

EP 1 777 523 discloses methods for determining the outcome of a cancer in a patient, which are based on the quantification of one or several biological markers that are indicative of the presence of, or alternatively the level of, the adaptive immune response of said patient against said cancer.

5 After extensive researches, for patients without distant metastasis (Stage IV), the inventors have found :

1. That a patient with low expression levels for the genes of the immune adaptive response and high expression levels for the genes representative of the immunosuppressive response not only will have a bad prognosis (e.g. a short disease-free survival time) but also
10 will not significantly improve his survival in case of treatment.

2. that a patient with high expression levels for the genes of the immune adaptive response and low expression levels for the genes representative of the immunosuppressive response will not only have a good prognosis (e.g. a long disease-free survival time) but also will not significantly improve his survival in case of treatment.

15 3. that a patient with high expression levels for the genes of the immune adaptive response and high expression levels for the genes representative of the immunosuppressive response will show an intermediate prognosis, but an antitumoral treatment will a significant impact on his survival (e.g from an intermediate prognosis to a good prognosis).

The two first groups of patients are to be considered as "bad responders" (i.e. the treatment
20 will have a limited (or moderate) impact on their survival), whereas the third group of patients is to be considered as "good responders".

SUMMARY OF THE INVENTION:

A subject of the present disclosure is therefore a method and kits for screening patients
25 with a cancer, particularly for determining whether a patient with a cancer would benefit of an antitumoral treatment.

~~In a first aspect, there is provided a method for screening patients with a cancer~~
comprising: i) determining in a tumor sample obtained from a patient an expression level
ELA₁ - ELA_n of one or several genes GA₁ - GA_n representative of human adaptive immune
30 response and an expression level ELI₁ - ELI_n of one or several genes GI₁ - GI_n representative
of human immunosuppressive response, ii) comparing the expression levels ELA₁ - ELA_n
and ELI₁ - ELI_n determined at step i) with predetermined reference values ELRA₁ - ELRA_n
and ELRI₁ - ELRI_n selected such as said predetermined reference values separate a panel of
patients with a cancer into two groupings according to the expression level of said genes and

to survival of patients according to Kaplan Meier curves analyses and associated logrank p values; iii) concluding whether the patient has a good (level higher than the predetermined reference value) or a bad (level lower than the predetermined reference value) adaptive immune response and a good or a bad immunosuppressive response.

Another ~~aspect~~-embodiment relates to a kit comprising nucleic acids which may be used as primers or probes for implementing the step of determination of an expression level $ELA_1 - ELA_n$ of one or several genes $GA_1 - GA_n$ representative of human adaptive immune response and the expression level $ELI_1 - ELI_n$ of one or several genes $GI_1 - GI_n$ representative of human immunosuppressive response of the method disclosed herein.

A further ~~aspect~~-embodiment relates to a chemo therapeutic agent, an immunotherapeutic agent or a radiotherapeutic agent for use in the treatment of a cancer patient who is considered as responder to antitumoral treatment according to the method disclosed herein.

Yet another ~~aspect~~-embodiment relates to a method of selecting a patient for the treatment of cancer comprising the steps consisting of:

- screening a patient with a cancer according to a method disclosed herein,
- concluding that the patient is a good responder for each of human adaptive immune response and human immunosuppressive response,
- selecting the patient for an appropriate anti cancer treatment.

In one aspect, there is provided a method of assessing the responsiveness of a patient suffering from solid cancer to antitumoral treatment, the method comprising the steps of:

i) determining, in a tumor sample obtained from a patient, an expression level of at least one gene representative of human adaptive immune response and an expression level of at least one gene representative of human immunosuppressive response,

ii) comparing the expression levels of said at least one gene representative of human adaptive immune response and said at least one gene representative of human immunosuppressive response determined at step i) with predetermined reference values selected such as said predetermined reference values separate a panel of patients with a cancer into two groupings according to the expression level of said genes and to survival of patients according to Kaplan Meier curves analyses and associated logrank p values;

iii) concluding whether the patient has a good (level higher than the predetermined reference value) or a bad (level lower than the predetermined reference value) adaptive immune response and a good or a bad immunosuppressive response;

iv) assessing that:

a) when the tumour of the patient is a stage I-III tumour, the patient:

- will advantageously receive an antitumoral treatment (responder to antitumoral treatment) if he is a good responder for each of human adaptive immune response and human immunosuppressive response; or
- will not advantageously receive an antitumoral treatment if he is not a good responder for both of human adaptive immune response and human immunosuppressive response;

b) when the tumour of the patient is a stage IV tumour, the patient:

- will advantageously receive an antitumoral treatment (responder to antitumoral treatment) if he is not a good responder for each of human adaptive immune response and human immunosuppressive response; or
- will not advantageously receive an antitumoral treatment if he is a good responder for both of human adaptive immune response and human immunosuppressive response;

wherein the at least one gene representative of human adaptive immune response is selected from the group consisting of

<u>CCL5</u>
<u>CCR2</u>
<u>CD247</u>
<u>CD3E</u>
<u>CD3G</u>
<u>CD8A</u>
<u>CX3CL1</u>
<u>CXCL11</u>
<u>GZMA</u>
<u>GZMB</u>
<u>GZMH</u>
<u>GZMK</u>

<u>IFNG</u>
<u>IL15</u>
<u>IRF1</u>
<u>ITGAE</u>
<u>PRF1</u>
<u>STAT1</u>
<u>TBX21</u>

and said at least one gene representative of human immunosuppressive response is selected from the group consisting of

<u>CD274</u>
<u>CTLA4</u>
<u>IHH</u>
<u>IL17A</u>
<u>PDCD1</u>
<u>PF4</u>
<u>PROM1</u>
<u>REN</u>
<u>TSLP</u>
<u>VEGF</u>

Another aspect relates to a kit comprising nucleic acids which may be used as primers or probes, when used for implementing the step of determination of an expression level of at least one gene representative of human adaptive immune response and the expression level of at least gene representative of human immunosuppressive response of the disclosed method.

Yet another aspect relates to the use of a chemotherapeutic agent, an immunotherapeutic agent or a radiotherapeutic agent for the manufacture of a medicament for the treatment of a cancer in a patient in need thereof, wherein said patient has been considered as responder to antitumoral treatment according to the disclosed method.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to a method for screening patients with a cancer comprising

CLAIMS

1. A method assessing the responsiveness of a patient suffering from solid cancer to antitumoral treatment comprising the steps of:

5 i) determining, in a tumor sample obtained from a patient, an expression level of at least one gene representative of human adaptive immune response and an expression level of at least one gene representative of human immunosuppressive response,

10 ii) comparing the expression levels of said at least one gene representative of human adaptive immune response and said at least one gene representative of human immunosuppressive response determined at step i) with predetermined reference values selected such as said predetermined reference values separate a panel of patients with a cancer into two groupings according to the expression level of said genes and to survival of patients according to Kaplan Meier curves analyses and associated logrank p values;

15 iii) concluding whether the patient has a good (level higher than the predetermined reference value) or a bad (level lower than the predetermined reference value) adaptive immune response and a good or a bad immunosuppressive response;

iv) assessing that:

a) when the tumour of the patient is a stage I-III tumour, the patient:

- 20 - will advantageously receive an antitumoral treatment (responder to antitumoral treatment) if he is a good responder for each of human adaptive immune response and human immunosuppressive response; or
- 25 - will not advantageously receive an antitumoral treatment if he is not a good responder for both of human adaptive immune response and human immunosuppressive response;

b) when the tumour of the patient is a stage IV tumour, the patient:

- 30 - will advantageously receive an antitumoral treatment (responder to antitumoral treatment) if he is not a good responder for each of human adaptive immune response and human immunosuppressive response; or

- will not advantageously receive an antitumoral treatment if he is a good responder for both of human adaptive immune response and human immunosuppressive response;

5 wherein the at least one gene representative of human adaptive immune response is selected from the group consisting of

CCL5
CCR2
CD247
CD3E
CD3G
CD8A
CX3CL1
CXCL11
GZMA
GZMB
GZMH
GZMK
IFNG
IL15
IRF1
ITGAE
PRF1
STAT1
TBX21

and said at least one gene representative of human immunosuppressive response is selected from the group consisting of

CD274
CTLA4
IHH
IL17A
PDCD1
PF4
PROM1
REN
TSLP
VEGF

2. The method according to claim 1, wherein a single gene representative of the adaptive immune response and a single gene representative of the immunosuppressive response are used in step i).

- 5 3. A method according to one of claim 1 or 2, wherein a pair of gene representative of human adaptive immune response and of gene representative of human immunosuppressive response is selected from the group consisting in:

CCL5 - REN
CCR2 - IL17A
CCR2 - REN
CD247 - IL17A
CD247 - REN
CD3E - REN
CD3G - IL17A
CD3G - REN
CD8A - IL17A
CD8A - REN
CX3CL1 - CTLA4
CX3CL1 - IHH
GZMA - PF4
GZMA - PROM1
GZMA - REN
GZMA - TSLP
GZMA - VEGF
GZMB - REN
GZMH - IL17A
GZMH - REN
GZMK - REN
IFNG - IL17A
IL15 - CD274
IL15 - CTLA4
IL15 - IHH
IL15 - TSLP
IL15 - VEGF
IRF1 - REN
ITGAE - IL17A
ITGAE - REN
PRF1 - REN
STAT1 - REN

TBX21 - REN
GZMK - PDCD1
CD247 - CD274
PRF1 - PDCD1
CCR2 - PF4
CD247 - PDCD1
CD3E - PDCD1
CCR2 - CD274
CCR2 - PDCD1
CCL5 - PDCD1
CD3G - PDCD1
CD8A - PDCD1
TBX21 - PDCD1
CD3G - IHH
CD3G - PF4
CD3G - PROM1
GZMB - PDCD1
GZMH - PDCD1
STAT1 - PDCD1
STAT1 - PF4
CD247 - PF4
CD247 - CTLA4
CD3G - CTLA4
CD8A - CTLA4
PRF1 - CTLA4
CCL5 - CTLA4
TBX21 - CTLA4
STAT1 - IHH
CD247 - IHH
CD247 - PROM1
PRF1 - PF4
CD3G - TSLP
CCR2 - IHH
CCR2 - CTLA4
IFNG - CD274
GZMB - CTLA4
IFNG - PDCD1
CXCL11 - IL17A
CXCL11 - REN

4. A kit comprising nucleic acids which may be used as primers or probes, when used for implementing the step of determination of an expression level of at least one gene representative of human adaptive immune response and the expression level of at least gene representative of human immunosuppressive response of the method of one of claims 1-3.

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5. Use of a chemotherapeutic agent, an immunotherapeutic agent or a radiotherapeutic agent for the manufacture of a medicament for the treatment of a cancer in a patient in need thereof, wherein said patient has been considered as responder to antitumoral treatment according to the method of claim 1.

CLAIMS

1. A method for screening patients with a cancer comprising

5 i) determining in a tumor sample obtained from a patient an expression level $ELA_1 - ELA_n$ of one or several genes $GA_1 - GA_n$ representative of human adaptive immune response and an expression level $ELI_1 - ELI_n$ of one or several genes $GI_1 - GI_n$ representative of human immunosuppressive response,

10 ii) comparing the expression levels $ELA_1 - ELA_n$ and $ELI_1 - ELI_n$ determined at step i) with predetermined reference values $ELRA_1 - ELRA_n$ and $ELRI_1 - ELRI_n$ selected such as said predetermined reference values separate a panel of patients with a cancer into two groupings according to the expression level of said genes and to survival of patients according to Kaplan Meier curves analyses and associated logrank p values

15 iii) concluding whether the patient has a good (level higher than the predetermined reference value) or a bad (level lower than the predetermined reference value) adaptive immune response and a good or a bad immunosuppressive response.

2. A method according to claim 1, wherein the tumour of the patient is a stage I-III tumour.

20 3. A method according to claim 2, further comprising the step of concluding that a patient would advantageously receive an antitumoral treatment (responder to antitumoral treatment) if the patient is a good responder for each of human adaptive immune response and human immunosuppressive response.

25 4. A method according to claim 2, further comprising the step of concluding that a patient would not advantageously receive an antitumoral treatment if the patient is not a good responder for both of human adaptive immune response and human immunosuppressive response.

5. A method according to claim 1, wherein the tumour of the patient is a stage IV tumour.

30 6. A method according to claim 5, further comprising the step of concluding that a patient would advantageously receive an antitumoral treatment (responder to antitumoral treatment) if the patient is not a good responder for each of human adaptive immune response and human immunosuppressive response.

7. A method according to claim 5, further comprising the step of concluding that a patient would not advantageously receive an antitumoral treatment if the patient is a good

responder for both of human adaptive immune response and human immunosuppressive response.

8. A method according to one of claims 1 to 7, wherein genes $GA_1 - GA_n$ representative of human adaptive immune response are selected from the group consisting in:

CCL5
CCR2
CD247
CD3E
CD3G
CD8A
CX3CL1
CXCL11
GZMA
GZMB
GZMH
GZMK
IFNG
IL15
IRF1
ITGAE
PRF1
STAT1
TBX21

9. A method according to one of claims 1 to 8, wherein genes $GI_1 - GI_n$ representative of human immunosuppressive response are selected from the group consisting in:

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CD274
CTLA4
IHH
IL17A
PDCD1
PF4
PROM1
REN
TSLP
VEGF

10. A method according to one of claims 1 to 9, wherein a single gene representative of the adaptive immune response and a single gene representative of the immunosuppressive response are used in step i).

- 5 11. A method according to one of claims 1 to 10, wherein a pair of genes $GA_1 - GA_n$ representative of human adaptive immune response and of genes $GI_1 - GI_n$ representative of human immunosuppressive response are selected from the group consisting in:

CCL5 - REN
CCR2 - IL17A
CCR2 - REN
CD247 - IL17A
CD247 - REN
CD3E - REN
CD3G - IL17A
CD3G - REN
CD8A - IL17A
CD8A - REN
CX3CL1 - CTLA4
CX3CL1 - IHH
GZMA - PF4
GZMA - PROM1
GZMA - REN
GZMA - TSLP
GZMA - VEGF
GZMB - REN
GZMH - IL17A
GZMH - REN
GZMK - REN
IFNG - IL17A
IL15 - CD274
IL15 - CTLA4
IL15 - IHH
IL15 - TSLP
IL15 - VEGF
IRF1 - REN
ITGAE - IL17A
ITGAE - REN
PRF1 - REN
STAT1 - REN

TBX21 - REN
GZMK - PDCD1
CD247 - CD274
PRF1 - PDCD1
CCR2 - PF4
CD247 - PDCD1
CD3E - PDCD1
CCR2 - CD274
CCR2 - PDCD1
CCL5 - PDCD1
CD3G - PDCD1
CD8A - PDCD1
TBX21 - PDCD1
CD3G - IHH
CD3G - PF4
CD3G - PROM1
GZMB - PDCD1
GZMH - PDCD1
STAT1 - PDCD1
STAT1 - PF4
CD247 - PF4
CD247 - CTLA4
CD3G - CTLA4
CD8A - CTLA4
PRF1 - CTLA4
CCL5 - CTLA4
TBX21 - CTLA4
STAT1 - IHH
CD247 - IHH
CD247 - PROM1
PRF1 - PF4
CD3G - TSLP
CCR2 - IHH
CCR2 - CTLA4
IFNG - CD274
GZMB - CTLA4
IFNG - PDCD1
CXCL11 - IL17A
CXCL11 - REN

12. A kit comprising nucleic acids which may be used as primers or probes for implementing the step of determination of an expression level $ELA_1 - ELA_n$ of one or several

genes $GA_1 - GA_n$ representative of human adaptive immune response and the expression level $ELI_1 - ELI_n$ of one or several genes $GI_1 - GI_n$ representative of human immunosuppressive response of the method, of one of claims 1-11.

5 13. A kit according to claim 12, wherein the nucleic acids are selected from nucleic acids specific of the genes of one of claims 8, 9 and 10.

14. A chemo therapeutic agent, an immunotherapeutic agent or a radiotherapeutic agent for use in the treatment of a cancer patient who is considered as responder to antitumoral
10 treatment according to the method of one of claims 3 and 8 to 11.

15. A method of selecting a patient for the treatment of cancer comprising the steps consisting in

- screening a patient with a cancer according to a method of one of claims 1 to 10,
- 15 - concluding that the patient is a good responder for each of human adaptive immune response and human immunosuppressive response,
- selecting the patient for an appropriate anti cancer treatment.