

## PREDICTIVE MIRNAS FOR RESPONSE TO CANCER THERAPY

The present invention relates to a method of predicting a response to cancer therapy of a patient suffering from cancer. Further, the present invention relates to a method of providing a survival prognosis to a patient suffering from cancer. Furthermore, the present invention relates to a method of determining whether to treat a patient suffering from cancer. In addition, the present invention relates to a method of selecting a patient suffering from cancer to benefit from immunochemotherapy or immunotherapy. Moreover, the present invention relates to a kit for carrying out these methods.

### BACKGROUND OF THE INVENTION

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. The abnormal cells are termed cancer cells, malignant cells, or tumor cells. Cancer cells can proliferate uncontrollably and form a mass of cancer cells.

Cancer therapy includes, but is not limited to, immunotherapy, chemotherapy, radiation therapy, vaccination therapy, stem cell therapy, anti-hormonal therapy, immunosuppressive therapy, antibody therapy, and surgical therapy. Standard clinical parameters such as tumor size, grade, lymph node involvement and tumor-node-metastasis (TNM) staging may correlate with outcome and severity to stratify patients with respect to cancer therapy regimens. However, stage-matched tumors grouped by histological subtypes may respond differently to the same treatment regimen.

Immunotherapy has recently gained recognition as highly effective therapy in late-stage cancers. Drugs used in immunotherapy work by blocking the mechanisms by which tumors commonly evade detection and unleashing the immune system to fight the cancer. Alongside the remarkable benefits, immunotherapies can be associated with characteristic (and sometimes severe) side effects. For this reason, it is required to administer immunotherapies only to those patients in which the benefits are predicted to outweigh the risks. The mainstay of response prediction to immunotherapies in the PD-1 inhibitor class (Nivolumab and Pembrolizumab) is the quantification of tumor PD-L1 expression. However, still only approximately 30% of patients will achieve a positive response.

In addition, in late-stage cancers, immunotherapy (IO) alone or immunotherapy in combination with chemotherapy (i.e. immunochemotherapy (ICT)) are recommended as treatment

## CLAIMS

1. A method of predicting a response to lung cancer immunotherapy of a patient suffering from lung cancer comprising the step of:
  - 5 determining the level of at least one miRNA in a blood sample of a patient suffering from lung cancer, wherein the at least one miRNA has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 44, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
- 10 2. The method of claim 1, wherein the at least one miRNA has a nucleotide sequence selected from
  - (i) the group consisting of SEQ ID NO: 1 to SEQ ID NO: 8, a fragment thereof, and a sequence having at least 80% sequence identity thereto, and/or
  - (ii) the group consisting of SEQ ID NO: 1, SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID  
15 NO: 34, SEQ ID NO: 39, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
3. The method of claim 1 or 2, wherein the level of the at least one miRNA is compared to a reference level of said at least one miRNA, wherein the reference level is the level of the  
20 at least one miRNA determined empirically by measuring a number of reference blood samples from subjects suffering from lung cancer who are non-responders to said lung cancer immunotherapy, and wherein  
the level of the at least one miRNA having a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 26, SEQ  
25 ID NO: 28 to SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39 to SEQ ID NO: 42, SEQ ID NO: 44, a fragment thereof, and a sequence having at least 80% sequence identity thereto below the reference level indicates that the patient will respond to said lung cancer immunotherapy, or  
the level of the at least one miRNA having a nucleotide sequence selected from the group  
30 consisting of SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO 38, SEQ ID NO: 43, a fragment thereof, and a sequence having at least 80% sequence identity thereto above the reference level indicates that the patient will respond to said lung cancer immunotherapy.

4. The method of any one of claims 1 to 3, wherein the level of the at least one miRNA is compared to an empirically determined cut-off score which allows to classify the patient as patient who will respond to lung cancer immunotherapy or as a patient who will not respond to lung cancer immunotherapy, and wherein the cut-off score is determined by the weighted sum of one or more miRNAs selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID NO: 34, SEQ ID NO: 39, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
5. The method of any one of claims 1 to 4, wherein the lung cancer immunotherapy is accompanied with the administration of at least one immune checkpoint inhibitor selected from the group consisting of an inhibitor targeting PD-1, PD-L1, PD-L2, CTLA-4, LAG3, TIGIT, CD73 and intrinsic checkpoint blockades.
6. The method of any one of claims 1 to 5, wherein the lung cancer is non-small-cell lung carcinoma (NSCLC).
7. A method of providing a survival prognosis to a patient suffering from lung cancer who is eligible to immunotherapy comprising the step of:  
determining the level of at least one miRNA in a blood sample of a patient suffering from lung cancer, wherein the at least one miRNA has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 44, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
8. The method of claim 7, wherein the at least one miRNA has a nucleotide sequence selected from
  - (i) the group consisting of SEQ ID NO: 1 to SEQ ID NO: 8, a fragment thereof, and a sequence having at least 80% sequence identity thereto, and/or
  - (ii) the group consisting of SEQ ID NO: 1, SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID NO: 34, SEQ ID NO: 39, a fragment thereof, and a sequence having at least 80% sequence identity thereto.

9. The method of claim 7 or 8, wherein the level of the at least one miRNA is compared to a reference level of said at least one miRNA, wherein the reference level is the level of the at least one miRNA determined empirically by measuring a number of reference blood samples from subjects suffering from lung cancer having a known poor survival prognosis, and wherein
- 5 the level of the at least one miRNA having a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28 to SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39 to SEQ ID NO: 42, SEQ ID NO: 44, a fragment thereof, and a sequence having at least 80% sequence identity thereto below the reference level indicates that the patient has a good survival prognosis, or
- 10 the level of the at least one miRNA having a nucleotide sequence selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO 38, SEQ ID NO: 43, a fragment thereof, and a sequence having at least 80% sequence identity thereto above the reference level indicates that the patient has a good survival prognosis.
- 15
10. The method of any one of claims 7 to 9, wherein the level of the at least one miRNA is compared to an empirically determined cut-off score which allows to classify the patient as having a good or poor survival prognosis, wherein the cut-off score is determined by the weighted sum of one or more miRNAs selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 7, SEQ ID NO: 13, SEQ ID NO: 34, SEQ ID NO: 39, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
- 20
11. The method of any one of claims 7 to 10, wherein the patient is a patient to whom a lung cancer immunotherapy will be administered.
- 25
12. The method of claim 11, wherein the lung cancer immunotherapy is accompanied with the administration of at least one immune checkpoint inhibitor selected from the group consisting of an inhibitor targeting PD-1, PD-L1, PD-L2, CTLA-4, LAG3, TIGIT, CD73 and intrinsic checkpoint blockades.
- 30
13. The method of any one of claims 7 to 12, wherein the lung cancer is non-small-cell lung carcinoma (NSCLC).

14. A method of determining whether to treat a patient suffering from lung cancer with immunotherapy comprising the steps of:
- 5 (i) carrying out the method of any one of claims 1 to 6, thereby identifying the patient as patient who will respond to lung cancer immunotherapy, or carrying out the method of any one of claims 7 to 13, thereby identifying the patient as patient having a good survival prognosis under immunotherapy, and
- (ii) assigning the patient to (said) lung cancer immunotherapy.
- 10 15. The method of claim 14, wherein the lung cancer is non-small-cell lung carcinoma (NSCLC).
- 15 16. Use of a kit for predicting a response to lung cancer immunotherapy of a patient suffering from lung cancer, for providing a survival prognosis to a patient suffering from lung cancer who is eligible to immunotherapy, or for determining whether to treat a patient suffering from lung cancer with immunotherapy, wherein said kit comprises:
- means for determining the level of at least one miRNA having a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 44, a fragment thereof, and a sequence having at least 80% sequence identity thereto.
- 20 17. A method of selecting a patient suffering from lung cancer to benefit from immunochemotherapy or immunotherapy comprising the step of:
- determining the level of at least one miRNA in a blood sample of a patient suffering from lung cancer,
- wherein the at least one miRNA has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 (miR-2115-3p), SEQ ID NO: 39 (miR-218-5p), SEQ ID NO: 13 (miR-224-5p), SEQ ID NO: 7 (miR-4676-3p), SEQ ID NO: 34 (miR-6503-5p), a fragment thereof, and a sequence having at least 80% sequence identity thereto.
- 25 18. The method of claim 17, wherein the lung cancer is non-small-cell lung carcinoma (NSCLC).
- 30

19. A method of determining whether to treat a patient suffering from lung cancer with immunochemotherapy or immunotherapy comprising the steps of:

(i) carrying out the method of claim 17 or 18, thereby identifying the patient as patient who will benefit from immunochemotherapy or immunotherapy, and

(ii) assigning the patient to said therapy.

20. Use of a kit for selecting a patient suffering from lung cancer to benefit from immunochemotherapy or immunotherapy, wherein said kit comprises:

means for determining the level of at least one miRNA having a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 (miR-2115-3p), SEQ ID NO: 39 (miR-218-5p), SEQ ID NO: 13 (miR-224-5p), SEQ ID NO: 7 (miR-4676-3p), SEQ ID NO: 34 (miR-6503-5p), a fragment thereof, and a sequence having at least 80% sequence identity thereto in a blood sample of a patient suffering from lung cancer.