cells within tumours, alone or in combination with other antibodies or other anti-cancer compounds. As discussed above the infiltration of Treg cells in tumors, and in particular a low ratio of Teff cells to Treg cells, can lead to poor clinical outcome. CD25 has been identified as a Treg marker and could thus be an interesting target for therapeutic antibodies aiming at depleting Treg. Importantly, CD25 is the alpha subunit of the receptor for IL-2 and IL-2 is a key cytokine for Teff responses. Anti-CD25 antibodies that have undergone clinical testing so far, whilst depleting Treg cells also block IL-2 signalling via CD25. The present inventors have now found that such a blockade of IL-2 signalling limits Teff responses and that an anti-CD25 antibody that does not block the IL2 signalling can effectively deplete Treg cells, whilst still allowing IL-2 to stimulate Teff cells, providing antibodies that exhibit a strong anti-cancer effect. Thus, there is a need in the art for a method of treating cancer involving depletion of Tregs, particularly while still allowing IL-2 to stimulate Teff cells, in particular by using appropriate anti-CD25 antibodies.

SUMMARY OF THE DISCLOSURE

[08] The present disclosure provides anti-CD25 antibodies and uses of anti-CD25 antibodies that are characterized by structural elements that allow both binding CD25 without substantially blocking the binding of Interleukin 2 (IL-2) to CD25 or signalling of IL-2 via CD25, and depleting efficiently Tregs, in particular within tumours. The structural and functional features of 7D4-IgM (as described with respect to mouse CD25) have been modified in order to provide antibodies that present surprisingly improved features in terms of use for depleting Tregs and efficacy against tumours, alone or in combination with other anti-cancer agents. Structural and functional features of further anti-CD25 antibodies which do not block the binding of interleukin 2 to CD25 (and do not block the signalling of IL2 via CD25) and efficiently deplete Tregs have also been characterised. These findings can be used for defining and generating further anti-human CD25 antibodies that provide comparable effects against tumours in human subjects. References herein to “anti-CD25 antibodies” and the like include antigen-binding fragments thereof, as well as variants (including affinity matured variants), unless the context implies otherwise.

[08a] In one aspect, the present disclosure provides a use of an anti-CD25 antibody, wherein said antibody does not inhibit the binding of Interleukin-2 (IL-2) to CD25 in the manufacture of a medicament for treating cancer in a human subject ~~who has cancer~~, wherein said subject has a solid tumour, and wherein the medicament is to be administered to the subject.

~~[08b] In another aspect, the present disclosure provides an anti-CD25 antibody for use in medicine, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25.~~

[~~08c~~08b] In another aspect, the present disclosure provides a combination of an anti-CD25 antibody, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, and a further therapeutic agent for use in the treatment of cancer in a human subject, wherein said subject has a solid tumour and the anti-CD25 antibody and the further therapeutic agent are administered simultaneously, separately or sequentially.

[~~08d~~08c] In another aspect, the present disclosure provides a kit for use in the treatment of cancer in a human subject, wherein the subject has a solid tumour, comprising an anti-CD25 antibody wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, and a further therapeutic agent for use in combination therapy.

~~[08e] In another aspect, the present disclosure provides a pharmaceutical composition comprising an anti-CD25 antibody wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25 in a pharmaceutically acceptable medium.~~

~~[08f] In another aspect, the present disclosure provides a bispecific antibody comprising: (a) a first antigen binding moiety that binds to CD25; and (b) a second antigen binding moiety that binds to an immune checkpoint protein; wherein the CD25 binding moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Recepto, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.~~

[~~08g~~08d] In another aspect, the present disclosure provides a use of a bispecific antibody in the manufacture of a medicament for treating cancer in a subject, wherein the subject has a solid tumour, wherein the bispecific antibody comprises (a) a first antigen binding moiety that binds to CD25; and (b) a second antigen binding moiety that binds to an immune checkpoint protein; wherein the CD25 binding moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Receptor, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.

~~[08h] In another aspect, the present disclosure provides a bispecific antibody, for use in the treatment of cancer in a subject, wherein the bispecific antibody comprises (a) a first antigen binding moiety that binds to CD25; and (b) a second antigen binding moiety that binds to an immune checkpoint protein; wherein the CD25 binding moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Receptor, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.~~

[~~08i~~08e] In another aspect, the present disclosure provides a use of an anti-CD25 antibody, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, in the manufacture of a medicament for treating cancer in a human subject, wherein said subject has a solid tumour, wherein the medicament is to be administered with a further therapeutic agent and wherein the medicament and further therapeutic agent are to be administered simultaneously, separately or sequentially.

[09] In a main example, the present disclosure provides a method of treating a human subject who has cancer comprising the step of administering an anti-CD25 antibody to a subject, wherein said subject has a tumour (preferably a solid tumour), wherein said antibody does not inhibit the binding of Interleukin-2 (IL-2) to CD25.

[10] References to “does not block”, “non-blocking”, “non-IL-2 blocking”, “without blocking” and similar terminology herein (with respect to the non-blocking of IL-2 binding to CD25 in the presence of the anti-CD25 antibody) include examples wherein the anti-CD25 antibody does not block the signalling of IL-2 via CD25. That is, the anti-CD25 antibody of the disclosure inhibits less than 50% of IL-2

Claims

1. Use of an anti-CD25 antibody, wherein said antibody does not inhibit the binding of Interleukin-2 (IL-2) to CD25 in the manufacture of a medicament for treating ~~a human subject who has~~ cancer in a human subject, wherein said subject has a solid tumour, and wherein the medicament is to be administered to the subject.

2. The use according to claim 1 wherein the anti-CD25 antibody competes with the antibody 7G7B6 for binding to human CD25; and/or competes with the antibody MA251 for binding to human CD25.

3. The use according to claim 1 or claim 2 wherein the anti-CD25 antibody binds to the same epitope recognised by antibody 7G7B6 and/or the epitope recognised by antibody MA251.

4. The use according to any one of claims 1 to 3 wherein the anti-CD25 antibody specifically binds to an epitope of human CD25 wherein the epitope comprises one or more amino acid residues comprised in one or more of the amino acid stretches selected from amino acids 150-163 of SEQ ID NO:1 (YQCVQGYRALHRGP), amino acids 166-186 of SEQ ID NO:1 (SVCKMTHGKTRWTQPQLICTG), amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 70-88 of SEQ ID NO:1 (NSSHSSWDNQCQCTSSATR).

5. The use according to claim 4 wherein the anti-CD25 antibody specifically binds to an epitope of human CD25 wherein the epitope comprises at least one sequence selected from: amino acids 150-158 of SEQ ID NO:1 (YQCVQGYRA), amino acids 176-180 of SEQ ID NO:1 (RWTQP), amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 74-84 of SEQ ID NO:1 (SSWDNQCQCTS).

6. The use according to claim 4 wherein the anti-CD25 antibody specifically binds to an epitope of human CD25 wherein the epitope comprises at least one sequence selected from: amino acids 150-158 of SEQ ID NO:1 (YQCVQGYRA), amino acids 166-180 of SEQ ID NO:1 (SVCKMTHGKTRWTQP), amino acids 176-186 of SEQ ID NO:1 (RWTQPQLICTG), amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 74-84 of SEQ ID NO:1 (SSWDNQCQCTS).

7. The use according to claim 4 wherein the anti-CD25 antibody specifically binds to an epitope of human CD25 wherein the epitope comprises at least one sequence selected from: amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR), amino acids 70 to 84 of SEQ ID NO: 1 (NSSHSSWDNQCQCTS) and amino acids 150 to 158 of SEQ ID NO: 1 (YQCVQGYRA).

8. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR).

9. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 150-160 of SEQ ID NO:1 (YQCVQGYRALH).

10. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 74-84 of SEQ ID NO:1 (SSWDNQCQCTSSATR).

11. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 150-163 of SEQ ID NO:1 (YQCVQGYRALHRGP), amino acids 166-180 of SEQ ID NO:1 (SVCKMTHGKTRWTQP), amino acids 42-56 of SEQ ID NO:1 (KEGTMLNCECKRGFR) and amino acids 74-84 of SEQ ID NO:1 (SSWDNQCQCTSSATR).

12. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 150-158 of SEQ ID NO:1 (YQCVQGYRA) and amino acids 176-180 of SEQ ID NO:1 (RWTQP).

13. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 150-158 of SEQ ID NO:1 (YQCVQGYRA) and amino acids 176-186 of SEQ ID NO:1 (RWTQPQLICTG).

14. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 150-163 of SEQ ID NO:1 (YQCVQGYRALHRGP) and amino acids 166-180 of SEQ ID NO:1 (SVCKMTHGKTRWTQP)

15. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 74-84 of SEQ ID NO:1 (SSWDNQCQCTS).

16. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 70-84 of SEQ ID NO:1 (NSSHSSWDNQCQCTS).

17. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 176-180 of SEQ ID NO:1 (RWTQP).

18. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids amino acids 166-180 of SEQ ID NO:1 (SVCKMTHGKTRWTQP).

19. The use according to claim 4 wherein the anti-CD25 antibody binds to an epitope comprising the sequence of amino acids 176-186 of SEQ ID NO:1 (RWTQPQLICTG).

20. The use according to any one of claims 1 to 19 wherein the anti-CD25 antibody is an IgG1 antibody that binds to at least one activating Fcγ receptor selected from FcγRI, FcγRIIc, and/or FcγRIIIa with high affinity, and depletes tumour-infiltrating regulatory T cells.

21. The use according to any one of claims 1 to 19, wherein the anti-CD25 antibody~~:~~ ~~(a) binds to Fcγ receptors with an activatory to inhibitory ratio (A/I) superior to 1; and/or (b)~~ binds to FcγRI, FcγRIIc, and/or FcγRIIIa with higher affinity than it binds to FcγRIIb.

22. The use according to any one of claims 1 to 21 wherein the antibody is selected from the group consisting of:

(a) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 3 and the light chain comprising the amino acid sequence of SEQ ID NO:4;

(b) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 5 and the light chain comprising the amino acid sequence of SEQ ID NO: 6;

(c) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 10 and the light chain comprising the amino acid sequence of SEQ ID NO: 14;

(d) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 18 and the light chain comprising the amino acid sequence of SEQ ID NO: 22;

(d) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 and the light chain comprising the amino acid sequence of SEQ ID NO: 25;

(e) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 and the light chain comprising the amino acid sequence of SEQ ID NO: 26;

(f) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 24 and the light chain comprising the amino acid sequence of SEQ ID NO: 25;

(g) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 24 and the light chain comprising the amino acid sequence of SEQ ID NO: 26;

(h) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 27 and the light chain comprising the amino acid sequence of SEQ ID NO: 30;
(i) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 27 and the light chain comprising the amino acid sequence of SEQ ID NO: 31;
(j) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 27 and the light chain comprising the amino acid sequence of SEQ ID NO: 32;
(k) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 27 and the light chain comprising the amino acid sequence of SEQ ID NO: 33;
(l) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and the light chain comprising the amino acid sequence of SEQ ID NO: 30;
(m) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and the light chain comprising the amino acid sequence of SEQ ID NO: 31;
(n) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and the light chain comprising the amino acid sequence of SEQ ID NO: 32;
(o) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and the light chain comprising the amino acid sequence of SEQ ID NO: 33;
(p) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 29 and the light chain comprising the amino acid sequence of SEQ ID NO: 30;
(q) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 29 and the light chain comprising the amino acid sequence of SEQ ID NO: 31;
(r) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 29 and the light chain comprising the amino acid sequence of SEQ ID NO: 32; and
(s) an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 29 and the light chain comprising the amino acid sequence of SEQ ID NO: 33.

23. The use according to any one of claims 1 to 22 wherein the anti-CD25 antibody is a human IgG2 antibody.

24. The use according to any one of claims 1 to 23, wherein the anti-CD25 antibody has a dissociation constant (Kd) for CD25 in the range of ~~less than~~ 10^{-7} M to 10^{-13} M.

25. The use according to any one of claims 1 to 24 wherein the anti-CD25 antibody inhibits IL-2 signalling by less than 50%.

26. The use according to any one of claims 1 to 25, wherein the anti-CD25 antibody is a monoclonal antibody.

27. The use according to any one of claims 1 to 26, wherein the anti-CD25 antibody is a human, chimeric, or humanized antibody.

28. The use according to any one of claims 1 to 27, wherein the antibody is an affinity matured variants thereof.

29. The use according to any one of claims 1 to 28, wherein the antibody the antibody is a humanised and/or affinity matured variant of 7G7B6 or MA251.

30. The use according to any one of claims 1 to 29, wherein the anti-CD25 antibody elicits an enhanced CDC, ADCC and/or ADCP response, preferably an increased ADCC and/or ADCP response, more preferably an increased ADCC response.

31. The use according to any one of claims 1 to 30 wherein the anti-CD25 antibody is to be administered to a subject who has an established tumour.

32. The use according to any one of claims 1 to 31 further comprising the step of identifying a subject who has a solid tumour.

33. The use according to any one of claims 1 to 32 wherein an immune checkpoint inhibitor is to be further administered to said subject.

34. The use according to claim 33 wherein said immune checkpoint inhibitor is a PD-1 antagonist.

35. The use according to claim 34 wherein said PD-1 antagonist is an anti-PD-1 antibody or an anti-PD-L1 antibody.

36. The use according to any one of claims 1 to 32 wherein a cancer vaccine is to be further administered.

37. The use according to claim 36 wherein the cancer vaccine is a GVAX cancer vaccine.

~~38. An anti-CD25 antibody for use in medicine, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25.~~

~~39~~38. ~~An anti-CD25 antibody for use according to claim 38 or t~~The use of an anti-CD25 antibody according to any one of claims 1-30 wherein the antibody is for administration in combination with a further therapeutic agent.

~~40. An anti-CD25 antibody for use according to claim 39 or the use of an anti-CD25 antibody according to claim 39, wherein the further therapeutic agent is an immune checkpoint inhibitor.~~

~~41. An anti-CD25 antibody for use according to claim 40 or the use according to claim 40, wherein the immune checkpoint inhibitor is a PD-1 antagonist.~~

~~42. An anti-CD25 antibody for use according to claim 39 or the use of an anti-CD25 antibody according to claim 39, wherein the further therapeutic agent is a cancer vaccine.~~

~~43~~39. A combination of an anti-CD25 antibody, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, and a further therapeutic agent for use in the treatment of cancer in a human subject, wherein said subject has a solid tumour and the anti-CD25 antibody and the further therapeutic agent are administered simultaneously, separately or sequentially.

~~44~~40. A kit for use in the treatment of cancer in a human subject, wherein the subject has a solid tumour, comprising an anti-CD25 antibody, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, and a further therapeutic agent for use in combination therapy.

~~45. A pharmaceutical composition comprising an anti-CD25 antibody , wherein the antibody is an anti-Cd25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, in a pharmaceutically acceptable medium.~~

~~46. A pharmaceutical composition according to claim 45 further comprising a further therapeutic agent.~~

~~47~~41. The combination for use as claimed in claim ~~43~~39, or the kit of claim ~~44~~40, ~~or the pharmaceutical composition according to claim 46,~~ wherein the further therapeutic agent is an immune checkpoint inhibitor.

~~48~~42. The combination for use as claimed in claim ~~47~~41, or the kit of claim ~~47~~41, ~~or the pharmaceutical composition according to claim 47,~~ wherein the immune checkpoint inhibitor is a PD-1 antagonist.

4943. The combination for use as claimed in claim 4741, or the kit of claim 4741, or the pharmaceutical composition according to claim 47, wherein the further therapeutic agent is a cancer vaccine.

50. A bispecific antibody comprising:
(a) a first antigen binding moiety that binds to CD25; and
(b) a second antigen binding moiety that binds to an immune checkpoint protein;
wherein the CD25 binding moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Receptor, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.

51. The bispecific antibody according to claim 50, wherein the immune checkpoint protein is selected from the group consisting of PD-1, CTLA-4, BTLA, KIR, LAG3, VISTA, TIGIT, TIM3, PD-L1, B7H3, B7H4, PD-L2, CD80, CD86, HVEM, LLT1, GAL9, GITR, OX40, CD137, and ICOS.

52. The bispecific antibody according to claim 50 or 51, wherein the immune checkpoint protein is expressed on a tumour cell.

53. The bispecific antibody according to claim 50 or 51, wherein the immune checkpoint protein is PD-L1.

54. The bispecific antibody according to claim 53, wherein the second antigen binding moiety that binds to PD-L1 is comprised in Atezolizumab.

5544. Use of a bispecific antibody in the manufacture of a medicament for treating cancer in a subject, wherein the subject has a solid tumour, wherein the bispecific antibody comprises (a) a first antigen binding moiety that binds to CD25; and (b) a second antigen binding moiety that binds to an immune checkpoint protein; wherein the CD25 binding moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Receptor, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.

56. The use according to claim 55, wherein the subject has a solid tumour.

57. A bispecific antibody, for use in the treatment of cancer in a subject, wherein the bispecific antibody comprises (a) a first antigen binding moiety that binds to CD25; and (b) a second antigen binding moiety that binds to an immune checkpoint protein; wherein the CD25 binding

~~moiety does not inhibit the binding of Interleukin-2 (IL-2) to CD25, and preferably the bispecific antibody is an IgG1 antibody that binds to at least one activating Fcγ Receptor, selected from FcgRI, FcgRIIa, FcgRIII with a high affinity and depletes tumour-infiltrating regulatory T cells.~~

~~58. The bispecific antibody for use according to claim 57, wherein the subject has a solid tumour.~~

~~59~~45. Use of an anti-CD25 antibody, wherein the antibody is an anti-CD25 antibody that does not inhibit the binding of Interleukin-2(IL-2) to CD25, in the manufacture of a medicament for treating cancer in a human subject, wherein said subject has a solid tumour, wherein the medicament is to be administered with a further therapeutic agent and wherein the medicament and further therapeutic agent are to be administered simultaneously, separately or sequentially.