

CLAIMS

- 5 1. A method for
- (i) determining the prognosis of a cancer subject after treatment removing the primary tumor,
 - (ii) determining the need for additional medical treatment for a cancer subject after treatment removing the primary tumor,
 - 10 (iii) selecting a cancer subject for additional cancer treatment after treatment removing the primary tumor, or
 - (iv) determining, after treatment removing the primary tumor, if the cancer of a cancer subject is metastatic,
- comprising the steps of:
- 15 a. measuring the pre-treatment level of methylated genomic DNA of a gene, or a fragment thereof, in a biological sample obtained from the subject prior to removing the primary tumor (pre-treatment sample);
 - b. measuring the post-treatment level of methylated genomic DNA of the gene or a fragment thereof, in a biological sample obtained from the subject when the primary tumor has been removed (post-treatment sample),
- 20 whereby an increased or equivalent amount a presence of the methylated genomic DNA or fragment in the post-treatment sample compared to the pre-treatment sample after removal of the primary tumor indicates
- (i) a bad prognosis,
 - 25 (ii) the need for additional cancer treatment for the subject,
 - (iii) to select the cancer subject for additional treatment,
 - (iv) that the cancer is metastatic.
- 30 2. ~~The method according to claim 1, wherein an increased amount of the methylated genomic DNA or fragment in the post-treatment sample compared to the pre-treatment sample indicates that the cancer is aggressive or has metastatic potential or reduced survival time for the subject.~~
- 35 3. ~~A method for determining medical treatment for a cancer subject, comprising the steps of:~~
- ~~a. measuring the pre-treatment level of methylated genomic DNA of a gene, or a fragment thereof, in a biological sample obtained from the subject; and~~
 - ~~b. measuring the post-treatment level of methylated genomic DNA of the gene or a fragment thereof, in a biological sample obtained from the subject,~~
 - 40 ~~whereby an increased or equivalent amount of the methylated genomic DNA or fragment in the post-treatment sample compared to the pre-treatment sample indicates additional cancer treatment for the subject.~~
- 45 4. ~~A method for determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject comprising:~~
- ~~a. measuring the pre-treatment level of methylated genomic DNA of a gene, or a fragment thereof, in a biological sample obtained from the subject; and~~
 - 50 ~~b. measuring the post-treatment level of methylated genomic DNA of the gene or a fragment thereof, in a biological sample obtained from the subject,~~
 - ~~whereby an increased or equivalent amount of the methylated genomic DNA or fragment in the post-treatment sample compared to the pre-treatment sample~~

~~indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject.~~

5. ~~A method for detecting an aggressive form of cancer in a subject, comprising~~
a. ~~measuring the pre-treatment level of methylated genomic DNA of a gene, or a~~
~~fragment thereof, in a biological sample obtained from the subject; and~~
b. ~~measuring the post-treatment level of methylated genomic DNA of the gene or a~~
~~fragment thereof, in a biological sample obtained from the subject,~~
whereby an increased amount of the methylated genomic DNA or fragment in the
post-treatment sample compared to the pre-treatment sample indicates that the
tumor is an aggressive form.

6. ~~A method for selecting a cancer subject for cancer treatment comprising:~~
a. ~~measuring the pre-treatment level of methylated genomic DNA of a gene, or a~~
~~fragment thereof, in a biological sample obtained from the subject; and~~
b. ~~measuring the post-treatment level of methylated genomic DNA of the gene or a~~
~~fragment thereof, in a biological sample obtained from the subject,~~
whereby an increase in the amount of methylated genomic DNA of the gene in
the post-treatment sample compared to the pre-treatment sample indicates
additional cancer treatment.

7. ~~A method for determining tumor load or cancer burden in a subject comprising:~~
a. ~~measuring the pre-treatment level of methylated genomic DNA of a gene, or a~~
~~fragment thereof, in a biological sample obtained from the subject; and~~
b. ~~measuring the post-treatment level of methylated genomic DNA of the gene or a~~
~~fragment thereof, in a biological sample obtained from the subject;~~
whereby an increase in the amount of methylated genomic DNA of the gene in
the post-treatment sample compared to the pre-treatment sample indicates that
the subject has increased or equivalent tumor load or cancer burden or that the
tumor load or cancer burden has not been diminished by the treatment.

8. ~~A method for determining tumor load or cancer burden in a subject comprising~~
~~comparing the post-treatment level of methylated genomic DNA of a gene or a fragment~~
~~thereof, in a biological sample obtained from the subject with the pre-treatment level of~~
~~methylated genomic DNA of the gene or fragment whereby an increase in the amount of~~

~~methyated genomic DNA of the gene in the post-treatment sample compared to the pre-treatment sample indicates that the subject has increased or equivalent tumor load or cancer burden or that the tumor load or cancer burden has not been diminished by the treatment.~~

92. The method according to ~~any of claims 1 to 8~~claim 1, wherein the gene is SEPTIN9 (SEQ ID NO:1) or RASSF2a (SEQ ID NO:2).

~~103.~~ The method according to claim 92, wherein the methylation of the genomic DNA is measured at least one cytosine selected from the group consisting of positions 21, 28, 30, 37 and 39 of SEQ ID NO: 32 and positions 25, 29, 46, 52, 58, 70, 74, 79 and 89 of SEQ ID NO: 34.

~~114.~~ The method according to claim 92, wherein the gene is SEPTIN9 (SEQ ID NO:1).

~~125.~~ The method according to any of claims 1 to ~~114~~, wherein the cancer is selected from the group consisting of:

- a. Colon cancer; and
- b. Colorectal cancer.

~~136.~~ The method according to claim ~~125~~, wherein the stage of the cancer is Stage I colorectal cancer.

~~147.~~ The method according to claim ~~125~~, wherein the stage of the cancer is Stage II colorectal cancer.

~~158.~~ The method according to claim ~~125~~, wherein the stage of the cancer is Stage III colorectal cancer.

~~169.~~ The method according to any of claims 1-~~125~~, wherein the treatment removing the primary tumor is selected from the group consisting of:

- a. Surgery or resection;
- b. Immunotherapy;
- c. Chemotherapy;
- d. Radiation therapy;
- e. Therapy targeting solid tumors;

- f. Therapy targeting soft-tissue tumors; and
- g. Therapy targeting blood cancers.

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~~1710~~. The method of any of claims 1 to ~~169~~, wherein the treatment is localized to the region of cancer in the subject.

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~~1811~~. The method of any of claims 1 to ~~1610~~, wherein the treatment is not localized to the region of cancer in the subject.

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~~1912~~. The method according to any of claims 1 to ~~1811~~, wherein the biological sample is selected from the group consisting of:

- a. tissue;
- b. urine;
- c. stool;
- d. lavage fluid; and
- e. blood.

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~~2013~~. The method according to any of claims 1 to ~~1912~~, wherein the sample is serum or plasma.

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~~2114~~. The method according to any of claims 1 to ~~2013~~, wherein methylated genomic DNA or fragment is measured quantitatively, or quantitatively in part, qualitatively, and/or qualitatively in part.

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~~2215~~. The method according to any of claims 1 to ~~2013~~, wherein methylated genomic DNA or fragment is measured quantitatively in part and qualitatively in part or semiquantitatively.

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16. The method to any of claims 1 to 6, wherein methylated genomic DNA or fragment is measured by MethyLight™, HeavyMethyl™ or methylation specific PCR.

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~~2317~~. The method according to any of claims 1 to ~~2216~~, wherein measuring the methylated genomic DNA or fragment comprises contacting genomic DNA from the biological sample with at least one reagent, or series of reagents that distinguishes between methylated and non-methylated CpG dinucleotides within at least one target region of the genomic DNA, wherein the target region comprises, or hybridizes under stringent conditions to a sequence of at least 9, at least 16 or at least 25 contiguous nucleotides of SEQ ID NO: 1, wherein said contiguous nucleotides comprise at least one CpG dinucleotide sequence.

~~2418~~. The method of claim ~~2317~~, wherein contacting the genomic DNA, or the fragment thereof

in b), comprises use of a reagent selected from the group comprising of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

2519. The method according to any of claims 1 to 2418, comprising:

- a. extracting or otherwise isolating the genomic DNA or fragment thereof from the biological samples;
- b. treating the extracted or isolated genomic DNA or a fragment thereof with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties;
- c. contacting the treated genomic DNA or treated fragment, with an amplification enzyme and at least one primer comprising, a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under moderately stringent or stringent conditions to a the treated sequence or to a complement thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplicate, or is not amplified; and
- d. determining, based on a presence, absence or amount of, or on a property of said amplicate, the methylation state or level of at least one CpG dinucleotide of the gene, or an average, or a value reflecting an average methylation state or level of a plurality of CpG dinucleotides of the gene.

2620. The method according to any of claims 1 to 2418, comprising:

- a. extracting or otherwise isolating the genomic DNA or fragment thereof from the biological samples;
- b. digesting the extracted or isolated genomic DNA or a fragment thereof with one or more methylation sensitive restriction enzymes;
- c. contacting the DNA restriction enzyme digest of b), with an amplification enzyme and at least two primers suitable for the amplification of a sequence comprising at least one CpG dinucleotide of the gene; and
- d. determining, based on a presence, absence or class of an amplicate the methylation state or level of at least one CpG dinucleotide of the gene.

21. The method according to any of claims 1 to 8, wherein the cancer is colorectal cancer or colon cancer, the treatment removing the primary tumor is surgery or resection, the biological sample is blood, serum, plasma or stool, and the gene is SEPTIN9 (SEQ ID NO:1) or RASSF2a (SEQ ID NO:2), and preferably wherein the methylated genomic DNA or fragment is measured by MethyLight™, HeavyMethyl™ or methylation specific PCR.

2722. A-Use of a methylated genomic SEPTIN9 nucleic acid or a fragment comprising at least 9, at least 16, at least 25, or at least 50 contiguous nucleotides of the nucleic acid and sequences complementary thereto for use in the determination of prognosis of a cancer subject when the primary tumor has been removed.

~~2823.~~ The ~~methyated genomic SEPTIN9 nucleic acid~~use according to claim ~~2722~~, wherein the subject has colorectal cancer.

~~2924.~~ Use of methylated genomic SEPTIN9 nucleic acid or a fragment comprising at least 9, at least 16, at least 25, or at least 50 contiguous nucleotides of the nucleic acid and sequences complementary thereto for determining the prognosis of a cancer subject, determining medical treatment for a cancer subject, determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, detecting an aggressive form of cancer in a subject, selecting a cancer subject for cancer treatment, or determining tumor load or cancer burden in a subject ~~comprising of a cancer subject when the primary tumor has been removed.~~

~~3025.~~ The use according to claim ~~2924~~, wherein the subject has colorectal cancer or colon cancer.

~~31.~~ A ~~bisulfite treated genomic SEPTIN9 DNA nucleic acid~~ comprising at least 9, at least 16, at least 25, or at least 50 contiguous nucleotides, or complement thereto for use in ~~determining the prognosis of a cancer subject, determining medical treatment for a cancer subject, determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, detecting an aggressive form of cancer in a subject, selecting a cancer subject for cancer treatment, or determining tumor load or cancer burden in a subject.~~

~~3226.~~ The ~~nucleic acid~~use of any of claims ~~27, 28, or 3122 or 23~~, wherein the contiguous base sequence comprises at least one CpG, TpG or CpA dinucleotide sequence.

~~3327.~~ A ~~Use of a kit~~ for determining the prognosis of a cancer subject, determining medical treatment for a cancer subject, for determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, for detecting an aggressive form of cancer in a subject, for selecting a cancer subject for cancer treatment, or for determining tumor load or cancer burden in a subject, when the primary tumor has been removed, the kit comprising:

- a. a plurality of oligonucleotides or polynucleotides able to hybridise under stringent or moderately stringent conditions to the transcription products of the gene or methylated genomic DNA; and

b. means to detect the hybridisation.

5 | 3428. The kit-use according to claim 3327, wherein the gene or methylated genomic DNA is SEPTIN9.

10 | 3529. ~~A-Use of a kit for determining the prognosis of a cancer subject, determining medical treatment for a cancer subject, for determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, for detecting an aggressive form of cancer in a subject, for selecting a cancer subject for cancer treatment, or for determining tumor load or cancer burden in a subject, when the primary tumor has been removed, the kit comprising:~~
 15 | (a) a bisulfite reagent;
 (b) at least one set of oligonucleotides containing two oligonucleotides whose sequences in each case are identical, are complementary, or hybridize under stringent or highly stringent conditions to an at least 9 or an at least 18 base long segment of a SEPTIN9 sequence.

20 | 36. ~~The use of a method according to any of claims 1-26, the nucleic acid according to any of claims 27, 28 or 31, and/or a kit according to claims 33 or 34 for determining the prognosis of a cancer subject, determining medical treatment for a cancer subject, determining if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, detecting an aggressive form of cancer in a subject, selecting a cancer subject for cancer treatment, or determining tumor load or cancer burden in a subject.~~

30 | 3730. A method for
 (i) determining the prognosis of a cancer subject after treatment removing the primary tumor,
 (ii) determining the need for additional medical treatment for a cancer subject after treatment removing the primary tumor,
 (iii) selecting a cancer subject for additional cancer treatment after treatment removing the primary tumor, or
 35 | (iv) determining after treatment removing the primary tumor, if the cancer of a cancer subject is metastatic if a tumor from a cancer subject indicates that the tumor is aggressive or has metastatic potential or indicates a reduced survival time for the subject, detecting an aggressive form of cancer in a subject, selecting a cancer subject for cancer treatment, or determining tumor load or cancer burden in a subject, comprising the steps of:
 40 | a. measuring the pre-treatment level of the Septin 9 gene expression and/or its promoter or regulatory elements or a fragment thereof, or of the RASSF2A gene and/or its promoter or regulatory elements or a fragment thereof, in a biological sample obtained from the subject prior to removing the primary tumor (pre-treatment sample);
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- b. measuring the ~~post-treatment~~ level of the Septin 9 gene expression and/or its promoter or regulatory elements or a fragment thereof, or of the RASSF2A gene and/or its promoter or regulatory elements or a fragment thereof, in a biological sample obtained from the subject when the primary tumor has been removed (post-treatment sample);
 whereby a ~~decreased or equivalent amount~~ presence of Septin 9 or RASSF2A gene expression in the post-treatment sample ~~compared to the pre-treatment sample~~ indicates
- (i) a bad prognosis,
 - (ii) the need for additional cancer treatment for the subject,
 - (iii) to select the cancer subject for additional cancer treatment,
 - (iv) that the cancer metastatic ~~additional cancer treatment for the subject.~~

~~3831.~~ A-Use of a kit for determining ~~carrying out~~ the method of claim ~~3730~~, the kit comprising:

- a. a plurality of oligonucleotides or polynucleotides able to hybridise under stringent or moderately stringent conditions to the transcription products of the Septin 9 gene and/or its promoter or regulatory elements (including all transcript variants thereof), or of the RASSF2A gene and/or its promoter or regulatory elements (including all transcript variants thereof); and
- b. means to detect the hybridisation.
- c.

~~3932.~~ The method according to any of claim ~~3730~~, wherein said expression level is determined by detecting the presence, absence or level of a polypeptide encoded by said gene or sequence thereof.

~~4033.~~ A-Use of a kit for determining ~~carrying out~~ the method of claim ~~3630~~, comprising:

- a. a means for detecting polypeptides of Septin9 or RASSF2A, wherein the means can form complexes with the polypeptides; and
- b. a means to detect the complexes of a).

~~4134.~~ The method according to claim ~~3932~~ or the ~~kit-use~~ according to claim ~~4033~~, wherein said polypeptide is detected by ~~one or more means selected from the group comprising~~ western blot analysis, chromatography, immunoassay, ELISA immunoassay, radioimmunoassay, antibody ~~and/or~~ combinations thereof.

~~4235.~~ A method of detecting metastasis in a cancer subject or in tissue from the cancer subject, comprising detecting methylated genomic DNA from the subject ~~after~~ when the primary tumor has been removed from the subject.

~~4336.~~ The method according to claim ~~4535~~, wherein the methylated genomic DNA is detected in blood or plasma from the subject.