

Claims

1. A method for determining the susceptibility of a patient suffering from a proliferative disease to treatment using an agent which targets a component of the PD1/PD-L1 pathway, said method comprising:
 - a) measuring both the PD-L1 and PD-L2 copy mutation variant,
 - b) determining in a tumour sample from said patient at least three biomarkers, wherein at least one of the biomarkers detected is directly associated with PD-1/PD-L1 pathway, at least one of the biomarkers is not directly associated with the PD1/PD-L1 pathway but is listed in Tables 3A-3D, and at least one biomarker is the tumour mutational burden, and
 - c) relating overexpression of mRNA and presence of more than one of said biomarkers as an indicator of susceptibility to said agent.
2. A method according to claim 1 wherein at least 10 of the biomarkers in Tables 3A-3D are determined.
3. A method according to claim 2 wherein all of the biomarkers in Tables 3A-3D are determined.
4. A method according to any one of the preceding claims wherein the proliferative disease is cancer.
5. A method according to any one of the preceding claims wherein the biomarker is identified using DNA or RNA analysis or a combination thereof.
6. A method according to any one of the preceding claims wherein targeted semiconductor sequencing is applied to cover the entire coding regions of the said biomarkers.
7. A method according to any one of the preceding claims wherein levels of RNA of at least one biomarker is measured, wherein a change in the expression level as compared to wild type is indicative of the PD-1/PD-L1 pathway.
8. A method according to claim 7 wherein the said biomarker is selected from the group consisting of CD273 (PD-L2), CD274 (PD-L1) and CD279 (PD-1).

9. A method according to claim 8 wherein analysis of gene expression at multiple exon-intron loci across PD-L1 and PD-1 mRNAs is carried out, and this is related to normalised gene expression so as to quantitate measurements of PD-1 and PD-L1 RNA expression levels.
10. A method according to any one of claims 7 to 9 wherein elevated levels of RNA of NFATC1 (Nuclear Factor Of Activated T-Cells 1), PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) PIK3CD (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta), PRDM1 (PR domain zinc finger protein 1), PTEN, (Phosphatase and tensin homolog), PTPN11 (Tyrosine-protein phosphatase non-receptor type 11), MTOR (mechanistic target of rapamycin), HIF1a (Hypoxia-inducible factor 1-alpha) and FOXO1m (forkhead box class O1 mutant) are determined.
11. A method according to any one of claims 7 to 10 wherein a loss of gene expression of the mismatch repair genes MLH1, PMS2, MSH6 and MLH2 is determined.
12. A method according to any one of the preceding claims wherein at least one biomarker comprises detection of a mutation in a gene, wherein said mutation impacts on expression or function of the gene or gene product.
13. A method according to claim 12 wherein the a mutation is present in a gene selected from the group consisting of ALK (anaplastic lymphoma kinase gene), BRAF (B-Raf gene), CD274 (PD-L1 gene), EGFR (epidermal growth factor receptor gene), ERBB2 (Receptor tyrosine-protein kinase erbB-2 or human epidermal growth factor receptor 2 gene), FGFR (fibroblast growth factor receptor gene), KIT (KIT proto-oncogene receptor tyrosine kinase gene), KRAS (K-ras gtpase gene), MET (MET proto-oncogene, receptor tyrosine kinase) or NRAS (N-ras protooncogene, GTPase gene).
14. A method according to any one of the preceding claims wherein a biomarker resulting from gene rearrangement leading to aberrant gene fusions is detected.
15. A method according to claim 14 wherein the gene fusion is a ALK fusion, an FGFR fusion or a MET gene fusion.
16. A method according to any one of the preceding claims wherein a biomarker detected comprises the presence of copy number variant of a gene.

17. A method according to claim 16 wherein the gene is selected from the group consisting of ERBB2, FGFR, FGFR1, FCGR2, FGFR3, FGFR4, CD273 (PD-L2 gene) or CD273.

18. A method according to any one of the preceding claims wherein the biomarkers are determined using a high-throughput assay platform.

19. A method according to any one of the preceding claims wherein an algorithm indicative of the level of susceptibility is applied to the results obtained.

20. A method according to claim 19 wherein a score of '0' is applied to results which show no changes over wild type or normal expression profiles of the various biomarkers, whereas a score of at least 1 is applied to any mutations or variations noted.

21. A method according to claim 20, wherein

- (i) a score of '1' is applied in the case of the presence of an oncogenic mutation in a biomarker gene, or to the presence of 2-3 additional copies of a biomarker gene, or for the presence of an oncogenic gene fusion, or to a small (i.e. 0-500 nRPM) change in RNA expression of a biomarker gene, or for a TMB of <6 mutations/Megabase;
- (ii) a score of '2' is applied in the case of the presence of from 4-8 additional copies of a biomarker gene; or to intermediate (500-1500 nRPM) change in RNA expression of a biomarker gene, or for a TMB of from 6-19 mutations/Megabase;
- (iii) a score of '3' is applied in the case of the presence of from more than 8 additional copies of a biomarker gene; or to a high (>1500 nRPM) change in RNA expression of a biomarker gene, or for a or for a TMB of >20 mutations/Megabase;

wherein an overall score of 1-2 is indicative of a minimum susceptibility to an agent which targets the PD-1/PD-L1 pathway, a score of 3-5 is indicative of a moderate susceptibility to such an agent and a score in excess of 6 is indicative of susceptibility to such an agent.

22. Apparatus arranged to carry out the method according to any one of claims 1 to 21 which comprises means for carrying out DNA and/or RNA analyses and a computer programmed to implement the algorithm according to any one of claims 19 to 21.

23. A computer or a machine-readable cassette programmed to implement the algorithm according to any one of claims 20 to 22.

24. A system for identifying patients suffering from proliferative disease who would respond to treatment using an agent which targets a component of the PD1/PD-L1 pathway, said system comprising:

a processor; and

a memory that stores code of an algorithm that, when executed by the processor, causes the computer system to:

receive input levels of a plurality of biomarkers selected from those listed in Tables 3A – 3D

identified in a sample of a tumour of a patient;

receive a further input level relating to the tumour mutational burden of nucleic acid in said sample;

analyze and transform the input levels via an algorithm to provide an output indicative of the level of susceptibility of said patient to treatment using an agent which targets a component of the PD1/PD-L1 pathway;

display the output on a graphical interface of the processor.

25. A system according to claim 24 wherein the memory further comprises code to provide a customized recommendation for the treatment of the patient, based upon the input levels.

26. A non-transitory computer-readable medium storing instructions that, when executed by a processor, cause a computer system to identify patients suffering from proliferative disease who would respond to treatment using an agent which targets the PD1/PD-L1 pathway, by:

receiving input levels of a plurality of biomarkers selected from those listed in Tables 3A-3D

identified in a tumour sample of a patient;

receiving a further input level relating to the tumour mutational burden of nucleic acid in said sample;

analyzing and transforming the input levels via an algorithm to provide an output indicating the the level of susceptibility of said patient to treatment using an agent which targets a component of the PD1/PD-L1 pathway;

displaying the output on a graphical interface of the processor.

27. A non-transitory computer-readable medium according to claim 26 further storing instructions for developing a customized recommendation for treatment of the patient based

upon the input levels and displaying the customized recommendation on a graphical interface of the processor.