

and more than half of all human genes have multiple promoters, which can be selectively activated as a consequence of normal biological function or disease state. The use of alternative promoters can produce distinct 5' untranslated regions (UTRs) and first exons, enhancing mRNA and protein isoform diversity. In cancer, alternative promoters can generate cancer-specific isoforms with oncogenic properties. Recently, studies have demonstrated that tumours may utilize alternative promoters as a mechanism of immune-editing and evasion. Epigenetically-driven alternative promoter utilization is thus a potential mechanism of resistance to ICI therapy.

[0006] There is therefore a need to develop alternative methods based on alternative promoter utilization to predict patient response to immunotherapy or ICI therapy.

SUMMARY

[0007] In one aspect, there is provided a method of identifying a patient suffering from cancer as benefiting or not benefiting from immune checkpoint inhibition (ICI) therapy comprising the steps of:

- a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient;
- b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a);
- c) calculating an alternative promoter usage score;
- d) identifying the patient as benefiting or not benefiting from ICI therapy using the alternative promoter usage score.

[0007a] In one aspect, there is provided a method of identifying a patient suffering from cancer as benefiting or not benefiting from immune checkpoint inhibition (ICI) therapy comprising the steps of:

- a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient, wherein the preselected marker is a marker that is associated with a somatic promoter region in a cancerous biological sample;
- b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a) relative to a non-cancerous biological sample, wherein an increase in the expression level of the one or

more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a gained promoter indicates a differentially expressed alternative promoter, and wherein a decrease in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a lost promoter indicates a differentially expressed alternative promoter;

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- c) calculating an alternative promoter usage score by (i) determining the sum of the number of gained promoters with at least a 4-fold increase in the expression level of transcripts and the number of lost promoters with an expression level lower than 0.25-fold when compared to the median expression level of transcripts or (ii) determining the sum of the number of gained promoters with a relative promoter activity greater than median and the number of lost promoters with relative promoter activity less than median;
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- d) identifying the patient as benefiting or not benefiting from ICI therapy using the alternative promoter usage score by comparing the alternative promoter usage score calculated in step (c) with a reference score to identify the patient as benefiting or not benefiting from ICI therapy, wherein an increased alternative promoter usage score compared to the reference score identifies the patient as not benefiting from ICI therapy, wherein the reference score is the alternative promoter usage score from one or more non-cancerous biological samples.
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[0008] In another aspect, there is provided a method of determining the prognosis of a patient suffering from cancer comprising the steps of:

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- a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient;
- b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a);
- c) calculating an alternative promoter usage score;
- d) determining the prognosis of the patient using the alternative promoter usage score.

30 [0008a] In one aspect, there is provided a method of determining the prognosis of a patient who is undergoing or has undergone ICI therapy, the method comprising the steps of:

- a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient, wherein the preselected marker is a marker that is associated with a somatic promoter region in a cancerous biological sample;
- 5 b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a) relative to a non-cancerous biological sample, wherein an increase in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a gained promoter indicates a differentially expressed alternative promoter, and wherein a decrease in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a lost promoter indicates a differentially expressed alternative promoter;
- 10 c) calculating an alternative promoter usage score by (i) determining the sum of the number of gained promoters with at least a 4-fold increase in the expression level of transcripts and the number of lost promoters with an expression level lower than 0.25-fold when compared to the median expression level of transcripts or (ii) determining the sum of the number of gained promoters with a relative promoter activity greater than median and the number of lost promoters with relative promoter activity less than median;
- 15 d) determining the prognosis of the patient using the alternative promoter usage score, wherein an increased alternative promoter usage score compared to a reference score indicates a poorer prognosis, wherein the reference score is the alternative promoter usage score from one or more non-cancerous biological samples.
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[0009] In another aspect, there is provided a biomarker for excluding a patient suffering from cancer from ICI therapy, wherein the biomarker is an alternative promoter usage score

Claims

1. A method of identifying a patient suffering from cancer as benefiting or not benefiting from immune checkpoint inhibition (ICI) therapy comprising the steps of:
 - a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient, wherein the preselected marker is a marker that is associated with a somatic promoter region in a cancerous biological sample;
 - b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a) relative to a non-cancerous biological sample, wherein an increase in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a gained promoter indicates a differentially expressed alternative promoter, and wherein a decrease in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a lost promoter indicates a differentially expressed alternative promoter;
 - c) calculating an alternative promoter usage score by (i) determining the sum of the number of gained promoters with at least a 4-fold increase in the expression level of transcripts and the number of lost promoters with an expression level lower than 0.25-fold when compared to the median expression level of transcripts or (ii) determining the sum of the number of gained promoters with a relative promoter activity greater than median and the number of lost promoters with relative promoter activity less than median;
 - d) identifying the patient as benefiting or not benefiting from ICI therapy using the alternative promoter usage score by comparing the alternative promoter usage score calculated in step (c) with a reference score to identify the patient as benefiting or not benefiting from ICI therapy, wherein an increased alternative promoter usage score compared to the reference score identifies the patient as not benefiting from ICI therapy, wherein the reference score is the alternative promoter usage score from one or more non-cancerous biological samples.
2. The method of claim 1, wherein the preselected marker is a nucleic acid molecule or a peptide.

~~3. The method of claim 1, wherein the preselected marker is a marker that is associated with a somatic promoter region in a cancerous biological sample.~~

~~4. The method of any one of claims 1 to 3, wherein the differentially expressed alternative promoter in step (b) is identified by comparing the expression level of the one or more preselected markers in the cancerous biological sample with the expression level of the said one or more preselected markers in one or more reference samples to determine an increase or decrease in expression level of said preselected markers between the cancerous biological sample and the one or more reference samples.~~

~~5. The method of claim 4, wherein an increase in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the one or more preselected markers in the one or more reference samples for a gained promoter indicates a differentially expressed alternative promoter, and wherein a decrease in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the one or more preselected markers in the one or more reference samples for a lost promoter indicates a differentially expressed alternative promoter.~~

~~6.3.~~ The method of claim ~~5~~1 or 2, wherein the gained promoter is a promoter that is gained or increased in a cancerous biological sample compared to a non-cancerous biological sample and wherein the lost promoter is a promoter that is lost or decreased in a cancerous biological sample compared to a non-cancerous biological sample.

~~7.4.~~ The method of any one of claims ~~4~~1 to ~~3~~6, wherein the expression level of the one or more preselected markers in the cancerous biological sample is compared with a percentile expression level of said one or more preselected markers in the one or more non-cancerous biological samples~~reference samples~~, or the mean expression level of said one or more preselected markers in the one or more non-cancerous biological samples~~reference samples~~, or both.

5 ~~8. The method of claim 7, wherein i) an increase in expression level of a preselected marker compared with the median expression level for a gained promoter, or ii) a decrease in expression level of a preselected marker compared with the median expression level for a lost promoter indicates a differentially expressed alternative promoter.~~

~~9. The method of any one of claims 1 to 8, wherein the alternative promoter usage score in step (c) is calculated by determining the sum of the differentially expressed alternative promoters identified in step (b).~~

10 ~~10. The method of any one of claims 1 to 9, wherein the alternative promoter usage score in step (c) is calculated by determining the sum of the number of gained promoters with at least a 4 fold increase in the expression level of transcripts and the number of lost promoters with an expression level lower than 0.25 fold when compared to the median expression level of transcripts.~~

15 ~~11. The method of any one of claims 1 to 9, wherein the alternative promoter usage score in step (c) is calculated by determining the sum of the number of gained promoters with a relative promoter activity greater than median and the number of lost promoters with relative promoter activity less than median.~~

20 ~~12. The method of any one of claims 1 to 11, further comprising the step of comparing the alternative promoter usage score calculated in step (c) with a reference score to identify the patient as benefiting or not benefiting from ICI therapy, wherein an increased alternative promoter usage score compared to the reference score identifies the patient as not benefiting from ICI therapy.~~

25 ~~13. The method of claim 12, wherein the reference score is the alternative promoter usage score from one or more reference samples.~~

30 ~~14.5.~~ The method of any one of claims 12 or 13, wherein the increased alternative promoter usage score is a score above the 66th percentile of alternative promoter usage scores from one or more non-cancerous biological samples~~reference samples~~.

~~15.6.~~ The method of claim ~~5~~14, wherein the increased alternative promoter usage score is a score above the 80th percentile of alternative promoter usage scores from one or more non-cancerous biological samples~~reference samples~~.

- 5 ~~16.7.~~ A method of determining the prognosis of a patient who is undergoing or has undergone ICI therapy, the method comprising the steps of:
- a) measuring an expression level of one or more preselected markers in a cancerous biological sample obtained from the patient, wherein the preselected marker is a marker that is associated with a somatic promoter region in a cancerous biological sample;
 - 10 b) identifying a differentially expressed alternative promoter based on the expression level of the one or more preselected markers measured in step (a) relative to a non-cancerous biological sample, wherein an increase in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a gained promoter indicates a differentially expressed alternative promoter, and wherein a decrease in the expression level of the one or more preselected markers in the cancerous biological sample compared to the expression level of the non-cancerous sample for a lost promoter indicates a differentially expressed alternative promoter;
 - 15 c) calculating an alternative promoter usage score by (i) determining the sum of the number of gained promoters with at least a 4-fold increase in the expression level of transcripts and the number of lost promoters with an expression level lower than 0.25-fold when compared to the median expression level of transcripts or (ii) determining the sum of the number of gained promoters with a relative promoter activity greater than median and the number of lost promoters with relative promoter activity less than median;
 - 20 d) determining the prognosis of the patient using the alternative promoter usage score, wherein an increased alternative promoter usage score compared to a reference score indicates a poorer prognosis, wherein the reference score is the alternative promoter usage score from one or more non-cancerous biological samples.
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~~17. The method of claim 16, further comprising the step of comparing the alternative promoter usage score calculated in step (c) with a reference score to determine the~~

~~prognosis of the patient, wherein an increased alternative promoter usage score compared to a reference score indicates a poorer prognosis.~~

~~18. The method of claim 17, wherein the reference score is the alternative promoter usage score from one or more reference samples.~~

~~19.8.~~ 8. The method of claim ~~7~~17 or ~~18~~, wherein the increased alternative promoter usage score is a score above the 66th percentile of alternative promoter usage scores from one or more non-cancerous biological samples~~reference samples~~.

~~20.9.~~ 9. The method of claim ~~8~~19, wherein the increased alternative promoter usage score is a score above the 80th percentile of alternative promoter usage scores from one or more non-cancerous biological samples~~reference samples~~.

~~21. A biomarker for excluding a patient suffering from cancer from ICI therapy, wherein the biomarker is an alternative promoter usage score that is above the alternative promoter usage score of one or more reference samples, wherein the alternative promoter usage score is the sum of the differentially expressed alternative promoters at each alternative promoter site.~~

~~22. The biomarker of claim 21, wherein the biomarker is an alternative promoter usage score that is above the 66th percentile of alternative promoter usage scores of one or more reference samples.~~

~~23. The biomarker of claim 21, wherein the biomarker is an alternative promoter usage score that is above the 80th percentile of alternative promoter usage scores of one or more reference samples.~~