

Claims:

1. A method of treating cancer in a subject, the method comprising:
 - (a) measuring CXCR3 expression in the tumor sample obtained from the subject that has been previously treated with at least one dose of a checkpoint inhibitor and/or at least one dose of an immunostimulatory cytokine;
 - (b) determining whether CXCR3 expression is increased in the tumor sample relative to CXCR3 expression in a predetermined control; and
 - (c) if CXCR3 expression is increased in the tumor sample relative to CXCR3 expression in the predetermined control, then administering at least one additional dose of the checkpoint inhibitor and/or at least one additional dose of the immunostimulatory cytokine to the subject, or if CXCR3 expression is not increased in the tumor sample relative to CXCR3 expression in the predetermined control, then administering at least one pharmaceutically effective dose of CXCL9 and/or CD3 half-BiTE and at least one additional dose of the checkpoint inhibitor and/or at least one additional dose of the immunostimulatory cytokine to the subject.
2. The method of claim 1, wherein the subject has been previously treated with systemic administration of the at least one dose of the checkpoint inhibitor wherein the checkpoint inhibitor.
3. The method of claim 2, wherein the checkpoint inhibitor comprises an anti-PD-1 antibody, an anti-PD-L1 antibody, nivolumab, pembrolizumab, pidilizumab, or atezolizumab.
4. The method of claim 1, wherein the subject has been previously treated with the at least one dose of the immunostimulatory cytokine wherein the immunostimulatory cytokine was administered by intratumoral electroporation of a nucleic acid encoding the immunostimulatory cytokine.
5. The method of claim 4, wherein the immunostimulatory cytokine comprises IL-12 or IL-15.
6. The method of claim 5, wherein the nucleic acid encoding IL-12 comprises a first nucleic acid sequence encoding an IL-12 p35 subunit and a second nucleic acid sequence

encoding an IL-12 p40 subunit wherein the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES) or a 2A translation modification element.

7. The method of any one of claims 1-6, wherein measuring CXCR3 expression in the tumor sample comprises:

- (a) measuring CXCR3 mRNA in the tumor sample;
- (b) measuring CXCR3 protein in the tumor sample; or
- (c) measuring a number of CXCR3⁺ T cells in the tumor sample.

8. The method of claim 7, wherein the predetermined control comprises:

- (a) a tumor sample obtained from the subject prior to step (a); or
- (b) a standard derived from a population of known responders and/or

known non-responders to checkpoint inhibitor and/or an immunostimulatory cytokine therapy.

9. The method of any one of claims 1-8, wherein administering at least one pharmaceutically effective dose of CXCL9 and/or CD3 half-BiTE comprises intratumoral electroporation of one or more nucleic acids encoding one or more of CXCL9, CD3 half-BiTE, CXCL9 and IL-12, or CD3 half-BiTE and IL-12.

10. The method of claim 1, wherein administering at least one additional dose of the checkpoint inhibitor and/or the least one additional dose of the immunostimulatory cytokine comprises: administering the at least one additional dose of an anti-PD-1 or anti-PD-L1 antibody by systemic administration, administering at least one additional dose of a nucleic acid encoding IL-12 by intratumoral electroporation, or administering of at least one additional dose of an anti-PD-1 or anti-PD-L1 antibody by systemic administration and administering at least one additional dose of a nucleic acid encoding IL-12 by intratumoral electroporation.

11. The method of claim 10, wherein the nucleic acid encoding the IL-12 comprises a first nucleic acid sequence encoding an IL-12 p35 subunit and a second nucleic acid sequence encoding an IL-12 p40 subunit wherein the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES) or a 2A translation modification element.

12. The method of any one of claims 1-11, wherein the cancer is melanoma, basal cell carcinoma, breast cancer, ER positive breast cancer, ER negative breast cancer triple negative breast cancer, or head and neck cancer.

13. A method of identifying a subject with cancer at risk of not responding to checkpoint inhibitor and/or immunostimulatory cytokine therapy, the method comprising:
measuring a level of CXCR3 in a tumor sample obtained from the subject that has been administered at least one dose of a checkpoint inhibitor and/or at least one dose of an immunostimulatory cytokine,

wherein the level of CXCR3 in the tumor sample less than a predetermined control indicates the subject is at risk of not responding to the checkpoint inhibitor and/or immunostimulatory cytokine therapy, and the level of CXCR3 in the tumor sample more than a predetermined control indicates the subject is likely to respond to the checkpoint inhibitor and/or immunostimulatory cytokine therapy.

14. The method of claim 13, wherein measuring the level of CXCR3 in the tumor sample comprises

- (a) measuring CXCR3 mRNA in the tumor sample;
- (b) measuring CXCR3 protein in the tumor sample; or
- (c) measuring a number of CXCR3⁺ T cells in the tumor sample.

15. The method of claim 13 or 14, wherein the predetermined control comprises:

- (a) a tumor sample obtained from the subject prior to the subject being administered the at least one pharmaceutically effective dose of the checkpoint inhibitor and/or the immunostimulatory cytokine; or
- (b) a standard derived from a population of known responders and/or known non-responders to checkpoint inhibitor and/or an immunostimulatory cytokine therapy.

16. A nucleic acid encoding CXCL9 and/or CD3 half-BiTE for use in a method of treating cancer, the method comprising:

- (a) measuring CXCR3 expression in a tumor sample obtained from the subject;
- (b) determining whether CXCR3 expression is increased in the tumor sample relative to CXCR3 expression in a predetermined control; and

(c) administering at least one pharmaceutically effective dose of CXCL9 and/or CD3 half-BiTE and at least one pharmaceutically effective dose of a checkpoint inhibitor and/or at least one pharmaceutically effective dose of an immunostimulatory cytokine to the subject if CXCR3 expression is not increased in the tumor sample relative to CXCR3 expression in the predetermined control.

17. The nucleic acid for use in the method of claim 16, wherein measuring CXCR3 expression in the tumor sample comprises

- (a) measuring CXCR3 mRNA in the tumor sample;
- (b) measuring CXCR3 protein in the tumor sample; or
- (c) measuring a number of CXCR3⁺ T cells in the tumor sample.

18. The nucleic acid for use in the method of claim 17, wherein the subject previously received at least one dose of a checkpoint inhibitor and/or at least one dose of an immunostimulatory cytokine prior to the tumor sample being obtained.

19. The nucleic acid for use in the method of claim 16, wherein the predetermined control comprises:

- (a) a tumor sample obtained from the subject prior to the subject being administered the at least one dose of the checkpoint inhibitor and/or the at least one dose of the immunostimulatory cytokine; or
- (b) a standard derived from a population of known responders and/or known non-responders to checkpoint inhibitor therapy and/or an immunostimulatory cytokine therapy.

20. The nucleic acid for use in the method claim 16, wherein the checkpoint inhibitor comprises an anti-PD-1 antibody, an anti-PD-L1 antibody, nivolumab, pembrolizumab, pidilizumab, or atezolizumab.

21. The nucleic acid for use in the method of claim 20, wherein the checkpoint inhibitor is administered systemically.

22. The nucleic acid for use in the method of claim 16, wherein administering at least one pharmaceutically effective dose of the immunostimulatory cytokine comprises administering a pharmaceutically effective dose of a nucleic acid encoding the immunostimulatory cytokine by intratumoral electroporation.

23. The nucleic acid for use in the method of claim 22, wherein the immunostimulatory cytokine comprises IL-12 or IL-15.

24. The nucleic acid for use in the method of claim 23, wherein the nucleic acid encoding IL-12 comprises a first nucleic acid sequence encoding an IL-12 p35 subunit and a second nucleic acid sequence encoding an IL-12 p40 subunit wherein the first and second nucleic acid sequences are separated by an internal ribosome entry site (IRES) or a 2A translation modification element.

25. The nucleic acid for use in the method of any one of claims 16-24, wherein administering at least one pharmaceutically effective dose of CXCL9 and/or CD3 half-BiTE comprises intratumoral electroporation of one or more nucleic acids encoding one or more of CXCL9, CD3 half-BiTE, CXCL9 and IL-12, or CD3 half-BiTE and IL-12.

26. The nucleic acid for use in the method of any one of claims 25, wherein the one or more nucleic acids encoding one or more of CXCL9, CD3 half-BiTE, CXCL9 and IL-12, and/or CD3 half-BiTE and IL-12 are administered on days 1, 5, and 8 of at least one three or six week cycle.

27. The nucleic acid for use in the method of claim 26, wherein the checkpoint inhibitor is administered on day 1 of at least one three week cycle.

28. The method of any one of claims 1-15 wherein administering at least one pharmaceutically effective dose of CXCL9 and/or CD-3 half-BiTE results in increased numbers of CXCR3⁺ T cells in the tumor.