul;84(1):18-28. doi: 10.1016/j.gie.2016.02.028. Epub 2016 Mar 3.

The currently available non-invasive tests like pepsinogen, *H. pylori* serology, etc do not provide the requisite specificity and sensitivity.

We therefore provide for a non-invasive methodology that is an improvement over those currently available, and which would be useful for diagnosing gastric cancer in the population at large.

In one aspect, the present disclosure refers to a method of diagnosing a gastric cancer, in which the method comprises detecting, in a sample obtained from or of an individual, wherein the expression level of an miRNA selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p; in which an altered expression level of the miRNA as compared to the expression level of the miRNA in a sample from or of an individual known not to be suffering from gastric cancer indicates that the individual is suffering, or is likely to be suffering, from gastric cancer; and in which the miRNA is detected in an extracellular vesicle (EV) and:

(a) hsa-miR-484 comprises a polynucleotide sequence having miRBase Accession Number MIMAT0002174 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-484 activity and in which the altered expression level comprises an increased expression level of hsa-miR-484;

(b) hsa-miR-320d comprises a polynucleotide sequence having miRBase Accession Number MIMAT0006764 or a variant, homologue, derivative or fragment thereof such as a

sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320d activity and in which the altered expression level comprises an increased expression level of hsa-miR-320d

(c) (h) hsa-miR-423-5p comprises a polynucleotide sequence having miRBase

5 Accession Number MIMAT0004748 or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-423-5p activity

(d) hsa-miR-186-5p comprises a polynucleotide sequence having miRbase Accession Number MIMAT0000456 or a variant, homologue, derivative or fragment thereof such as a

10 sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-186-5p activity and in which the altered expression level comprises an increased expression level of hsa-miR-186-5p;

(e) hsa-miR-142-5p comprises a polynucleotide sequence having miRBase Accession Number MIMAT0000433 or a variant, homologue, derivative or fragment thereof such as a

15 sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-142-5p activity and in which the altered expression level comprises a decreased expression level of hsa-miR-142-5p;

(f) hsa-miR-320a comprises a polynucleotide sequence having miRBase Accession Number MI0000542 or a variant, homologue, derivative or fragment thereof such as a sequence

20 having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320a activity and in which the altered expression level comprises an increased expression level of hsa-miR-320a;

(g) hsa-miR-320b comprises a polynucleotide sequence having miRBase Accession Number MIMAT0005792 or a variant, homologue, derivative or fragment thereof such as a

25 sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320b activity and in which the altered expression level comprises an increased expression level of hsa-miR-320b; or

(h) hsa-miR-17-5p comprises a polynucleotide sequence having miRBase Accession Number MIMAT0000070 or a variant, homologue, derivative or fragment thereof such as a

30 sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-17-5p activity and in which the altered expression level comprises a decreased expression level of hsa-miR-17-5p.

In another aspect, the present disclosure refers to a combination of two or more nucleic acids specified herein or probes capable of binding specifically thereto, such as a combination of nucleic acids immobilised on a substrate, preferably in the form of a microarray or as a multiplex polymerase chain reaction (PCR) kit. In some examples, a positive diagnosis using this test may lead to further confirmatory tests using the more invasive gold-standard tests such as endoscopy or biopsy. This would also reduce the number of patients needing to undergo such invasive and costly tests in the first place.

Accordingly, disclosed herein is a method of diagnosing a gastric cancer. The method may include detecting the expression level of an miRNA in a sample from or of an individual.

The miRNA may be selected from the group consisting of: hsa-miR-484, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320d, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p. The method may be such that an altered expression level of the miRNA as compared to the expression level of the miRNA in a sample from or of an individual known not to be suffering from gastric cancer indicates that the individual is suffering, or is likely to be suffering, from gastric cancer.

The expression level of the miRNA may be detected in an extracellular vesicle (EV) in a sample from or of the individual.

vesicle (EV) in or of the sample, from or of an individual known not to be suffering from gastric cancer, is detected.

Gastric cancer may be indicated where an expression level of hsa-miR-17-5p of -0.37 or less, measured as $\log_2(\text{expression level of individual} / \text{expression level of control})$, in which
 5 control designates the expression level of that miRNA in a sample, such as in an extracellular vesicle (EV) in or of the sample, from or of an individual known not to be suffering from gastric cancer, is detected.

Gastric cancer may be indicated where an expression level of hsa-miR-423-5p of 0.54 or more, measured as $\log_2(\text{expression level of individual} / \text{expression level of control})$, in
 10 which control designates the expression level of that miRNA in a sample, such as in an extracellular vesicle (EV) in or of the sample, from or of an individual known not to be suffering from gastric cancer, is detected.

The sample may comprise a bodily fluid sample. The sample may comprise a nasopharyngeal secretion, urine, serum, lymph, saliva, anal and vaginal secretions,
 15 perspiration or semen of the individual.

The extracellular vesicle (EV) in or of the individual may be from a sample in or of the individual. The extracellular vesicle (EV) may be isolated from the sample using polymer based precipitation.

Expression of miRNA may be detected by any means. For example, the miRNA
 20 detection may comprise use of a polymerase chain reaction, such as real-time polymerase chain reaction (RT-PCR), multiplex polymerase chain reaction (multiplex PCR). The miRNA detection may be by means of Northern Blot, RNase protection, microarray hybridisation or RNA sequencing.

Disclosed herein is a combination of two or more nucleic acids specified above or
 25 probes capable of binding specifically thereto. This may comprise a combination of nucleic acids immobilised on a substrate. The combination may be in the form of a microarray or as a multiplex polymerase chain reaction (PCR) kit.

The combination may comprise probes capable of binding specifically thereto to each of hsa-miR-484 (miRBase Accession Number MIMAT0002174), hsa-miR-186-5p (miRbase
 30 Accession Number MIMAT0000456), hsa-miR-142-5p (miRBase Accession Number

MIMAT0000433), hsa-miR-320d (miRBase Accession Number MIMAT0006764), hsa-miR-320a (miRBase Accession Number MI0000542), hsa-miR-320b (miRBase Accession Number MIMAT0005792), hsa-miR-17-5p (miRBase Accession Number MIMAT0000070) and hsa-miR-423-5p (miRBase Accession Number MIMAT0004748).

5 In one aspect, the present disclosure refers to an miRNA selected from the group consisting of hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto for use in a method of detecting or determining the severity of gastric
10 cancer.

 In one aspect, the present disclosure refers to a pharmaceutical composition comprising two or more miRNAs selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b, hsa-miR-17-5p, and hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence
15 having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto together with a pharmaceutically acceptable excipient, carrier or diluent.

 In yet another aspect, the present disclosure refers to a diagnostic kit for gastric cancer, the kit comprising sequences capable of binding to two or more miRNAs selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p,
20 hsa-miR-320a, hsa-miR-320b, hsa-miR-17-5p and hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto together with instructions for use.

 Further described herein is a method of treatment of a gastric cancer in an individual, the method comprising performing a method as set out above and, where the individual is determined
25 to be suffering from, or likely to suffer from, gastric cancer, administering to the individual a treatment for gastric cancer.

 Described herein is a method of treating gastric cancer in an individual, the method comprising: (a) receiving results of an assay that measures

the expression level of an miRNA selected from the group consisting of: hsa-miR-484, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320d, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p, optionally together with hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto in a sample of or from an individual, in which the results show the expression level of the miRNA in the sample; (b) if the expression of the miRNA in the sample is modulated compared to a reference expression level of an miRNA, the reference expression level being the expression level of the miRNA in a sample of an individual known not to be suffering from gastric cancer, thereby providing or predicting an indication of gastric cancer in the individual, administering a treatment for gastric cancer. The expression level of the or each miRNA may be measured in an extracellular vesicle (EV) of the or each sample.

Also described herein is a method for treating gastric cancer in an individual, comprising: (a) obtaining the results of an analysis of the expression level of an miRNA selected from the group consisting of: hsa-miR-484, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320d, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p, optionally together with hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto in a sample of or from an individual; and (b) administering a treatment for gastric cancer to the individual if the expression level of the miRNA is modulated compared to a reference expression level, the reference expression level being the expression level of the miRNA in a sample of or from an individual known not to be suffering from gastric cancer. The expression level of the or each miRNA may be measured in an extracellular vesicle (EV) of the or each sample.

The practice of this invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ*

CLAIMS

1. A method of diagnosing a gastric cancer, in which the method comprises detecting, in a sample obtained from or of an individual, wherein the expression level of an miRNA selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p; in which an altered expression level of the miRNA as compared to the expression level of the miRNA in a sample from or of an individual known not to be suffering from gastric cancer indicates that the individual is suffering, or is likely to be suffering, from gastric cancer; and in which the miRNA is detected in an extracellular vesicle (EV) and:
 - 10 (a) hsa-miR-484 comprises a polynucleotide sequence having miRBase Accession Number MIMAT0002174 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-484 activity and in which the altered expression level comprises an increased expression level of hsa-miR-484;
 - 15 (b) hsa-miR-320d comprises a polynucleotide sequence having miRBase Accession Number MIMAT0006764 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320d activity and in which the altered expression level comprises an increased expression level of hsa-miR-320d
 - 20 (c) (h) hsa-miR-423-5p comprises a polynucleotide sequence having miRBase Accession Number MIMAT0004748 or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-423-5p activity
 - 25 (d) hsa-miR-186-5p comprises a polynucleotide sequence having miRbase Accession Number MIMAT0000456 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-186-5p activity and in which the altered expression level comprises an increased expression level of hsa-miR-186-5p;
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- (e) hsa-miR-142-5p comprises a polynucleotide sequence having miRBase Accession Number MIMAT0000433 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-142-5p activity and in which the altered expression level comprises a decreased expression level of hsa-miR-142-5p;
- (f) hsa-miR-320a comprises a polynucleotide sequence having miRBase Accession Number MI0000542 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320a activity and in which the altered expression level comprises an increased expression level of hsa-miR-320a;
- (g) hsa-miR-320b comprises a polynucleotide sequence having miRBase Accession Number MIMAT0005792 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-320b activity and in which the altered expression level comprises an increased expression level of hsa-miR-320b; or
- (h) hsa-miR-17-5p comprises a polynucleotide sequence having miRBase Accession Number MIMAT0000070 or a variant, homologue, derivative or fragment thereof such as a sequence having 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto and comprising hsa-miR-17-5p activity and in which the altered expression level comprises a decreased expression level of hsa-miR-17-5p.
2. The method according to Claim 1, in which the method comprises detecting the expression level in a sample, of two or more such miRNAs, for example, three miRNAs, four miRNAs, five miRNAs, six miRNAs or seven miRNAs in the group.
3. The method according to any preceding claim, in which:
- (a) an expression level of hsa-miR-484 of 0.39 or more;
 - (b) an expression level of hsa-miR-186-5p of 0.15 or more;
 - (c) an expression level of hsa-miR-142-5p of -0.35 or less;
 - (d) an expression level of hsa-miR-320d of 0.48 or more;

- (e) an expression level of hsa-miR-320a of 0.49 or more;
- (f) an expression level of hsa-miR-320b of 0.4 or more;
- (g) an expression level of hsa-miR-17-5p of -0.37 or less;
- (h) an expression level of hsa-miR-423-5p of 0.54 or more;

5 each measured as $\log_2(\text{expression level of individual} / \text{expression level of control})$, in which control designates the expression level of that miRNA in a sample, such as in an extracellular vesicle (EV) in or of the sample, from or of an individual known not to be suffering from gastric cancer, is indicative of gastric cancer.

4. The method according to any preceding claim, in which the sample comprises a bodily
10 fluid sample such as a nasopharyngeal secretion, urine, serum, lymph, saliva, anal and vaginal secretions, perspiration or semen.

5. The method according to any preceding claim, in which extracellular vesicles (EV) are isolated from the sample using polymer based precipitation.

6. The method according to any preceding claim, in which the detection comprises
15 polymerase chain reaction, such as real-time polymerase chain reaction (RT-PCR), multiplex polymerase chain reaction (multiplex PCR), Northern Blot, RNase protection, microarray hybridisation or RNA sequencing.

7. A combination of two or more nucleic acids specified in any preceding claim or probes capable of binding specifically thereto, such as a combination of nucleic acids immobilised on
20 a substrate, preferably in the form of a microarray or as a multiplex polymerase chain reaction (PCR) kit.

8. The combination according to Claim 7, comprising probes capable of binding specifically thereto to each of hsa-miR-484 (miRBase Accession Number MIMAT0002174), hsa-miR-186-5p (miRbase Accession Number MIMAT0000456), hsa-miR-142-5p (miRBase
25 Accession Number MIMAT0000433), hsa-miR-320d (miRBase Accession Number MIMAT0006764), hsa-miR-320a (miRBase Accession Number MI0000542), hsa-miR-320b (miRBase Accession Number MIMAT0005792), hsa-miR-17-5p (miRBase Accession Number MIMAT0000070) and hsa-miR-423-5p (miRBase Accession Number MIMAT0004748).

9. An miRNA selected from the group consisting of hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b and hsa-miR-17-5p or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto for use in a method of detecting or determining the severity of gastric cancer.
10. A pharmaceutical composition comprising two or more miRNAs selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b, hsa-miR-17-5p and hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto together with a pharmaceutically acceptable excipient, carrier or diluent.
11. A diagnostic kit for gastric cancer, the kit comprising sequences capable of binding to two or more miRNAs selected from the group consisting of: hsa-miR-484, hsa-miR-320d, hsa-miR-423-5p, hsa-miR-186-5p, hsa-miR-142-5p, hsa-miR-320a, hsa-miR-320b, hsa-miR-17-5p and hsa-miR-423-5p, or a variant, homologue, derivative or fragment thereof such as a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity thereto together with instructions for use.