

CLAIMS

1. A pair of oligonucleotide, primers or probe comprising or consisting essentially of:

(i) SEQ ID NOs. 1 and 2;

(ii) SEQ ID NOs. 3 and 4;

(iii) SEQ ID NOs. 5 and 6; or

(iv) SEQ ID NOs. 7 and 8;

~~or consisting of the nucleotide sequence of any of SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 61, 62 or 63 which~~ pair of oligonucleotide, primers or probe is useful for the detection of the methylation status of a the CTAG1B gene.

~~2. An oligonucleotide, primer or probe according to claim 1 comprising, consisting essentially of or consisting of the nucleotide sequence of any of SEQ ID NO. 9 to 12 and SEQ ID NO. 21 to 40 which oligonucleotide, primer or probe is useful for the detection of the methylation status of a gene.~~

~~32. An oligonucleotide, primer or probe~~ comprised within the pair of oligonucleotide primers according to claim 1 comprising or consisting essentially of or consisting of the following contiguous sequences in 5' to 3' order:

(a) a first nucleotide sequence of between approximately 6 and 30 nucleotides, wherein a nucleotide within said first nucleotide sequence is labelled with a first moiety selected from the donor moiety and the acceptor moiety of a molecular energy transfer pair, wherein the donor moiety emits fluorescence at one or more particular wavelengths when excited, and the acceptor moiety absorbs and/or quenches said fluorescence emitted by said donor moiety;

(b) a second, single-stranded nucleotide sequence comprising, consisting essentially of or consisting of between approximately 3 and 20 nucleotides;

(c) a third nucleotide sequence comprising, consisting essentially of or consisting of between approximately 6 and 30 nucleotides, wherein a nucleotide within said third nucleotide sequence is labelled with a second moiety selected from said donor moiety and said acceptor moiety, and said second moiety is the member of said group not labelling said first nucleotide sequence, wherein said third nucleotide sequence is complementary in reverse order to said first nucleotide sequence such that a duplex can form between said first nucleotide sequence and said third nucleotide sequence such that said first moiety and second moiety are in proximity such that, when the donor moiety is excited and emits

fluorescence, the acceptor moiety absorbs and quenches said fluorescence emitted by said donor moiety; and

(d) at the 3' end of the primer, a fourth, single-stranded nucleotide sequence comprising, consisting essentially of or consisting of between approximately 8 and 40 nucleotides that comprises or consists essentially of or consists of at its 3' end a sequence of ~~any of SEQ ID NO. 2, 4, 6, or 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 61 or 63~~ (and able to prime synthesis by a nucleic acid polymerase of a nucleotide sequence complementary to a nucleic acid strand comprising the portion of the unmethylated DNA of the CTAG1B, CTAG2, MageA3 and/or PRAME gene);

wherein when said duplex is not formed, said first moiety and said second moiety are separated by a distance that prevents molecular energy transfer between said first and second moiety.

~~4. Primer pair comprising a primer according to any preceding claim.~~

~~5. Primer pair according to claim 4 comprising a primer according to claim 3.~~

~~6. Primer pair comprising or consisting essentially of or consisting of the nucleotide sequence of SEQ ID NO. 9 and SEQ ID NO. 10; SEQ ID NO. 11 and SEQ ID NO. 12; SEQ ID NO. 21 and SEQ ID NO. 22; SEQ ID NO. 23 and SEQ ID NO. 24; SEQ ID NO. 25 and SEQ ID NO. 26; SEQ ID NO. 27 and SEQ ID NO. 28; SEQ ID NO. 29 and SEQ ID NO. 30; SEQ ID NO. 31 and SEQ ID NO. 32; SEQ ID NO. 33 and SEQ ID NO. 34; SEQ ID NO. 35 and SEQ ID NO. 36; SEQ ID NO. 37 and SEQ ID NO. 38; SEQ ID NO. 39 and SEQ ID NO. 40; or SEQ ID NO. 61 and 62.~~

~~7. A kit for detecting the methylation status of a the CTAG1B gene comprising at least one oligonucleotide, primer or probepair as defined in any one of claims 1 to 3 or 2 a primer pair as defined in any one of claims 4 to 6.~~

~~8. An oligonucleotide, primer or probe as defined in any one of claims 1 to 3 or a primer pair as defined in any one of claims 4 to 6 or a kit as defined in claim 7, wherein said gene is the CTAG1B, CTAG2, MageA3 and/or PRAME gene.~~

94. Method of detecting the presence and/or amount of unmethylated CTAG1B, ~~CTAG2, MageA3 and/or PRAME~~ gene in a DNA-containing sample, comprising:

(a) contacting/treating the DNA-containing sample with a reagent which selectively modifies unmethylated cytosine residues in the DNA to produce detectable modified residues but which does not modify methylated cytosine residues

(b) amplifying at least a portion of the unmethylated gene of interest using at least one primer pair, at least one primer of which is designed to bind only to the sequence of unmethylated DNA following treatment with the reagent.

10. ~~Method according to claim 9 wherein at least one primer in the primer pair comprises, consists essentially of, or consists of the nucleotide sequences of:~~

(i) SEQ ID NOs. 1 and 2;

(ii) SEQ ID NOs. 3 and 4;

(iii) SEQ ID NOs. 5 and 6; or

(iv) SEQ ID NOs. 7 and 8

~~any of SEQ ID NO. 1 to 40 or SEQ ID NO. 61 to 63.~~

115. Method of diagnosing cancer or predisposition to cancer comprising detecting the methylation status of ~~at least one of the CTAG1B, CTAG2, MageA3 and/or PRAME~~ gene in a sample by using an oligonucleotide, primer ~~or probe~~ pair as defined in ~~any one of claims 1 to 3 or 82, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 83 or a method according to any of claims 9 to 104,~~ wherein the presence of unmethylated CTAG1B, CTAG2, ~~MageA3 and/or PRAME~~ in the sample is indicative for cancer or predisposition to cancer.

126. Method for identifying and/or selecting a patient suitable for treatment with a CTAG1B, CTAG2, ~~MageA3 and/or PRAME~~ immunotherapeutic comprising detecting the methylation status of the CTAG1B, CTAG2, ~~MageA3 and/or PRAME~~ gene in a sample of the patient by using an oligonucleotide, primer ~~or probe~~ pair as defined in ~~any one of claims 1 to 3 or 82, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 83 or a method according to any of claims 9 to 104,~~ wherein if the CTAG1B, CTAG2, ~~MageA3 and/or PRAME~~ gene is unmethylated the subject is identified and/or selected for treatment with the CTAG1B, CTAG2 ~~MageA3 and/or PRAME~~ immunotherapeutic.

13. Method for predicting the likelihood of successful treatment of cancer comprising detecting the methylation status of the CTAG1B, CTAG2, MageA3 and/or PRAME gene in a sample of the patient by using an oligonucleotide, primer or probe as defined in any one of claims 1 to 3 or 8, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 8 or a method according to any of claims 9 to 10, wherein if the gene is unmethylated the likelihood of successful treatment with a CTAG1B, CTAG2, MageA3 and/or PRAME immunotherapeutic is higher than if the gene is methylated.

14. Method of selecting a suitable treatment regimen for cancer comprising detecting the methylation status of the CTAG1B, CTAG2, MageA3 and/or PRAME gene in a sample of the patient by using an oligonucleotide, primer or probe as defined in any one of claims 1 to 3 or 8, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 8 or a method according to any of claims 9 to 10, wherein if the gene is unmethylated, an immunotherapeutic is selected for treatment.

15. ~~Use of a composition comprising or encoding CTAG1B, CTAG2, MageA3 and/or PRAME in the manufacture of a medicament for the treatment of a patient suffering from a tumour, in which the patient has been selected for treatment on the basis of measuring the methylation status of a CTAG1B, CTAG2, MageA3 and/or PRAME gene, by using an oligonucleotide, primer or probe as defined in any one of claims 1 to 3 or 8, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 8 or a method according to any of claims 9 to 10.~~

16. ~~Use of a composition comprising or encoding CTAG1B, CTAG2, MageA3 and/or PRAME in the manufacture of a medicament for the treatment of a patient susceptible to recurrence of a CTAG1B, CTAG2, MageA3 and/or PRAME expressing tumour, in which a patient has been selected for treatment on the basis of measuring the methylation status of a CTAG1B, CTAG2, MageA3 and/or PRAME gene by using an oligonucleotide, primer or probe as defined in any one of claims 1 to 3 or 8, a primer pair as defined in any one of claims 4 to 6 or 8, a kit according to claim 7 or 8 or a method according to any of claims 9 to 10.~~

17. ~~A use according to any of claims 15 or 16, in which the composition comprises CTAG1B, CTAG2, MageA3 and/or PRAME comprises full length CTAG1B, CTAG2, MageA3 and/or PRAME, substantially full-length CTAG1B, CTAG2, MageA3 and/or PRAME or fragments of CTAG1B, CTAG2, MageA3 and/or PRAME, for example peptides of CTAG1B, CTAG2, MageA3 and/or PRAME.~~

18. Use according to claim 17, in which CTAG1B is a fragment of full length CTAG1B including binding motifs to HLA A2, selected from peptides 157-167, 157-165 and 155-163.

19. Use according to claim 17 wherein CTAG1B is a fragment of full length CTAG1B including one or more MHC Class 1 or Class 2 epitopes selected from A31, DR1, DR2, DR4, DR7, DP4, B35, B51, Cw3, Cw6 and A2 as disclosed in WO2008/089074.

20. Use according to claims 15-19, in which the CTAG1B, CTAG2 and/or PRAME protein, fragment or peptide is linked to a fusion partner protein.

21. Use according to claim 20, in which the fusion partner protein is protein D, a surface protein of the gram-negative bacterium, *Haemophilus influenza* B or a derivative thereof.

22. Use according to claim 20, in which the fusion partner protein is LytA or a derivative thereof comprising or consisting of the repeat portion of the LytA molecule found in the C-terminal end starting at residue 178, or comprising residues 188-305.

23. Use according to claim 20, in which the fusion partner protein is NS1 (hemagglutinin), or a derivative thereof comprising the N-terminal 81 amino acids of NS1.

24. Use according to any of claims 15-23, in which the composition comprises a nucleic acid molecule encoding the CTAG1B, CTAG2 and/or PRAME protein, fragment or peptide or fusion protein thereof.

25. Use according to claim 24, in which the nucleic acid molecule is provided within an expression vector.

26. Use according to any of claims 15-25, in which the CTAG1B, CTAG2 and/or PRAME protein, fragment or peptide containing composition, or nucleic acid containing composition, further comprises one or more of an adjuvant, an immunostimulatory cytokine and a chemokine.

27. Use according to claim 26, in which the adjuvant comprises one or more of monophosphoryl lipid A or a derivative thereof, a saponin or a derivative thereof and a TLR9 antagonist.

28. Use according to claim 27, in which the TLR9 agonist is a CpG-containing oligonucleotide.

29. Use according to any of claims 26-28, in which the adjuvant is formulated in an oil-in-water emulsion or a water-in-oil emulsion, optionally containing cholesterol and/or tocopherol, or is formulated in a liposomal composition.

30. A method according to any of claims 9 to 16, in which the gene is the MageA3 gene and the sample or tumor tissue is taken by fine needle biopsy.