

CIRCULATING MICRORNA PANEL FOR THE DETECTION OF NASOPHARYNGEAL CARCINOMA AND METHODS THEREOF

FIELD OF THE INVENTION

5

The present invention relates generally to the field of molecular biology. In particular, the present invention relates to biomarkers associated with nasopharyngeal carcinoma and methods of using the biomarkers to determine whether a subject suffers from, or is at risk of developing, nasopharyngeal carcinoma.

10

BACKGROUND OF THE DISCLOSURE

According to the global cancer statistics reported by the International Agency for Research on Cancer, over 84,000 new nasopharyngeal carcinoma (NPC) cases are diagnosed per year, of which more than 80% occur in Asia and 5% in Europe. NPC is highly prevalent in southern China and Southeast Asia, with an annual incidence of 15–50 cases per 100,000 population. Commonly, nasopharyngeal biopsy examination and radiological imaging are the most important tools to diagnose NPC, which provide the most definitive evidence to determine the clinical stage, prognosis, and treatment. However, these methods are invasive and painful diagnostic procedures, and are not suitable for early diagnosis or screening NPC in an endemic area. Thus, there is a need to provide for an alternative method of detecting NPC in a subject.

20

SUMMARY

25 In one aspect, the present disclosure relates to a method of determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma, wherein the method comprises detecting/determining the expression level of at least one miRNA from a biological sample obtained from the subject, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p; and wherein differential

35

expression levels of the miRNA, as compared to a control, is indicative of the subject having or is at risk of developing nasopharyngeal carcinoma.

In some embodiments, the method for determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma, comprising detecting/determining the expression level of at least one miRNA from a biological sample obtained from the subject, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p and ebv-miR-BART5-5p. In some embodiments, the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p and ebv-miR-BART9-5p.

In some embodiments, the method for determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma, comprising detecting/determining the expression level of at least one miRNA from a biological sample obtained from the subject, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p. In some embodiments, the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p and ebv-miR-BART2-3p.

In some embodiments, the method for determining whether a subject suffers from, or is at risk of developing NPC, comprising detecting/determining the expression level of at least one miRNA from a biological sample obtained from the subject, wherein the at least one miRNA is

selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p. In some embodiments, the method may comprise detecting/determining at least one additional miRNA selected from ebv-miR-BART15, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART17-3p, ebv-miR-BART2-3p, ebv-miR-BART5-5p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART18-5p, ebv-miR-BART8-5p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p.

Also provided are methods of treating NPC in a subject determined to be suffering from, or is at risk of developing NPC, comprising: (i) detecting/determining the expression levels of at least one or more miRNAs from a biological sample obtained from the subject, wherein the miRNAs are selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p in a biological sample obtained from said subject; and (ii) treating the subject determined to be suffering from, or is at risk of developing NPC. In some embodiments, the method further comprises detecting Epstein-Barr virus DNA (EBV DNA) in a biological sample obtained from the subject.

In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-

miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p is determined/detected/measured.

5 In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one or at least twenty-two miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p and ebv-miR-BART5-5p is determined/detected/measured.

15 In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two or at least twenty-three miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p is determined/detected/measured.

20 In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen or at least sixteen miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p is determined/detected/measured. In further embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at

least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve additional miRNAs selected from ebv-miR-BART15, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART17-3p, ebv-miR-BART2-3p, ebv-miR-BART5-5p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART18-5p, ebv-miR-BART8-5p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p is determined/detected/measured.

In some embodiments, the method comprises detecting expression levels of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven or at least twelve miRNAs.

In some embodiments, the methods above further comprise detecting Epstein-Barr virus DNA (EBV DNA) in a biological sample obtained from the subject. In further embodiment, detecting EBV DNA comprises detecting one or more nucleic acid relating to EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP1, LMP2A, LMP2B, BMRF1, BALF2, BALF5, EBER1 and EBER 2. In another further embodiment, detecting EBV DNA comprises detecting one or more nucleic acid relating to EBNA-1.

In some embodiments, the expression level of the miRNA measured in a biological sample obtained from a subject is higher in a subject with nasopharyngeal carcinoma or is at risk of developing NPC, compared to the control.

In some embodiments, the control is a cancer-free subject, a nasopharyngeal carcinoma-free subject, a subject not suffering from, or not at risk of developing nasopharyngeal carcinoma.

In some embodiments, the biological sample obtained from the subject is a non-cellular bodily fluid. In further embodiments, the non-cellular bodily fluid is plasma and/or serum.

In certain embodiments, when said subject is determined to suffer from nasopharyngeal carcinoma, the method further comprises treating the subject with one or more therapy selected from the group consisting of an administration of an anti-cancer compound, surgery, immunotherapy, and radiation therapy.

In some embodiments, the method further comprises performing one or more clinical procedures for determining and/or confirming whether a subject is suffering from or is at risk of developing nasopharyngeal carcinoma. In further embodiments, the clinical procedures may be selected from an endoscopy (e.g. nasopharyngoscopy), a biopsy (e.g. nasopharyngeal biopsy

examination), a fine needle aspiration or a diagnostic imaging test (e.g. radiological imaging procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI) scan or positron emission tomography (PET) scan).

- 5 In some embodiments, a subject determined to be suffering from, or is at risk of developing nasopharyngeal carcinoma, may be further tested using nasopharyngoscopy, nasopharyngeal biopsy examination or radiological imaging procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI) scan or positron emission tomography (PET) scan.
- 10 Also provided are kits for determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma. The kits can, for example, comprise: (a) an isolated set of probes capable of detecting a panel of miRNAs comprising at least one miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p; and (b)
- 15 instructions for use.
- 20

In some embodiments, the kits comprise an isolated set of probes capable of detecting at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-

25 four, twenty-five, twenty-six, twenty-seven or twenty-eight miRNAs.

In some embodiments, the kit comprises an isolated set of probes capable of detecting a panel of miRNAs comprises at least one miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART21-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART14-5p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART19-5p, ebv-miR-BART18-5p and ebv-miR-BART5-5p. In certain

30 ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-

35

BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p and ebv-miR-BART9-5p.

- 5 In some embodiments, the kit comprises an isolated set of probes capable of detecting a panel of miRNAs comprises at least one miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p. In certain embodiments, the at least one miRNA may be selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p and ebv-miR-BART2-3p.

- In some embodiments, the kit comprises an isolated set of probes capable of detecting a panel of miRNAs comprises at least one miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p. In certain embodiments, the kit comprises an isolated set of probes capable of detecting at least one additional miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p and ebv-miR-BART2-3p.

- 30 In some embodiments, the kit may further comprise an isolated set of probes capable of detecting Epstein-Barr virus (EBV DNA). In further embodiment, EBV DNA may comprise one or more nucleic acid relating to EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP1, LMP2A, LMP2B, BMRF1, BALF2, BALF5, EBER1 and EBER 2. In another further embodiment, EBV DNA may comprise one or more nucleic acid relating to EBNA-1.

In some embodiments, the probe is selected from the group consisting of an aptamer, an antibody, an affibody, a peptide and a nucleic acid.

5 In some embodiments, the isolated set of probes is capable of determining the level of expression of the at least one miRNA. The level of expression of the at least one miRNA can, for example, be determined by methods selected from sequencing, nucleic acid hybridization, microarray and nucleic acid amplification (such as a quantitative reverse transcription-polymerase chain reaction (qRT-PCR), reverse transcription-polymerase chain reaction (RT-PCR), quantitative polymerase chain reaction (qPCR), a locked nucleic acid PCR, a clustered
10 regularly interspaced short palindromic repeat (CRISPR)-based assay, or isothermal amplification assay).

DEFINITIONS

15 As used herein, the term “miRNA” refers to microRNA, small non-coding RNA molecules, which in some examples contain about 19 to 25 nucleotides, and are found in plants, animals and some viruses. miRNAs are known to have functions in RNA silencing and post-transcriptional regulation of gene expression. These highly conserved RNAs regulate the expression of genes by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs. For
20 example, each miRNA is thought to regulate multiple genes, and since hundreds of miRNA genes are predicted to be present in higher eukaryotes. miRNAs tend to be transcribed from several different loci in the genome. These genes encode for long RNAs with a hairpin structure that when processed by a series of RNase III enzymes (including Drosha and Dicer) form a miRNA duplex of usually about 19 to 25 nucleotides long with 2nt overhangs on the 3' end.

25 As used herein, “nasopharyngeal carcinoma”, “nasopharyngeal cancer” or “NPC” refers to a disease in which malignant (cancer) cells form in the tissues of the nasopharynx, which is located behind the nose and above the back of the throat.

30 As used herein, “biomarker” or “marker” can refer to a gene, protein, or miRNA whose level of expression or concentration in a sample is altered compared to that of a control. As used herein, a control refers to a level of expression or concentration of a biomarker that is indicative or correlated with a different outcome compared to the outcome of interest. For example, a biomarker can be a miRNA whose level of expression or concentration is altered (e.g., increased
35 or decreased) compared to that of a control in a sample of a subject with a condition (i.e., NPC). It is understood by anyone with the relevant skill in the art that comparing the level of

expression in the control does not necessarily entail obtaining a sample from a subject without NPC and testing said sample at the same time as the test subject. In some embodiments, said control could be a control sample incorporated in the kit or a threshold set to represent the range of expression of the biomarker where expression levels falling into this range would identify a subject as suffering from, or is at risk of suffering from, NPC.

As used herein, “biological sample” is intended to include any sampling of cells, tissues, or bodily fluids in which expression of a biomarker can be detected. Examples of such biological samples include, but are not limited to, biopsies, smears, blood, lymph, urine, saliva, or any other bodily secretion or derivative thereof. Blood can, for example, include whole blood, plasma, serum, or any derivative of blood. In some embodiments, the biological sample is a liquid biological sample. In some embodiments, the biological sample is a non-cellular bodily fluid. In some embodiments, the non-cellular bodily fluid may comprise serum and/or plasma. Samples can be obtained from a subject by a variety of techniques, which are known to those skilled in the art.

As used herein, the term “differential expression” refers to the measurement of a cellular component in comparison to a control or another sample, and thereby determining the difference in, for example concentration, presence or intensity of said cellular component. The result of such a comparison can be given in the absolute, that is a component is present in the samples and not in the control, or in the relative, that is the expression or concentration of component is increased or decreased compared to the control. The terms “increased” and “decreased” in this case can be interchanged with the terms “upregulated” and “downregulated” which are also used in the present disclosure.

As used herein, “probe” refers to any molecule or agent that is capable of selectively detecting an intended target biomolecule, for example, by binding directly or indirectly to the target biomolecule. The target molecule can be a biomarker, for example, a nucleotide transcript or a protein encoded by or corresponding to a biomarker. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations, in view of the present disclosure. Probes can be specifically designed to be labelled. Examples of molecules that can be utilized as probes include, but are not limited to, oligonucleotides, RNA, DNA (e.g., primers), proteins, peptides, antibodies, aptamers, affibodies, and organic molecules. In the case of a probe designed for the detection of a nucleic acid biomarker, such a probe may be directed to the target region, the complementary nucleic acid sequence on the reverse strand, or copies of the same generated via an amplification process.

As used herein, the term “(statistical) classification” refers to the problem of identifying to which of a set of categories (sub-populations) a new observation belongs, on the basis of a training set of data containing observations (or instances) whose category membership is known. An example is assigning a diagnosis to a given patient as described by observed characteristics of the patient (gender, blood pressure, presence or absence of certain symptoms, etc.). In the terminology of machine learning, classification is considered an instance of supervised learning, i.e., learning where a training set of correctly identified observations is available. The corresponding unsupervised procedure is known as clustering and involves grouping data into categories based on some measure of inherent similarity or distance. Often, the individual observations are analysed into a set of quantifiable properties, known variously as explanatory variables or features. These properties may variously be categorical (e.g., "A", "B", "AB" or "O", for blood type), ordinal (e.g., "large", "medium" or "small"), integer-valued (e.g., the number of occurrences of a part word in an email) or real-valued (e.g. a measurement of blood pressure). Other classifiers work by comparing observations to previous observations by means of a similarity or distance function. An algorithm that implements classification, especially in a concrete implementation, is known as a classifier. The term "classifier" sometimes also refers to the mathematical function, implemented by a classification algorithm, which maps input data to a category.

20

As used herein, the term “pre-trained” or “supervised (machine) learning” refers to a machine learning task of inferring a function from labelled training data. The training data can consist of a set of training examples. In supervised learning, each example is a pair consisting of an input object (typically a vector) and a desired output value (also called the supervisory signal). A supervised learning algorithm, that is the algorithm to be trained, analyses the training data and produces an inferred function, which can be used for mapping new examples. An optimal scenario will allow for the algorithm to correctly determine the class labels for unseen instances. This requires the learning algorithm to generalize from the training data to unseen situations in a "reasonable" way.

30

As used herein, the term “score” refers to an integer or number, that can be determined mathematically, for example by using computational models known in the art, which can include but are not limited to, SMV, as an example, and that is calculated using any one of a multitude of mathematical equations and/or algorithms known in the art for the purpose of statistical classification. Such a score is used to enumerate one outcome on a spectrum of possible outcomes. The relevance and statistical significance of such a score depends on the

35

size and the quality of the underlying data set used to establish the results spectrum. For example, a blind sample may be input into an algorithm, which in turn calculates a score based on the information provided by the analysis of the blind sample. This results in the generation of a score for said blind sample. Based on this score, a decision can be made, for example, how likely the patient, from which the blind sample was obtained, has cancer or not. The ends of the spectrum may be defined logically based on the data provided, or arbitrarily according to the requirement of the experimenter. In both cases the spectrum needs to be defined before a blind sample is tested. As a result, the score generated by such a blind sample, for example the number “45” may indicate that the corresponding patient has cancer, based on a spectrum defined as a scale from 1 to 50, with “1” being defined as being cancer-free and “50” being defined as having cancer.

DETAILED DESCRIPTION OF DISCLOSURE

MiRNAs are evolutionary conserved, single-stranded non-coding RNAs of 19 to 25 nucleotides which primarily function in mediating the degradation or translational repression of mRNA targets. Under normal physiological conditions, miRNAs are key components of feedback mechanisms for a wide range of biological pathways such as cell proliferation, differentiation, and apoptosis. Conversely, dysregulated miRNAs have been implicated in the hallmarks of cancer including supporting tumour growth by inhibiting growth suppression, sustaining proliferative signalling and resisting cell death, activating invasion and metastasis, and promoting angiogenesis. It is now known that miRNAs regulate oncogenesis through their tumour suppressor or oncogenic activities, with increasing evidence of aberrant miRNA expression in a variety of malignancies.

The diagnosis of NPC is commonly via nasopharyngoscopy, nasopharyngeal biopsy examination or radiological imaging procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI) scan or positron emission tomography (PET) scan. Cancers of the nasopharynx are notorious for being submucosal, located deep in the pharyngeal recess, or masked by adenoidal hyperplasia in the nasopharyngeal roof. Accordingly, endoscopic tumour detection is challenging at this site, not only for asymptomatic patients in the screening setting but also for symptomatic patients in the clinical setting. The use of radiological imaging procedures is both costly and exposes the subject to radiation and hence are not suitable for use in screening for NPC in the general population. Blood tests such as assays for EBV antibodies or EBV DNA do not provide sufficient accuracy for use in the clinical setting.

Up to 80% of NPC patients are diagnosed at advanced stages (clinical stages III and IV) and 10% with distant metastasis, which is associated with unfavourable outcome and poor prognosis. This is mainly due to NPC being commonly asymptomatic in its early stages, its high metastatic rate, and its inaccessibility for examination, whereby examination of the local primary tumour in the small, curved structure of the nasal cavity is difficult. It is also known in the art that early detection of cancer significantly improves the survival rate compared to subjects where the cancer is detected in the late stage. For NPC, the 10-year overall survival rate for stage I patients is as high as 98% and hence, highlighting the importance of early detection. As such, an effective liquid biopsy test able to detect NPC with high specificity and sensitivity would greatly assist early detection of the disease without the discomfort, costs or radiation exposure that limits the utility of present diagnostics methods for screening and early detection of NPC.

MiRNAs are deemed suitable as biomarkers because of altered miRNA expression profiles in cancer that reflect disease development, as well as the stability and the accessibility of circulating miRNAs in a myriad of body fluids including blood, urine and saliva. Minimally invasive methods, such as miRNA-based liquid biopsies, can potentially overcome these disadvantages and improve overall detection accuracy.

At the same time, the Epstein-Barr virus (EBV) is a herpes virus that is closely associated with the initiation and development of nasopharyngeal carcinoma (NPC), lymphoma and other malignant tumours. EBV encodes 44 mature microRNAs (miRNAs) that regulate viral and host cell gene expression and plays a variety of roles in biological functions and the development of cancer.

The present invention relates to methods of determining whether a subject suffers from, or is at risk of developing NPC, comprising the detection and/or measurement and/or determination of the expression level of one or more biomarkers present in a biological sample obtained from the subject, specifically EBV-specific miRNAs (listed in **Table 1**). In some examples, miRNAs that had been identified to be useful for this purpose include, but not limited to, ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-

BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p, ebv-miR-BHRF1-2-3p, and the like.

Table 1: Sequences of the miRNA biomarkers described herein (from miRbase).

SEQ ID NO.	miRNA	Accession number	Sequence (5' to 3')
1	ebv-miR-BART21-3p	MIMAT0010131	CUAGUUGUGCCCACUGGUGUUU
2	ebv-miR-BART14-5p	MIMAT0003425	UACCCUACGCUGCCGAUUUACA
3	ebv-miR-BART19-5p	MIMAT0004836	ACAUUCCCCGCAAACAUGACAUG
4	ebv-miR-BART1-5p	MIMAT0000999	UCUUAGUGGAAGUGACGUGCUGUG
5	ebv-miR-BART19-3p	MIMAT0003718	UUUUGUUUGCUUGGGAAUGCU
6	ebv-miR-BART7-3p	MIMAT0003416	CAUCAUAGUCCAGUGUCCAGGG
7	ebv-miR-BART8-3p	MIMAT0003418	GUCACAAUCUAUGGGGUCGUAGA
8	ebv-miR-BART13-3p	MIMAT0003424	UGUAACUUGCCAGGGACGGCUGA
9	ebv-miR-BART10-3p	MIMAT0003420	UACAUAAACCAUGGAGUUGGCUGU
10	ebv-miR-BART3-3p	MIMAT0003411	CGCACCACUAGUCACCAGGUGU
11	ebv-miR-BART11-5p	MIMAT0003421	UCAGACAGUUUGGUGCGCUAGUUG
12	ebv-miR-BART16	MIMAT0003714	UUAGAUAGAGUGGGUGUGUGCUCU
13	ebv-miR-BART17-5p	MIMAT0003715	UAAGAGGACGCAGGCAUACAAG
14	ebv-miR-BART11-3p	MIMAT0003422	ACGCACACCAGGCUGACUGCC

SEQ ID NO.	miRNA	Accession number	Sequence (5' to 3')
15	ebv-miR-BART9-5p	MIMAT0004816	UACUGGACCCUGAAUUGGAAAC
16	ebv-miR-BART2-5p	MIMAT0001000	UAUUUUCUGCAUUCGCCCCUUGC
17	ebv-miR-BART17-3p	MIMAT0003716	UGUAUGCCUGGUGUCCCCUAGU
18	ebv-miR-BART4-5p	MIMAT0003412	GACCUGAUGCUGCUGGUGUGCU
19	ebv-miR-BART21-5p	MIMAT0010130	UCACUAGUGAAGGCAACUAAAC
20	ebv-miR-BART9-3p	MIMAT0003419	UAACACUUCAUGGGUCCCGUAGU
21	ebv-miR-BART18-5p	MIMAT0003717	UCAAGUUCGCACUUCCUAUACA
22	ebv-miR-BART5-5p	MIMAT0003413	CAAGGUGAAUAUAGCUGCCCAUCG
23	ebv-miR-BART15	MIMAT0003713	GUCAGUGGUUUUGUUUCCUUGA
24	ebv-miR-BART8-5p	MIMAT0003417	UACGGUUUCCUAGAUUGUACAG
25	ebv-miR-BART3-5p	MIMAT0003410	ACCUAGUGUUAGUGUUGUGCU
26	ebv-miR-BART2-3p	MIMAT0004744	AAGGAGCGAUUUGGAGAAAAUAAA
27	ebv-miR-BART4-3p	MIMAT0009204	CACAUCACGUAGGCACCAGGUGU
28	ebv-miR-BHRF1-2-3p	MIMAT0000997	UAUCUUUUGCGGCAGAAAUUGA

In certain embodiments of the invention, the methods of determining whether a subject suffers from, or is at risk of developing NPC, comprises detecting and/or measuring and/or determining the expression level of one or more miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-

BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p and ebv-miR-BART5-5p.

5

In certain embodiments, the methods of determining whether a subject suffers from, or is at risk of developing NPC, comprises detecting and/or measuring and/or determining the expression level of one or more miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p.

15

In certain embodiments, the methods of determining whether a subject suffers from, or is at risk of developing NPC, comprises detecting and/or measuring and/or determining the expression level of one or more miRNAs selected from miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p.

20

Further to this, the present disclosure also discloses the utility of combining the measurement of EBV-encoded miRNA with the measurement of EBV DNA in a biological sample obtained from the test subject in improving the accuracy of the method to determine whether said subject is suffering from or is at risk of developing NPC. In some embodiments, measuring EBV DNA for this purpose comprises detecting one or more nucleic acid relating to EBV genes, antigens, antibodies and/or proteins, which include, but not limited to, EBV Nuclear Antigen (EBNA) 1 (EBNA-1), EBV Nuclear Antigen 2 (EBNA-2), EBV Nuclear Antigen 3A (EBNA-3A), EBV Nuclear Antigen 3B (EBNA-3B), EBV Nuclear Antigen 3C (EBNA-3C), EBV Nuclear Antigen Leader Protein (EBNA-LP), Latent Membrane Protein 1 (LMP1), Latent Membrane Protein 2A (LMP2A), Latent Membrane Protein 2B (LMP2B), BMRF1, BALF2, BALF5, EBV-encoded RNA 1 (EBER 1) and EBV-encoded RNA 2 (EBER 2). In some embodiments, detecting and/or measuring and/or determining the level of EBNA-1 comprises detecting one or more nucleic acid relating to EBNA-1. For the avoidance of doubt, EBV DNA (Epstein-Barr

25

30

35

virus DNA) may be measured directly or indirectly, such as measuring the level/concentration of EBV DNA, determining the Ct (threshold cycle) values (also may be known as Cq or quantification cycle), and/or detecting the presence or absence of EBV DNA. The EBV DNA may be determined by methods such as sequencing, nucleic acid hybridization, microarray and nucleic acid amplification (e.g. quantitative polymerase chain reaction). A person skilled in the art will understand that the detection of EBV DNA may be carried out before, after or concurrently with the detection of EBV miRNAs and such analysis may be carried out in the same sample, different aliquots of the same sample or samples obtained separately from the same subject.

10

In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p is determined. In some embodiments, measurement of the one or more miRNAs above may be combined with detection of EBV DNA in the same subject.

25

In some embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one or at least twenty-two miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p and ebv-miR-BART5-5p is

35

determined and/or detected and/or measured. In some embodiments, detection/measurement/determination of the one or more miRNAs above may be combined with detection of EBV DNA in the same subject.

- 5 In some embodiments, the level of expression of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two or at least twenty-three miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p is determined and/or detected and/or measured. In some embodiments, detection/measurement/determination of the one or more miRNAs above may be combined with detection of EBV DNA in the same subject.

- 20 In certain embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen or at least sixteen miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p is determined and/or detected and/or measured. In further embodiments, the level of expression of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve additional miRNAs selected from ebv-miR-BART15, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART17-3p, ebv-miR-BART2-3p, ebv-miR-BART5-5p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART18-5p, ebv-miR-BART8-5p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p is determined and/or detected and/or measured. In some embodiments, detection/measurement/determination of the one or more miRNAs above may be combined with detection of EBV DNA in the same subject.

35

In some embodiments, the method as described herein comprises determining and/or detecting and/or measuring the level of expression of at least one or at least two or at least three miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, and ebv-miR-BART19-5p, and at least one or more miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p.

In some embodiments, the level of expression of miRNA panels as described in Table 8, Table 9, Table 11, Table 12, Table 14, or Table 15, or as described herein, is determined and/or detected and/or measured.

In some embodiments, detection/measurement/determination of the one or more miRNAs above may be combined with measurement of EBV DNA in the same subject.

In some embodiments, the level of expression of miRNA panels as described in Table 16a, Table 16b, or Table 16c, or as described herein, is determined and/or detected and/or measured.

In some embodiments, the present disclosure refers to a method of determining whether a subject suffers from, or is at risk of developing NPC, the method comprising: (i) detecting the presence of miRNA in a biological sample obtained from the subject; (ii) detection and/or measuring and/or determining the expression level of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-

miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p in the biological sample; and (iii) wherein the at least one miRNA is differentially expressed as compared to a control, said subject is
5 determined to suffer from NPC or to be at risk of developing NPC.

In some embodiments, the level of expression of miRNA panels as described in Table 8, Table 9, Table 11, Table 12, Table 14, or Table 15, or as described herein, is determined and/or detected and/or measured.

10

In some embodiments, the methods provided herein further comprises detecting the EBV DNA in a biological sample obtained from the subject. In the event wherein EBV DNA is detected and the miRNAs disclosed above are also determined to be differentially expressed in the sample from the subject compared to a control, said subject is determined to suffer from NPC
15 or to be at risk of developing NPC.

In some embodiments, the level of expression of miRNA panels as described in Table 16a, Table 16b, or Table 16c, or as described herein, is determined and/or detected and/or measured.

20 In some embodiments, a subject determined to be suffering from, or is at risk of developing NPC, may be further tested using endoscopy, biopsy, fine needle aspiration or diagnostic imaging tests including but not limited to nasopharyngoscopy, nasopharyngeal biopsy examination or radiological imaging procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI) scan or positron emission tomography (PET) scan.

25

In some embodiments, the present disclosure provides for a method of treating a subject determined to be suffering from NPC using the methods disclosed herein. Such a subject would be referred to a medical practitioner and, where determined to be appropriate, treated with a suitable anti-cancer compound, surgery, immunotherapy or radiation therapy or combinations
30 of these. Where a subject is diagnosed with NPC, treatment options may include surgery, radiation therapy (including but not limited to external radiation therapy, intensity-modulated radiation therapy, proton therapy, stereotactic radiosurgery or brachytherapy), chemotherapy (including but not limited to combinations of gemcitabine and cisplatin; docetaxel with cisplatin and 5-fluorouracil (5-FU); cisplatin and 5-fluorouracil; cisplatin and capecitabine; or docetaxel
35 and cisplatin; nedaplatin; carboplatin, or oxaliplatin, adjuvant chemotherapy including cisplatin, 5-fluorouracil, and/or carboplatin, targeted therapy (such as monoclonal antibodies

targeting the epidermal growth factor receptor (EGFR) such as cetuximab) or immunotherapy (e.g. PD-1 and PDL-1 inhibitors such as pembrolizumab or nivolumab).

In some embodiments, the one or more miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p are detected at higher levels in a subject with NPC or is at risk of developing NPC compared to a control.

In some embodiments, the method as described herein comprises determining and/or detecting and/or measuring the level of expression of at least one or at least two or at least three miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, and ebv-miR-BART19-5p, and at least one or more miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p.

In some embodiments, the level of expression of miRNA panels as described in Table 8, Table 9, Table 11, Table 12, Table 14, or Table 15, or as described herein, is determined and/or detected and/or measured. In some embodiments, the level of expression of miRNA panels as described in Table 16a, Table 16b, or Table 16c, or as described herein, is determined and/or detected and/or measured.

In some embodiments, the control may include a healthy subject, a non-diseased subject, a cancer-free subject, a NPC-free subject, a subject not suffering from, or not at risk of developing nasopharyngeal carcinoma.

In some embodiments, the present disclosure refers to a kit for use in determining whether a subject suffers from, or is at risk of developing NPC in a biological sample obtained from the

subject, the kit comprises: (a) an isolated set of probes capable of detecting at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least
 5 twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, , ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-
 10 BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p; and (b) instructions for use.

15

In some embodiments, the kit as described herein comprises probes capable of detecting at least one or at least two or at least three miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, and ebv-miR-BART19-5p, and at least one or more miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p.
 20
 25

In some embodiments, the kit comprises: the isolated set of probes capable of detecting the level of expression of miRNA panels as described in Table 8, Table 9, Table 11, Table 12, Table 14, or Table 15, or as described herein.

30

In some embodiments, said kit may further comprise an isolated set of probes capable of detecting EBV DNA. In some embodiments, EBV DNA may further comprise one or more nucleic acid relating to EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP1, LMP2A, LMP2B, BMRF1, BALF2, BALF5, EBER1 and EBER 2. In some
 35 embodiments, EBV DNA may further comprise one or more nucleic acid relating to EBNA-1.

In some embodiments, the kit comprises: the isolated set of probes capable of detecting the level of expression of miRNA panels as described in Table 16a, Table 16b, or Table 16c, or as described herein.

5 In some embodiments, the present disclosure also provides for compositions/agents/reagents for use in the methods disclosed herein. In some embodiments, such compositions/agents/reagents may include, but are not limited to, probes, antibodies, affibodies, nucleic acids, and/or aptamers. In some embodiments, the compositions can detect (or detects) the level of expression (e.g., miRNA) of a panel of biomarkers from a biological sample.

10

Any of the compositions can be provided in the form of a kit or a reagent mixture. By way of an example, labelled probes can be provided in a kit for the detection of a panel of biomarkers. Kits can include all components necessary or sufficient for assays, which can include, but is not limited to, target enrichment reagents, detection reagents (e.g., probes and/or fluorescent dyes),
15 buffers, control reagents (e.g., positive and negative controls), amplification reagents, solid supports, labels, instruction manuals, calibrators, and reference materials, etc. In certain embodiments, the kit comprises a set of probes for the panel of biomarkers and a solid support to immobilize the set of probes. In certain embodiments, the kit comprises a set of probes for the panel of biomarkers, a solid support, and reagents for processing the sample to be tested
20 (e.g., reagents to isolate the protein or nucleic acids from the sample).

In some embodiments, provided herein are DNA-, RNA-, and protein-based detection methods that either directly or indirectly detect the biomarkers described herein. The present invention also provides compositions, reagents, and kits for such diagnostic purposes. The diagnostic
25 methods described herein may be qualitative or quantitative. Quantitative diagnostic methods may be used, for example, to compare a detected biomarker level to a cut-off or threshold level. Where applicable, qualitative or quantitative diagnostic methods can also include amplification of target, signal, or intermediary.

30 In some embodiments, biomarkers are detected at the nucleic acid (e.g., DNA or RNA) level. For example, the amount of biomarker RNA (e.g., miRNA) present in a sample is determined (e.g., to determine the level of biomarker expression). Biomarker nucleic acid (e.g., miRNA, amplified cDNA, etc.) can be detected/quantified using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to, sequencing, nucleic acid
35 hybridization (e.g., northern blot), microarray and nucleic acid amplification (e.g., quantitative reverse transcription polymerase chain reaction (qRT-PCR), reverse transcription polymerase

chain reaction (RT-PCR), quantitative polymerase chain reaction (qPCR), a locked nucleic acid (LNA) real-time PCR, a CRISPR-based assay, or isothermal amplification assay. An isothermal amplification assay can, for example, include, but is not limited to, a nicking endonuclease amplification reaction (NEAR) assay, a transcription mediated amplification (TMA) assay, a
5 loop-mediated isothermal amplification (LAMP) assay, a helicase-dependent amplification (HDA) assay, a clustered regularly interspaced short palindromic repeat (CRISPR) assay, or a strand displacement amplification (SDA) assay. In some embodiments, the method used to detect miRNA biomarkers may comprise the use of the assay methodologies disclosed in WO2011159256A1 and a kit for the detection of miRNAs may comprise the stem-loop
10 oligonucleotides designed based on the teachings of WO2011159256A1, the disclosure of which is incorporated herein by reference.

The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for
15 example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the
20 invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

25
The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material
30 is specifically recited herein.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges.
35 Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example,

description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

5

Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

10

Exemplary embodiments of the present invention are provided in the following examples. While the exemplary embodiments are described in sufficient detail to enable those skilled in the art to practice the invention, it should be understood that the present invention is not limited to these examples.

15

Sample calculation of a prediction score or risk score for the risk of NPC

It is known in the art that, biomarkers (e.g., miRNAs) can be combined to form a biomarker panel to calculate the disease risk score, for example using a linear model. An example would be to calculate such a risk score using logistic regression, a form of linear model. The prediction score may also be calculated using a classification algorithm selected from the group comprising support vector machine algorithm, logistic regression algorithm, multinomial logistic regression algorithm, Fisher's linear discriminant algorithm, quadratic classifier algorithm, perceptron algorithm, k-nearest neighbours algorithm, artificial neural network algorithm, random forests algorithm, decision tree algorithm, naive Bayes algorithm, adaptive Bayes network algorithm, and ensemble learning method combining multiple learning algorithms.

The challenge in the field pertains to identifying relevant biomarkers, such as circulatory miRNAs, that could be applied to identify an individual at risk of a disease such as NPC. Where relevant miRNAs could be identified via exhaustive and well-designed studies, it would be within the skill of someone aware of the state of the art to apply the measured level of the relevant miRNAs in such statistical models to generate a score for the prediction of the risk of a subject having NPC.

35

Examples of such mathematical methods used to perform the calculations disclosed herein, for example, the calculation of a prediction score, can be, but are not limited to, support vector machine algorithm, logistic regression algorithm, multinomial logistic regression algorithm, Fisher's linear discriminant algorithm, quadratic classifier algorithm, perceptron algorithm, k-nearest neighbours algorithm, artificial neural network algorithm, random forests algorithm, decision tree algorithm, naive Bayes algorithm, adaptive Bayes network algorithm, and ensemble learning method combining multiple learning algorithms. In one example, the calculation of the prediction score is calculated using linear models and support vector machine algorithms.

As an illustrative example, the control and subjects with NPC have different disease risk score values calculated. Fitted probability distributions of the disease risk scores for the control and subjects with NPC show a separation between the two groups can be found. Based on this prior probability and the fitted probability distributions previously determined, the probability (risk) of an unknown subject having NPC can be calculated based on their disease risk score values. With higher score, the subject has higher risk of having NPC. Furthermore, the disease risk score can, for example, tell the fold change of the probability (risk) of an unknown subject having NPC compared to, for example, the NPC rate in high-risk population.

A requirement for the success of such process is the availability of high-quality data. The quantitative data of all the detected miRNAs in a large number of well-defined clinical samples including appropriate controls from symptomatic non-NPC patients (disease controls), not only improves the accuracy, as well as precision, of the result, but also ensures the consistency of the identified biomarker panels for further clinical application using, for example, quantitative polymerase chain reaction (qPCR).

As an example, Formula 1 below exemplifies the use of a linear model for NPC risk prediction, where the disease risk score (unique for each subject) indicates the likelihood of a subject having NPC. This is calculated by the summing the weighted measurements for, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 miRNAs.

$$\text{Formula 1 - disease risk score} = C + \sum_{i=1}^n K_i \times \log_2 \text{copy_miRNA}_i$$

Whereby $\log_2 \text{copy_miRNA}_i$ is the log transformed copy numbers in the volume of test sample of the n individual miRNAs (e.g., for the example of 12 miRNAs, n=12). Whereby, K_i - the coefficients used to weight multiple miRNA targets and C - constant, can be derived through

the application of a linear model. Subjects with disease risk score lower than 0 will be considered as 0 and subjects with disease risk score higher than 100 will be considered as 100. It would be within the understanding of someone skilled in the art, knowing the identity of the relevant miRNA biomarkers, to derive the relevant disease risk scores and cut-offs to identify a subject at risk of having NPC.

A further example of such an algorithm for risk score calculation includes the use of logistic regression models:

Formula 2 - $Score = \sum_{i=1}^n K_i Ct_i + constant$

Whereby, n is the number of individual miRNAs (e.g., for the example of 12 miRNAs, n = 12), K_i - the coefficients used to weight multiple miRNA targets, Ct - the cycle threshold value (generally defined as the number of cycles in a real-time PCR reaction required for the fluorescent signal to cross the threshold), and the constant can be derived through the application of a linear model.

A further example of such an algorithm further incorporates the use of a reference sample with known levels of assayed miRNAs to normalize the score for each assay run to account for run-to-run variations (which may be referred to as the Quantitative Reference (QR)), and in some embodiments, the mean value for the QR scores may be used to calculate the expected QR scores. Using the example provided above, an example of how such normalization may be performed is provided below. It would be within the understanding of someone skilled in the art, knowing the identity of the relevant miRNA biomarkers, to derive the relevant disease risk scores, QR scores and cut-offs to identify a subject at risk of having NPC.

Formula 3 - $Score = \sum_{i=1}^n K_i Ct_i + constant - QR_{run} + QR_{expected}$

Whereby, n is the number of individual miRNAs (e.g., for the example of 12 miRNAs, n = 12), K_i - the coefficients used to weight multiple miRNA targets, Ct - the cycle threshold value (generally defined as the number of cycles in a real-time PCR reaction required for the fluorescent signal to cross the threshold), and the constant can be derived through the application of a linear model, QR_{run} - the QR score obtained from the specific run and the $QR_{expected}$ is the mean value of QR scores from multiple validation runs.

EXAMPLES

Methods and Materials

RNA Extraction

- 5 Maxwell® RSC miRNA Plasma and Serum Kit (AS1680, Promega, USA) was used to purify total nucleic acid from plasma samples. A set of 3 proprietary spike-in controls (MiRXES, Singapore) representing high, medium, and low expression levels of miRNA were added into a mixture of 80 µL of Proteinase K and 230 µL of Lysis Buffer C. This mixture was transferred into 400 µL plasma sample, followed by vortexing and incubating the lysate at 37 °C for 15
10 minutes. Lysate was pipetted into Maxwell® RSC Cartridge and then automatically purified by the Maxwell® RSC Instrument using miRNA Plasma and Serum method and eluted in 60 µL of Nuclease-Free Water. RNA was stored at -80°C until further processing.

RT-qPCR Detection of miRNA Expression

- 15 Reverse Transcription and quantification of miRNA by qPCR: Multiplexed miRNA reverse transcription was carried out using MiRXES reagents and miRNA specific stem-loop primers. The program for reverse transcription involved 42 °C for 30 minutes, 95 °C for 5 minutes, then a hold at 25 °C on a Veriti™ Thermal Cycler (Thermo Fisher, USA). Pre-amplification prior to qPCR was applied by using multiplexed miRNA specific assays, the cycling condition is as
20 following: 95 °C for 5 minutes, 40 °C for 5 minutes, 9-14 cycles of 10 seconds at 95 °C, and 30 seconds at 60 °C on Veriti™ Thermal Cycler. Single-plexed SYBR green based qPCR was subsequently carried out in duplicate on the QuantStudio™ 5 Real-Time PCR System (Thermo Fisher, USA) with the following conditions: 95 °C for 5 minutes, 40 cycles of 10 seconds at 95°C, and 30 seconds at 60 °C.

25

Detection of EBV DNA

- Firstly, 200 µL of plasma was isolated using QIAamp DNA Mini Kit (Qiagen, #51304) and eluted in 50 µL nuclease free water. Quantification of EBV DNA was carried out using GeneProof Epstein-Barr Virus (EBV) PCR Kit, following the manufacturer's protocol. For
30 quantification using MiRXES EBV quantification PCR kit, 10 µL of the eluent was added into the probe-based PCR reaction. The copy number of the EBNA-1 gene was calculated using the standard curve of the positive control that was carried out on the same PCR plate.

Statistical analyses

- 35 Quantification cycle (Cq) was calculated using QuantStudio™ Design & Analysis Software with automatic baseline setting and a threshold at 0.4. Technical variations were normalized by

spike-in controls. Statistical analyses were undertaken using GraphPad Prism version 9.1.2. Differences in miRNA levels between NPC and healthy controls were assessed for significance using unpaired parametric multiple t-test with 1% FDR correction.

5 Multivariate analyses using logistic regression were undertaken to classify the biomarkers suitable to be combined into a panel for determining whether a subject is suffering from or is at risk of developing NPC. A five-fold cross-validation procedure that incorporated the sequential forward floating search (SFFS) algorithm and the logistic regression model was used for building and optimizing miRNA biomarker panels to discriminate between NPC cases and
10 non-cancer controls. The SFFS was used to select miRNA biomarkers for inclusion in each biomarker panel built. In each iteration of the five-fold cross validation procedure, the 40 (or 48 for batch 2) samples were randomly partitioned into two groups: Group A and Group B. During each iteration of cross-validation, Group A was first used as the training set for building an NPC prediction model while Group B was used as the test set. For every multi-miRNA
15 biomarker panel optimized in each iteration, a logistic regression prediction model was built, and the diagnostic ability of each panel was evaluated using the area under the curve of the receiver operating characteristics (AUC) analysis. The cross-validation procedure was carried out five times. The diagnostic power (AUC) of each optimized multi-miRNA panel for classifying NPC and non-cancer patient samples was then calculated and compared with other
20 panels optimized in each iteration. Using a logistic regression model incorporating multi-miRNA biomarker panel expression measurements, a prediction algorithm score could be calculated for each sample, with higher scores indicating increased risk of cancer. A prediction algorithm score cut-off was then used to predict NPC.

25 **Example 1. Discovery and validation of EBV miRNAs for the diagnosis of nasopharyngeal carcinoma**

The expression levels of EBV miRNAs are quantitated in study subjects from a cohort in two different batches (i.e., batch 1 and 2). The study subjects from batch 1 comprises 20 NPC
30 plasma samples and 20 healthy controls, while those from batch 2 comprises 24 NPC plasma samples and 24 healthy controls. All samples in this cohort were obtained from a single source and the clinical characteristics of these samples are as shown in **Table 2**.

For clarity, data from batch 1 and batch 2 were presented separately. For the avoidance of doubt,
35 data between the two batches may be combined, interpreted and analysed together, and/or used interchangeably, such that exemplary panels may be derived from batch 1 and/or batch 2.

Table 2. Clinical characteristics of NPC and control samples

Characteristic		Batch 1		Batch 2	
		NPC (n = 20)	Control (n = 20)	NPC (n = 24)	Control (n = 24)
Age	Median	43	44	42.5	41.5
	Range	18-71	31-72	18-71	20-65
Sex	Female	10 (50%)	14 (70%)	8 (33%)	10 (42%)
	Male	10 (50%)	6 (30%)	16 (67%)	14 (58%)
Clinical stage	I	3 (15%)		6 (25%)	
	II	1 (5%)		5 (21%)	
	III	6 (30%)		6 (25%)	
	IVA	7 (35%)		6 (25%)	
	IVB	1 (5%)		1 (4%)	
	IVC	1 (5%)		0	
	Recurrent	1 (5%)		0	

Among the total of 44 EBV miRNAs assayed, 40 differentially expressed miRNAs between healthy controls and NPC cases were identified. The ability of these 40 differentially expressed EBV miRNAs to differentiate between NPC cases and healthy controls were further assessed via the analysis of their respective AUCs. The 40 differentially expressed miRNAs all identified NPC cases with AUC >0.55.

10 Example 2. Optimization of miRNA biomarker panels

To identify an optimal panel with good performance while balancing the number of miRNAs included for practicality of clinical testing, multi-miRNA panels were assessed. The best-performing multi-miRNA panel comprising between one to fifteen miRNAs were formed from all 44 EBV miRNAs using a stratified five-fold cross-validation procedure that incorporated the Sequential Forward Floating Selection (SFFS) algorithm. AUC of the multi-miRNA panels in the training and test group was calculated for five iterations. The mean AUC for NPC prediction from five iterations of training and testing was calculated for each miRNA from the results of cross-validation experiments comprising two to eight miRNAs (Tables 3 and 4). The median AUC increased significantly ($p < 0.001$) with a mean AUC of >0.9 achievable with a panel comprising at least 2 miRNAs; hence indicating that a panel of at least two miRNAs is

useful for determining whether a subject is suffering from or is at risk of developing nasopharyngeal carcinoma.

Batch 1

5

Detection of circulating EBV DNA has been recognised as a useful tool for detection of NPC in subjects (Tan *et al*, 2020). Here, from batch 1 samples, we identified 22 miRNAs that, when used individually, was able to identify subjects suffering from, or is at risk of developing NPC with comparable or even higher AUC than EBV DNA (**Table 3a**). Combining two or more of these miRNAs further increased the AUC value. It can be seen from **Table 3a** that the AUC for most of the multivariate EBV miRNA panels can reach very high levels (>0.9) when three or more miRNAs are combined and generally, increasing the number of EBV miRNAs in the panel further no longer increase the AUC value appreciably.

It can also be seen that, in the case of ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p, the AUC of the miRNAs individually may be below that of EBV DNA, but the combinations of several of these miRNAs produces multivariate panels able to identify subjects with NPC, or at risk of developing NPC with AUC higher than measuring EBV DNA.

20

In a separate analysis detailed in **Table 3b**, it can be seen that even where ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p and ebv-miR-BHRF1-2-3p are only combined with each other or other EBV-encoded miRNAs with lower single feature AUCs in a multivariate panel, a panel comprising at least two of these miRNAs was able to identify subjects with NPC, or at risk of developing NPC with AUC comparable or higher than measuring EBV DNA. For ebv-miR-BART4-3p, combining this miRNA with another two or more other EBV-encoded miRNAs was able to have the same effect.

25

We also noted that the addition of one or more miRNAs selected from ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART21-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART14-5p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART19-5p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p and ebv-miR-BHRF1-2-3p was able to improve the AUC of a panel comprising other EBV

35

miRNAs (ebv-miR-BART1-3p, ebv-miR-BART13-5p, ebv-miR-BART6-5p, ebv-miR-BART6-3p, ebv-miR-BART14-3p, ebv-miR-BHRF1-2-5p, ebv-miR-BART22, ebv-miR-BART10-5p, ebv-miR-BART12, ebv-miR-BART5-3p, ebv-miR-BHRF1-1, ebv-miR-BART20-3p, ebv-miR-BART7-5p, ebv-miR-BART18-3p, ebv-miR-BART20-5p and ebv-miR-BHRF1-3) to achieve AUC values greater than from the detection of EBV DNA. Combining ebv-miR-BART4-3p with two or more miRNAs also was able to provide similar improvements in the AUC

In Table 3c, the presence of EBV DNA is also included as a feature in the analysis and it was found that the combination of the presence of EBV DNA with one or more EBV miRNA significantly improved the accuracy for determining whether a subject is suffering from or is at risk of developing NPC. Therefore, adding one or more miRNA to the measurement of circulating EBV DNA could improve the utility of this test to provide AUC values greater than the measurement of EBV DNA alone.

Table 3. Batch 1 mean AUC values for the use of: (a) miRNAs alone or in combinations of up to 8 miRNAs; (b) selected miRNAs with lower single-feature AUCs combined only with each other; and (c) miRNAs in combination with EBV DNA for diagnosing NPC.

3(a)

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART1-5p	0.91	0.94	0.94	0.96	0.97	0.97	0.97	0.98
ebv-miR-BART19-3p	0.90	0.93	0.94	0.95	0.95	0.96	0.96	0.96
ebv-miR-BART7-3p	0.89	0.91	0.94	0.95	0.95	0.95	0.95	0.95
ebv-miR-BART21-3p	0.88	0.93	0.94	0.94	0.95	0.96	0.96	0.96
ebv-miR-BART8-3p	0.88	0.94	0.96	0.95	0.95	0.96	0.96	0.96
ebv-miR-BART13-3p	0.87	0.89	0.90	0.90	0.94	0.94	0.95	0.96
ebv-miR-BART10-3p	0.87	0.93	0.94	0.95	0.96	0.96	0.96	0.96
ebv-miR-BART14-5p	0.86	0.90	0.94	0.95	0.97	0.97	0.97	0.98
ebv-miR-BART3-3p	0.86	0.88	0.93	0.94	0.96	0.97	0.97	0.96
ebv-miR-BART11-5p	0.84	0.91	0.94	0.96	0.97	0.97	0.97	0.98
ebv-miR-BART16	0.84	0.90	0.91	0.92	0.93	0.94	0.94	0.94
ebv-miR-BART17-5p	0.83	0.92	0.94	0.94	0.97	0.97	0.96	0.96
ebv-miR-BART11-3p	0.83	0.86	0.87	0.89	0.92	0.93	0.93	0.93

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART9-5p	0.82	0.89	0.89	0.91	0.92	0.93	0.94	0.93
ebv-miR-BART2-5p	0.82	0.86	0.88	0.91	0.92	0.94	0.94	0.94
ebv-miR-BART17-3p	0.82	0.84	0.86	0.88	0.90	0.91	0.92	0.92
ebv-miR-BART4-5p	0.81	0.84	0.89	0.91	0.94	0.94	0.95	0.95
ebv-miR-BART21-5p	0.80	0.89	0.92	0.93	0.94	0.95	0.94	0.94
ebv-miR-BART9-3p	0.80	0.85	0.87	0.89	0.90	0.91	0.93	0.93
ebv-miR-BART19-5p	0.80	0.91	0.91	0.91	0.92	0.92	0.92	0.91
ebv-miR-BART18-5p	0.79	0.80	0.85	0.87	0.89	0.90	0.90	0.90
ebv-miR-BART5-5p	0.78	0.83	0.87	0.88	0.90	0.90	0.91	0.91
ebv-miR-BART15	0.77	0.90	0.93	0.94	0.96	0.98	0.98	0.90
ebv-miR-BART8-5p	0.77	0.91	0.93	0.95	0.96	0.97	0.97	0.91
ebv-miR-BART3-5p	0.75	0.91	0.93	0.96	0.97	0.98	0.98	0.91
ebv-miR-BART2-3p	0.74	0.91	0.93	0.95	0.96	0.97	0.98	0.91
ebv-miR-BART4-3p	0.68	0.90	0.93	0.94	0.95	0.96	0.97	0.90
ebv-miR-BHRF1-2-3p	0.67	0.92	0.93	0.94	0.95	0.96	0.96	0.92

3(b)

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART15	0.77	0.84	0.87	0.88	0.89	0.90	0.93	0.93
ebv-miR-BART8-5p	0.77	0.82	0.88	0.90	0.92	0.92	0.93	0.92
ebv-miR-BART3-5p	0.75	0.88	0.90	0.91	0.92	0.92	0.91	0.92
ebv-miR-BART2-3p	0.74	0.87	0.89	0.90	0.91	0.91	0.91	0.91
ebv-miR-BART4-3p	0.68	0.77	0.79	0.81	0.82	0.83	0.83	0.83
ebv-miR-BHRF1-2-3p	0.67	0.80	0.82	0.82	0.82	0.82	0.82	0.81

3(c)

Feature name	Mean AUC							
	Number of miRNAs combined with EBV DNA in panel							
	0	1	2	3	4	5	6	7
EBV DNA	0.78	0.91	0.93	0.94	0.95	0.97	0.98	0.98

Batch 2

Similar trends were also observed in the batch 2 samples. We identified 12 miRNAs, when used individually, was able identify subjects suffering from, or is at risk of developing NPC with comparable or even higher AUC than EBV DNA (**Table 4a**). Combining two or more of these miRNAs further increased the AUC value. It can be seen from **Table 4a** that the AUC for most of the multivariate EBV miRNA panels can reach very high levels (>0.9) when three or more miRNAs are combined and generally, increasing the number of EBV miRNAs in the panel further no longer increase the AUC value appreciably.

It was also observed that, in the case of ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p, the AUC of the miRNAs individually may be below that of EBV DNA, but the combinations of several of these miRNAs produces multivariate panels able to identify subjects with NPC, or at risk of developing NPC with AUC higher than measuring EBV DNA.

In a separate analysis detailed in **Table 4b**, it can be seen that even where ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p are only combined with each other or other EBV-encoded miRNAs with lower single feature AUCs in a multivariate panel, a panel comprising at least two of these miRNAs is able to identify subjects with NPC, or at risk of developing NPC with AUC comparable or higher than measuring EBV DNA.

Further, it was also observed that the addition of one or more miRNAs selected from ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART21-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART14-5p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART8-5p was able to improve the AUC of a panel comprising other EBV miRNAs (ebv-miR-BART6-5p, ebv-miR-BART4-3p, ebv-miR-BART22, ebv-miR-BART21-5p, ebv-miR-BART17-3p, ebv-miR-BART1-3p, ebv-miR-BART18-5p, ebv-miR-BART7-5p, ebv-miR-BART14-3p, ebv-miR-BART10-5p, ebv-miR-

BART13-5p, ebv-miR-BART18-3p, ebv-miR-BART5-3p, ebv-miR-BART6-3p, ebv-miR-BART20-5p, ebv-miR-BART12, ebv-miR-BHRF1-2-3p, ebv-miR-BHRF1-3, ebv-miR-BHRF1-2-5p, ebv-miR-BHRF1-1 and ebv-miR-BART20-3p) to achieve AUC values greater than from the detection of EBV DNA.

5

In **Table 4c**, the presence of EBV DNA is also included as a feature in the analysis and it was found that the combination of the presence of EBV DNA with one or more EBV miRNA significantly improved the accuracy for determining whether a subject is suffering from or is at risk of developing NPC. Therefore, adding one or more miRNA to the measurement of circulating EBV DNA could improve the utility of this test to provide AUC values greater than the measurement of EBV DNA alone.

10

Table 4. Batch 2 mean AUC values for the use of: (a) miRNAs alone or in combinations of up to 8 miRNAs; (b) selected miRNAs with lower single-feature AUCs combined only with each other; and (c) miRNAs in combination with EBV DNA for diagnosing NPC.

15

4(a)

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART19-5p	0.87	0.92	0.94	0.96	0.97	0.97	0.97	0.97
ebv-miR-BART9-3p	0.86	0.88	0.90	0.92	0.93	0.95	0.97	0.98
ebv-miR-BART15	0.85	0.92	0.93	0.94	0.96	0.96	0.97	0.98
ebv-miR-BART13-3p	0.85	0.89	0.92	0.92	0.93	0.96	0.97	0.98
ebv-miR-BART21-3p	0.85	0.88	0.89	0.90	0.92	0.92	0.95	0.96
ebv-miR-BART3-3p	0.85	0.89	0.92	0.93	0.95	0.96	0.97	0.97
ebv-miR-BART11-3p	0.84	0.90	0.92	0.95	0.97	0.98	0.99	0.99
ebv-miR-BART14-5p	0.84	0.88	0.92	0.93	0.94	0.97	0.97	0.98
ebv-miR-BART2-5p	0.84	0.92	0.93	0.94	0.95	0.96	0.97	0.97
ebv-miR-BART8-3p	0.84	0.89	0.92	0.95	0.96	0.97	0.97	0.98
ebv-miR-BART19-3p	0.83	0.88	0.91	0.93	0.93	0.95	0.97	0.97
ebv-miR-BART3-5p	0.82	0.90	0.93	0.94	0.96	0.96	0.97	0.98
ebv-miR-BART9-5p	0.82	0.91	0.92	0.93	0.94	0.94	0.94	0.94
ebv-miR-BART10-3p	0.81	0.88	0.92	0.94	0.95	0.96	0.97	0.97
ebv-miR-BART2-3p	0.81	0.88	0.92	0.93	0.93	0.95	0.97	0.98
ebv-miR-BART7-3p	0.81	0.89	0.93	0.95	0.96	0.98	0.99	0.99

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART11-5p	0.80	0.87	0.90	0.92	0.93	0.93	0.96	0.97
ebv-miR-BART1-5p	0.80	0.86	0.93	0.94	0.95	0.96	0.97	0.98
ebv-miR-BART5-5p	0.80	0.90	0.93	0.94	0.95	0.98	0.98	0.98
ebv-miR-BART16	0.80	0.85	0.89	0.91	0.92	0.94	0.95	0.96
ebv-miR-BART17-5p	0.79	0.88	0.92	0.95	0.98	0.98	0.99	0.99
ebv-miR-BART4-5p	0.79	0.90	0.92	0.94	0.96	0.96	0.97	0.97
ebv-miR-BART8-5p	0.78	0.85	0.91	0.92	0.93	0.95	0.96	0.97

4(b)

miRNA name	Mean AUC							
	Number of miRNAs combined in panel							
	1	2	3	4	5	6	7	8
ebv-miR-BART9-5p	0.82	0.91	0.91	0.92	0.92	0.92	0.93	0.95
ebv-miR-BART10-3p	0.81	0.88	0.89	0.92	0.94	0.95	0.96	0.97
ebv-miR-BART2-3p	0.81	0.88	0.91	0.94	0.94	0.95	0.96	0.96
ebv-miR-BART7-3p	0.81	0.82	0.82	0.85	0.88	0.89	0.90	0.91
ebv-miR-BART11-5p	0.80	0.87	0.88	0.89	0.90	0.91	0.92	0.93
ebv-miR-BART1-5p	0.80	0.85	0.88	0.90	0.93	0.93	0.94	0.93
ebv-miR-BART5-5p	0.80	0.84	0.85	0.85	0.86	0.87	0.88	0.90
ebv-miR-BART16	0.80	0.85	0.86	0.88	0.89	0.90	0.92	0.93
ebv-miR-BART17-5p	0.79	0.84	0.89	0.90	0.93	0.93	0.92	0.92
ebv-miR-BART4-5p	0.79	0.83	0.84	0.85	0.89	0.92	0.92	0.93
ebv-miR-BART8-5p	0.78	0.83	0.87	0.88	0.90	0.91	0.92	0.93

4(c)

Feature name	Mean AUC							
	Number of miRNAs combined with EBV DNA in panel							
	0	1	2	3	4	5	6	7
EBV DNA	0.82	0.90	0.92	0.93	0.93	0.94	0.95	0.98

5

Example 3. Exemplary panels comprising EBV miRNAs for the diagnosis of nasopharyngeal carcinoma

To further examine the utility of the EBV miRNA and DNA panel, exemplary panels of both batches comprising miRNAs listed in above **Tables 3a** and **4a** alone and in combination with EBV DNA were analysed. The mean AUC, mean sensitivity, mean specificity, mean Positive Predictive Value (PPV) and mean Negative Predictive Value (NPV) were as shown in **Tables 5** and **6**, respectively, for the two batches.

These exemplary panels comprising 1-8 EBV encoded miRNAs or 1-7 EBV encoded miRNA with EBV DNA detectable in human plasma may be used for the use to detect NPC with the mean AUC ranging from 0.84 up to 0.94. A person skilled in the art, with knowledge of the identity of the miRNAs listed in **Tables 3** and **4**, and their utility disclosed herein, would be able to design other panels comprising any number of features wherein the measurement of any number of the miRNAs disclosed herein, whether measured alone or in combination with EBV DNA, shall yield a panel suitable for determining whether a subject is suffering from, or is at risk of developing NPC with similarly high performance.

Table 5. Batch 1 mean AUC, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) values from the combination of one to eight features where: (a) the features comprise the miRNAs listed in Table 3a); and (b) where the features further comprise EBV DNA.

(a)

No. of Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
1-feature	0.91	0.83	0.98	0.97	0.86
2-features	0.94	0.85	0.98	0.98	0.88
3-features	0.94	0.88	0.98	0.98	0.90
4-features	0.96	0.90	0.95	0.96	0.92
5-features	0.97	0.93	0.93	0.94	0.93
6-features	0.97	0.95	0.93	0.94	0.96
7-features	0.97	0.95	0.93	0.94	0.96
8-features	0.98	0.98	0.95	0.95	0.98

(b)

Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
EBV DNA only	0.78	0.55	1.00	1.00	0.69
EBV DNA + 1 miRNA	0.91	0.83	0.98	0.97	0.86
EBV DNA + 2 miRNAs	0.93	0.88	0.95	0.95	0.90
EBV DNA + 3 miRNAs	0.94	0.88	0.98	0.98	0.90
EBV DNA + 4 miRNAs	0.95	0.90	0.98	0.98	0.92
EBV DNA + 5 miRNAs	0.97	0.93	1.00	1.00	0.93
EBV DNA + 6 miRNAs	0.98	0.90	1.00	1.00	0.91
EBV DNA + 7 miRNAs	0.98	0.90	1.00	1.00	0.91

Table 6. Batch 2 mean AUC, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) values from the combination of one to eight features where: (a) the features comprise the miRNAs listed in Table 4(a); and (b) where the features further comprise EBV DNA.

5

(a)

No. of Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
1-feature	0.87	0.77	0.98	0.98	0.81
2-features	0.92	0.83	0.94	0.94	0.86
3-features	0.94	0.88	0.94	0.93	0.88
4-features	0.96	0.96	0.92	0.93	0.96
5-features	0.97	0.96	0.94	0.94	0.96
6-features	0.97	0.98	0.92	0.92	0.98
7-features	0.97	0.90	0.98	0.98	0.91
8-features	0.97	0.92	0.96	0.96	0.92

(b)

Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
EBV DNA only	0.82	0.66	0.98	0.98	0.75
EBV DNA + 1 miRNA	0.90	0.81	0.90	0.90	0.83
EBV DNA + 2 miRNAs	0.92	0.90	0.88	0.90	0.91
EBV DNA + 3 miRNAs	0.93	0.86	0.94	0.94	0.88
EBV DNA + 4 miRNAs	0.93	0.86	0.96	0.96	0.88
EBV DNA + 5 miRNAs	0.94	0.90	0.96	0.96	0.90
EBV DNA + 6 miRNAs	0.95	0.92	0.92	0.92	0.92
EBV DNA + 7 miRNAs	0.98	0.90	0.98	0.98	0.90

The generality of deriving exemplary miRNA panels (illustrated above) is further supported by the following non-limiting examples.

- 5 In one approach, top performing miRNAs from both batches were selected, followed by addition of other miRNAs to improve the AUC of the panels. As examples, these top performing miRNAs include, but not limited to, ebv-miR-BART21-3p, ebv-miR-BART14-5p and ebv-miR-BART19-5p, which demonstrated good performance in both batches. For the avoidance of doubt, one or more of these top miRNAs may be selected and may be combined
- 10 with other miRNAs to form a panel. The selection of these miRNAs is not limited to any order.

Exemplary panels with ebv-miR-BART21-3p

- In a scenario where ebv-miR-BART21-3p was chosen as the fixed miRNA, further addition of other features (EBV DNA and/or other miRNAs listed in **Table 4a**) clearly increased the AUCs
- 15 (**Table 7**). However, the increment was less noticeable going beyond around 10 features.

Exemplary panels with ebv-miR-BART21-3p as one of the miRNAs are as shown in **Tables 8** and **9**. With each successive addition of a miRNA, it was observed that the AUCs increased considerably, but the increment was less noticeable going beyond around 10 miRNAs.

- 5 **Table 7. Mean AUC, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) values from the combination of ebv-miR-BART-21-3p with one to fourteen features selected from EBV DNA and/or miRNAs listed in Table 4a.**

Features	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
ebv-miR-BART-21-3p only	0.850	0.76	0.96	0.95	0.82
ebv-miR-BART-21-3p + 1 feature	0.882	0.81	0.96	0.95	0.85
ebv-miR-BART-21-3p + 2 features	0.894	0.79	0.98	0.98	0.83
ebv-miR-BART-21-3p + 3 features	0.899	0.77	1.00	1.00	0.81
ebv-miR-BART-21-3p + 4 features	0.917	0.83	0.96	0.96	0.86
ebv-miR-BART-21-3p + 5 features	0.923	0.81	0.96	0.95	0.84
ebv-miR-BART-21-3p + 6 features	0.948	0.90	0.92	0.92	0.91
ebv-miR-BART-21-3p + 7 features	0.956	0.88	0.96	0.96	0.89
ebv-miR-BART-21-3p + 8 features	0.967	0.90	0.98	0.98	0.91
ebv-miR-BART-21-3p + 9 features	0.967	0.90	0.96	0.96	0.91
ebv-miR-BART-21-3p + 10 features	0.967	0.90	0.96	0.96	0.91
ebv-miR-BART-21-3p + 11 features	0.967	0.90	0.98	0.98	0.91

Features	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
ebv-miR-BART-21-3p + 12 features	0.978	0.96	0.96	0.96	0.96
ebv-miR-BART-21-3p + 13 features	0.994	0.96	0.98	0.98	0.96
ebv-miR-BART-21-3p + 14 features	0.996	0.96	1.00	1.00	0.96

Table 8. Exemplary panels of EBV miRNAs combinations with ebv-miR-BART21-3p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART21-3p	0.850	0.76	0.95	0.95	0.82
2	ebv-miR-BART21-3p, ebv-miR-BART3-5p	0.874	0.83	0.93	0.91	0.85
3	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART13-3p	0.869	0.79	0.96	0.96	0.83
4	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART13-3p, ebv-miR-BART15	0.858	0.79	0.94	0.92	0.82
5	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART13-3p, ebv-miR-BART15, ebv-miR-BART19-5p	0.883	0.77	0.98	0.98	0.81
6	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART13-3p, ebv-miR-BART15, ebv-miR-BART19-5p, ebv-miR-BART3-3p	0.889	0.75	0.96	0.95	0.8
7	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART13-3p, ebv-miR-BART15, ebv-miR-BART19-5p, ebv-miR-BART3-3p, ebv-miR-BART14-5p	0.896	0.79	1	1	0.83

5

Table 9. Further exemplary panels of EBV miRNAs combinations with ebv-miR-BART21-3p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART21-3p	0.850	0.76	0.96	0.95	0.82
2	ebv-miR-BART21-3p, ebv-miR-BART3-5p	0.874	0.83	0.92	0.91	0.85
3	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p	0.878	0.85	0.92	0.91	0.87
4	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p	0.871	0.85	0.90	0.89	0.87
5	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p	0.885	0.80	0.94	0.93	0.83
6	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p	0.922	0.83	0.94	0.95	0.86
7	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15	0.935	0.86	0.94	0.94	0.87
8	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p	0.948	0.90	0.94	0.94	0.91
9	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p	0.952	0.88	0.98	0.98	0.90
10	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p	0.957	0.90	0.98	0.98	0.91
11	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p,	0.957	0.90	0.98	0.98	0.91

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
	ebv-miR-BART2-3p, ebv-miR-BART8-3p, ebv-miR-BART1-5p					
12	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p, ebv-miR-BART1-5p, ebv-miR-BART11-5p	0.957	0.90	0.98	0.98	0.91
13	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p, ebv-miR-BART1-5p, ebv-miR-BART11-5p, ebv-miR-BART11-3p	0.957	0.92	0.96	0.96	0.93
14	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p, ebv-miR-BART1-5p, ebv-miR-BART11-5p, ebv-miR-BART11-3p, ebv-miR-BART13-3p	0.957	0.90	0.96	0.96	0.91
15	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p, ebv-miR-BART1-5p, ebv-miR-BART11-5p, ebv-miR-BART11-3p, ebv-miR-BART13-3p, ebv-miR-BART14-5p	0.957	0.90	0.98	0.98	0.91
16	ebv-miR-BART21-3p, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART4-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART8-3p,	0.959	0.88	0.96	0.96	0.89

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
	ebv-miR-BART1-5p, ebv-miR-BART11-5p, ebv-miR-BART11-3p, ebv-miR-BART13-3p, ebv-miR-BART14-5p, ebv-miR-BART5-5p					

Exemplary panels with ebv-miR-BART14-5p

In a second scenario where ebv-miR-BART14-5p was chosen as the fixed miRNA, further addition of other features (EBV DNA and/or other miRNAs listed in **Table 4a**) clearly increased the AUCs (**Table 10**).

Exemplary panels with ebv-miR-BART14-5p as one of the miRNAs are as shown in **Tables 11** and **12**. With each successive addition of a miRNA, it was observed that the AUCs increased accordingly.

Table 10. Mean AUC, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) values from the combination of ebv-miR-BART-14-5p with one to ten features selected from EBV DNA and/or miRNAs listed in Table 4a.

Features	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
ebv-miR-BART-14-5p only	0.843	0.70	1.00	1.00	0.78
ebv-miR-BART-14-5p + 1 feature	0.878	0.82	0.86	0.87	0.84
ebv-miR-BART-14-5p + 2 features	0.918	0.80	0.94	0.94	0.83
ebv-miR-BART-14-5p + 3 features	0.932	0.86	0.92	0.93	0.88
ebv-miR-BART-14-5p + 4 features	0.942	0.92	0.89	0.91	0.92
ebv-miR-BART-14-5p + 5 features	0.969	0.94	0.96	0.96	0.94

Features	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
ebv-miR-BART-14-5p + 6 features	0.970	0.90	0.96	0.96	0.91
ebv-miR-BART-14-5p + 7 features	0.980	0.92	0.98	0.98	0.92
ebv-miR-BART-14-5p + 8 features	0.982	0.94	0.96	0.96	0.94
ebv-miR-BART-14-5p + 9 features	0.985	0.96	0.96	0.96	0.96
ebv-miR-BART-14-5p + 10 features	0.987	0.98	0.96	0.96	0.98

Table 11. Exemplary panels of EBV miRNAs combinations with ebv-miR-BART14-5p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART14-5p	0.843	0.70	1.00	1.00	0.78
2	ebv-miR-BART14-5p, ebv-miR-BART3-5p	0.872	0.75	0.96	0.96	0.80
3	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p	0.883	0.84	0.92	0.92	0.86
4	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART15	0.892	0.84	0.96	0.96	0.86
5	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART15, ebv-miR-BART19-3p	0.900	0.81	0.98	0.98	0.85
6	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART3-3p	0.909	0.81	0.96	0.96	0.84

5

Table 12. Further exemplary panels of EBV miRNAs combinations with ebv-miR-BART14-5p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART14-5p	0.843	0.70	1.00	1.00	0.78
2	ebv-miR-BART14-5p, ebv-miR-BART3-5p	0.872	0.75	0.96	0.96	0.80
3	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p	0.883	0.84	0.92	0.92	0.86
4	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p	0.919	0.88	0.94	0.94	0.89
5	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART3-3p	0.943	0.86	0.96	0.96	0.88
6	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART3-3p, ebv-miR-BART2-5p	0.948	0.84	0.98	0.98	0.86
7	ebv-miR-BART14-5p, ebv-miR-BART3-5p, ebv-miR-BART19-5p, ebv-miR-BART7-3p, ebv-miR-BART3-3p, ebv-miR-BART2-5p, ebv-miR-BART17-5p	0.950	0.84	1.00	1.00	0.86

Exemplary panels with ebv-miR-BART19-5p

In a third scenario where ebv-miR-BART19-5p was chosen as the fixed miRNA, the further addition of other features (EBV DNA and/or other miRNAs listed in **Table 4a**) clearly increased the AUCs (**Table 13**).

Exemplary panels with ebv-miR-BART19-5p as one of the miRNAs are as shown in **Tables 14** and **15**. With each successive addition of a miRNA, it was observed that the AUCs increased accordingly.

Table 13. Mean AUC, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) values from the combination of ebv-miR-BART-19-5p with one to ten features selected from EBV/DNA and/or miRNAs listed in Table 4a.

Features	Mean AUC	Mean sensitivity	Mean specificity	Mean PPV	Mean NPV
ebv-miR-BART-19-5p only	0.873	0.77	0.98	0.98	0.81
ebv-miR-BART-19-5p + 1 feature	0.923	0.83	0.94	0.94	0.86
ebv-miR-BART-19-5p + 2 features	0.942	0.88	0.94	0.93	0.88
ebv-miR-BART-19-5p + 3 features	0.957	0.96	0.92	0.93	0.96
ebv-miR-BART-19-5p + 4 features	0.965	0.96	0.94	0.94	0.96
ebv-miR-BART-19-5p + 5 features	0.967	0.98	0.92	0.92	0.98
ebv-miR-BART-19-5p + 6 features	0.968	0.90	0.98	0.98	0.91
ebv-miR-BART-19-5p + 7 features	0.968	0.92	0.96	0.96	0.92
ebv-miR-BART-19-5p + 8 features	0.970	0.94	0.94	0.94	0.94
ebv-miR-BART-19-5p + 9 features	0.972	0.96	0.96	0.97	0.96
ebv-miR-BART-19-5p + 10 features	0.974	0.94	0.98	0.98	0.94

Table 14. Exemplary panels of EBV miRNAs combinations with ebv-miR-BART19-5p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART19-5p	0.873	0.77	0.98	0.98	0.81
2	ebv-miR-BART19-5p, ebv-miR-BART3-5p	0.898	0.84	0.94	0.94	0.86

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
3	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART15	0.908	0.84	0.96	0.96	0.86
4	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART3-3p	0.917	0.83	0.96	0.96	0.86
5	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART3-3p, ebv-miR-BART19-3p	0.920	0.81	0.96	0.96	0.84

Table 15. Further exemplary panels of EBV miRNAs combinations with ebv-miR-BART19-5p, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	ebv-miR-BART19-5p	0.873	0.77	0.98	0.98	0.81
2	ebv-miR-BART19-5p, ebv-miR-BART3-5p	0.898	0.84	0.94	0.94	0.86
3	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART7-3p	0.934	0.84	0.96	0.96	0.86
4	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART7-3p, ebv-miR-BART15	0.954	0.86	0.98	0.98	0.88
5	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p	0.961	0.88	0.98	0.98	0.89
6	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p	0.965	0.88	0.98	0.98	0.89
7	ebv-miR-BART19-5p, ebv-miR-BART3-5p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART2-3p, ebv-miR-BART17-5p	0.966	0.90	0.96	0.96	0.91

5

Exemplary panels with EBV DNA

Similarly, exemplary panels having EBV DNA with miRNAs are as shown in **Table 16**. With each successive addition of a miRNA, it was observed that the AUCs increased accordingly.

Table 16. Exemplary panels (a-c) of EBV miRNAs combinations with EBV DNA, and their respective AUC, sensitivity, specificity, PPV and NPV in identifying subjects with, or at risk of developing nasopharyngeal carcinoma.

16(a)

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART21-3p	0.856	0.77	0.96	0.95	0.81
2	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART21-3p, ebv-miR-BART19-5p	0.883	0.77	0.98	0.98	0.81
3	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART21-3p, ebv-miR-BART19-5p, ebv-miR-BART3-3p	0.885	0.75	0.96	0.95	0.80
4	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART21-3p, ebv-miR-BART19-5p, ebv-miR-BART3-3p, ebv-miR-BART14-5p	0.892	0.77	1.00	1.00	0.82

16(b)

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p	0.867	0.82	0.96	0.96	0.84
2	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p	0.912	0.81	0.94	0.94	0.84

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
3	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p, ebv-miR-BART15	0.935	0.81	0.96	0.96	0.84
4	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p	0.959	0.90	0.94	0.94	0.91
5	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART8-3p	0.958	0.88	0.96	0.96	0.89
6	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART8-3p, ebv-miR-BART10-3p	0.959	0.90	0.96	0.96	0.91
7	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART19-5p, ebv-miR-BART21-3p, ebv-miR-BART7-3p, ebv-miR-BART15, ebv-miR-BART19-3p, ebv-miR-BART8-3p, ebv-miR-BART10-3p, ebv-miR-BART2-3p	0.961	0.96	0.92	0.92	0.96

16(c)

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
1	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART14-5p, ebv-miR-BART7-3p, ebv-miR-BART19-5p, ebv-miR-BART2-5p, ebv-miR-BART3-3p	0.950	0.84	1.00	1.00	0.86

No.	Features	AUC	Sensitivity	Specificity	PPV	NPV
2	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART14-5p, ebv-miR-BART7-3p, ebv-miR-BART19-5p, ebv-miR-BART2-5p, ebv-miR-BART3-3p, ebv-miR-BART4-5p	0.950	0.84	1.00	1.00	0.86
3	EBV DNA, ebv-miR-BART3-5p, ebv-miR-BART14-5p, ebv-miR-BART7-3p, ebv-miR-BART19-5p, ebv-miR-BART2-5p, ebv-miR-BART3-3p, ebv-miR-BART4-5p, ebv-miR-BART9-3p	0.951	0.84	1.00	1.00	0.86

References

1. Tan LP, Tan GW, Sivanesan VM, Goh SL, Ng XJ, Lim CS, Kim WR, Mohidin TBBM, Mohd Dali NS, Ong SH, Wong CY, Sawali H, Yap YY, Hassan F, Pua KC, Koay CE, Ng CC, Khoo AS; Malaysian Nasopharyngeal Carcinoma Study Group. Systematic comparison of plasma EBV DNA, anti-EBV antibodies and miRNA levels for early detection and prognosis of nasopharyngeal carcinoma. *Int J Cancer*. 2020 Apr 15;146(8):2336-2347. doi: 10.1002/ijc.32656. Epub 2019 Oct 8. PMID: 31469434; PMCID: PMC7065012.
2. Too HP and Azlinda BA. Modified stem-loop oligonucleotide mediated reverse transcription and base-spacing constrained quantitative PCR. 2011 Dec 22. (WO2011159256A1).

CLAIMS

What is claimed is:

1. A method of determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma, wherein the method comprises detecting the expression level of at least one miRNA from a biological sample obtained from the subject,

wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p; and

wherein differential expression levels of the miRNA, as compared to a control, is indicative of the subject having or is at risk of developing nasopharyngeal carcinoma.

2. The method of claim 1, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p and ebv-miR-BART5-5p.

3. The method of claim 1 or 2, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p and ebv-miR-BART9-5p.

4. The method of claim 1, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-

BART9-5p, ebv-miR-BART10-3p, ebv-miR-BART2-3p, ebv-miR-BART7-3p, ebv-miR-BART11-5p, ebv-miR-BART1-5p, ebv-miR-BART5-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART4-5p and ebv-miR-BART8-5p.

5. The method of claim 1 or 4, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART9-3p, ebv-miR-BART15, ebv-miR-BART13-3p, ebv-miR-BART3-3p, ebv-miR-BART11-3p, ebv-miR-BART2-5p, ebv-miR-BART8-3p, ebv-miR-BART19-3p, ebv-miR-BART3-5p, ebv-miR-BART9-5p, ebv-miR-BART10-3p and ebv-miR-BART2-3p.

6. The method of claim 1, wherein the at least one miRNA is selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p and ebv-miR-BART9-3p.

7. The method of claim 6, wherein the method comprises detecting at least one additional miRNA selected from ebv-miR-BART15, ebv-miR-BART3-5p, ebv-miR-BART17-5p, ebv-miR-BART17-3p, ebv-miR-BART2-3p, ebv-miR-BART5-5p, ebv-miR-BART4-5p, ebv-miR-BART21-5p, ebv-miR-BART18-5p, ebv-miR-BART8-5p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p.

8. The method of any one of the preceding claims, wherein the method comprises detecting expression levels of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs.

9. The method of any one of the preceding claims, wherein the expression level of the miRNA is higher in a subject suffering from or is at risk of developing nasopharyngeal carcinoma compared to the control.

10. The method of any one of the preceding claims, wherein the control is a nasopharyngeal carcinoma-free subject, a subject not suffering from, or not at risk of developing nasopharyngeal carcinoma.
11. The method of any one of the preceding claims, wherein the biological sample is a non-cellular bodily fluid.
12. The method of any one of the preceding claims, wherein the biological sample is plasma and/or serum.
13. The method of any one of the preceding claims, wherein the method further comprises detecting an Epstein-Barr virus DNA (EBV DNA).
14. The method of claim 13, wherein detecting the EBV DNA comprises detecting one or more nucleic acid comprising EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP1, LMP2A, LMP2B, BMRF1, BALF2, BALF5, EBER1 and EBER 2.
15. The method of any one of the preceding claims, wherein the method further comprises performing one or more clinical procedures for determining and/or confirming whether a subject is suffering from or is at risk of developing nasopharyngeal carcinoma, optionally wherein the clinical procedures are selected from an endoscopy, a biopsy, a fine needle aspiration and a diagnostic imaging test.
16. The method of any one of the preceding claims, wherein when said subject is determined to suffer from nasopharyngeal carcinoma, the method further comprises treating the subject with one or more therapy selected from the group consisting of an administration of an anti-cancer compound, surgery, immunotherapy, and radiation therapy.
17. A kit for determining whether a subject suffers from, or is at risk of developing nasopharyngeal carcinoma, the kit comprising:
 - a) an isolated set of probes capable of detecting a panel of miRNAs comprising at least one miRNA selected from ebv-miR-BART21-3p, ebv-miR-BART14-5p, ebv-miR-BART19-5p, ebv-miR-BART1-5p, ebv-miR-BART19-3p, ebv-miR-BART7-3p, ebv-miR-BART8-3p, ebv-miR-BART13-3p, ebv-miR-BART10-3p, ebv-miR-BART3-3p, ebv-miR-BART11-5p, ebv-miR-BART16, ebv-miR-BART17-5p, ebv-miR-BART11-3p, ebv-miR-BART9-5p, ebv-miR-BART2-5p, ebv-miR-BART17-3p, ebv-miR-BART4-5p, ebv-miR-

BART21-5p, ebv-miR-BART9-3p, ebv-miR-BART18-5p, ebv-miR-BART5-5p, ebv-miR-BART15, ebv-miR-BART8-5p, ebv-miR-BART3-5p, ebv-miR-BART2-3p, ebv-miR-BART4-3p and ebv-miR-BHRF1-2-3p; and

b) instructions for use.

18. The kit of claim 17, wherein the isolated set of probes capable of detecting a panel of miRNAs comprises at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven or at least twenty-eight miRNAs.

19. The kit of claim 17 or 18, wherein the kit further comprises an isolated set of probes capable of detecting Epstein-Barr virus DNA (EBV DNA).

20. The kit of claim 19, wherein detecting the EBV DNA comprises one or more nucleic acid relating to EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP1, LMP2A, LMP2B, BMRF1, BALF2, BALF5, EBER1 and EBER 2.

21. The kit of any one of claims 17 to 20, wherein the probe is selected from the group consisting of an aptamer, an antibody, an affibody, a peptide and a nucleic acid.

22. The kit of any one of claims 17 to 21, wherein the at least one miRNA is detected by methods selected from sequencing, nucleic acid hybridization, microarray and nucleic acid amplification.