

[10a] In one aspect, provided herein is an antibody or antigen-binding fragment thereof, comprising:

- a) ~~aCD25-a-686-HCDR3 amino acid the~~ sequence ~~of~~ (SEQ ID NO: 4) as variable heavy chain complementarity determining region 3
- 5 b) a sequence selected from ~~aCD25-a-686-HCDR1 amino acid sequence~~ (SEQ ID NO: 2), ~~GTFSSLAIT~~ (SEQ ID NO: 10), ~~GTFSSALAI~~ (SEQ ID NO: 11) and ~~GTFSSLAIF~~ (SEQ ID NO: 12), as variable heavy chain complementarity determining region 1;
- 10 c) a sequence selected from ~~aCD25-a-686-HCDR2 amino acid sequence~~ (SEQ ID NO: 3), ~~AIIPVFGTASYAQKFQG~~ (SEQ ID NO: 13), ~~GIIPFGDASYAQKFQG~~ (SEQ ID NO: 14), ~~GIIPFGDANYAQKLQG~~ (SEQ ID NO: 15), ~~GIIPFGGRANYAQKFQG~~ (SEQ ID NO: 16) and ~~GIIPVFGQANYAQKFQG~~ (SEQ ID NO: 17), as variable heavy chain complementarity determining region 2;
- 15 d) ~~aCD25-a-686-LCDR1 amino acid the~~ sequence ~~of~~ SEQ ID NO: 6) as variable light chain complementarity determining region 1;
- e) ~~aCD25-a-686-LCDR2 amino acid the~~ sequence ~~of~~ SEQ ID NO: 7) as variable light chain complementarity determining region 2; and
- f) ~~aCD25-a-686-LCDR3 amino acid the~~ sequence ~~of~~ SEQ ID NO: 8) as variable light chain complementarity determining region 3.

20 [10b] In one aspect, provided herein is an affinity matured variant of an antibody or antigen-binding fragment thereof as described herein.

[10c] In one aspect, provided herein is a nucleic acid molecule encoding the antibody or antigen-binding fragment thereof as described herein.

25 [10d] In one aspect, provided herein is a nucleic acid vector comprising the nucleic acid molecule as described herein.

[10e] In one aspect, provided herein is a host cell comprising the nucleic acid vector as described herein.

30 [10f] In one aspect, provided herein is a method for producing an antibody or antigen-binding fragment thereof as described herein comprising culturing a host cell as described herein.

[10g] In one aspect, provided herein is a [pharmaceutical](#) composition comprising an antibody or antigen-binding fragment thereof as described herein and a pharmaceutically acceptable carrier or excipient.

[10h] In one aspect, provided herein is the pharmaceutical composition as described herein, wherein said composition is for use in therapy [in a subject](#).

[10i] In one aspect, provided herein is a use of an antibody or antigen-binding fragment thereof as described herein, or of a composition as described herein in the manufacture of a medicament for treating cancer [in a subject](#).

[10j] In one aspect, provided herein is a method of preparing an anti-CD25 antibody comprising providing an antibody as described herein, and subjecting the antibody to affinity maturation, wherein the anti-CD25 antibody produced has a greater affinity to CD25 than the parental antibody.

[10k] In one aspect, provided herein is a method of preparing a pharmaceutical composition comprising providing an antibody prepared according to a method as described herein and co-formulating the antibody with at least one of more pharmaceutically acceptable excipient.

CLAIMS

1. An antibody or antigen-binding fragment thereof, comprising:

- 5 a) ~~aCD25-a-686-HCDR3 amino acid the~~ sequence of (SEQ ID NO: 4) as variable heavy chain complementarity determining region 3;
- b) a sequence selected from ~~aCD25-a-686-HCDR1 amino acid sequence~~ (SEQ ID NO: 2), ~~GTFSSLAIT~~ (SEQ ID NO: 10), ~~GTFSSALAI~~ (SEQ ID NO: 11) and ~~GTFSSLAIF~~ (SEQ ID NO: 12), as variable heavy chain complementarity determining region 1;
- 10 c) a sequence selected from ~~aCD25-a-686-HCDR2 amino acid sequence~~ (SEQ ID NO: 3), ~~AHPVFGTASYAQKFQG~~ (SEQ ID NO: 13), ~~GIPIFGDASYAQKFQG~~ (SEQ ID NO: 14), ~~GIPIFGDANYAQKLQG~~ (SEQ ID NO: 15), ~~GIPLFGRANYAQKFQG~~ (SEQ ID NO: 16) and ~~GIIPVFGQANYAQKFQG~~ (SEQ ID NO: 17), as variable heavy chain complementarity determining region 2;
- 15 d) ~~aCD25-a-686-LCDR1 amino acid the~~ sequence of (SEQ ID NO: 6) as variable light chain complementarity determining region 1;
- e) ~~aCD25-a-686-LCDR2 amino acid the~~ sequence of (SEQ ID NO: 7) as variable light chain complementarity determining region 2; and
- f) ~~aCD25-a-686-LCDR3 amino acid the~~ sequence of (SEQ ID NO: 8) as variable light chain complementarity determining region 3.
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2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a variable heavy chain comprising a sequence selected from ~~aCD25-a-686-HCDR123 amino acid~~ ~~sequence~~ (SEQ ID NO: 5), ~~aCD25-a-686-m1-HCDR123 amino acid sequence~~ (SEQ ID NO: 18), ~~aCD25-a-686-m2-HCDR123 amino acid sequence~~ (SEQ ID NO: 19), ~~aCD25-a-686-m3-HCDR123 amino acid sequence~~ (SEQ ID NO: 20), ~~aCD25-a-686-m4-HCDR123 amino acid sequence~~ (SEQ ID NO: 21), and ~~aCD25-a-686-m5-HCDR123 amino acid sequence~~ (SEQ ID NO: 22).

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3. The antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the antibody or antigen-binding fragment thereof comprises a variable light chain comprising ~~aCD25-a-686-LCDR123 amino acid~~ ~~(the sequence of~~ SEQ ID NO: 9).

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4. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody, a domain antibody, a single chain antibody, a Fab fragment, a F(ab')₂ fragment, a

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single chain variable fragment (scFv), a scFv-Fc fragment, a single chain antibody (scAb), or a single domain antibody.

5. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof is a rabbit, mouse, chimeric, humanized or fully human antigen-binding antibody.

6. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4 isotype antibodies.

7. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof is comprised in a bispecific antibody, a multispecific antibody, or an immunoconjugate further comprising a therapeutic or diagnostic agent.

8. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof binds the extracellular domain of human CD25.

9. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment thereof binds cells expressing human CD25 on their surface and is an anti-CD25 Antibody Agent.

10. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment does not inhibit the binding of Interleukin-2 (IL-2) to CD25.

11. The antibody or antigen-binding fragment thereof of any preceding claim, wherein the antibody or antigen-binding fragment does not inhibit the signalling of Interleukin-2 (IL-2) via CD25.

12. The antibody or an antigen-binding fragment thereof of any preceding claim wherein the antibody or antigen-binding fragment thereof is afucosylated.

13. An affinity matured variant of an antibody or antigen-binding fragment thereof of any one of claims 1 to ~~16~~12.

14. An antibody or antigen-binding fragment of any of claims 1 to ~~15~~13, wherein the antibody or antigen-binding fragment thereof comprises:

- a) a variable heavy chain comprising ~~a aCD25-a-686-HCDR123 amino acid~~the sequence of {SEQ ID NO: 5} and a variable light chain comprising ~~a aCD25-a-686-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9};
- b) a variable heavy chain comprising ~~a aCD25-a-686-m1-HCDR123 amino acid~~the sequence of {SEQ ID NO: 18} and a variable light chain comprising ~~a aCD25-a-686-m1-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9};
- c) a variable heavy chain comprising ~~a aCD25-a-686-m2-HCDR123 amino acid~~the sequence of {SEQ ID NO: 19} and a variable light chain comprising ~~a aCD25-a-686-m2-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9};
- d) a variable heavy chain comprising ~~a aCD25-a-686-m3-HCDR123 amino acid~~the sequence of {SEQ ID NO: 20} and a variable light chain comprising ~~a aCD25-a-686-m3-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9};
- e) a variable heavy chain comprising ~~a aCD25-a-686-m4-HCDR123 amino acid~~the sequence of {SEQ ID NO: 21} and a variable light chain comprising ~~a aCD25-a-686-m4-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9}; or
- f) a variable heavy chain comprising ~~a aCD25-a-686-m5-HCDR123 amino acid~~the sequence of {SEQ ID NO: 22} and a variable light chain comprising ~~a aCD25-a-686-m5-LCDR123 amino acid~~the sequence of {SEQ ID NO: 9}.

15. A nucleic acid molecule encoding the antibody or antigen-binding fragment thereof of any of the Claims 1 to 14.

16. A nucleic acid vector comprising the nucleic acid molecule of claim 15.

17. A host cell comprising the nucleic acid vector of claim 16.

18. A method for producing an antibody or antigen-binding fragment thereof of any one of claims 1 to 14 comprising culturing a host cell of claim 17.

19. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof of any one of claims 1 to 14 and a pharmaceutically acceptable carrier or excipient.

20. The pharmaceutical composition of claim 19, wherein said composition is for use in therapy in a subject.

21. Use of an antibody or antigen-binding fragment thereof of any one of claims 1 to 14, or of a composition of claim 19- in the manufacture of a medicament for treating cancer in a subject.

22. The composition for use according to claim 20 or the use according to claim 21 wherein the antibody is to be administered simultaneously or sequentially in any order, with a second agent.

23. The composition for use according to claim 20 or 22 or the use according to claim 21 or 22 wherein the second agent is an immune checkpoint inhibitor or a cancer vaccine.

24. The composition for use according to claim 23 or the use according to claim 23 wherein the second agent is an immune checkpoint inhibitor, wherein the immune checkpoint inhibitor is a PD-1 antagonist.

25. The composition for use according to claim 24 or the use according to claim 24 wherein the PD-1 antagonist is an anti-PD-1 antibody or an anti-PD-L1 antibody.

26. The composition for use according to claim 20 or 22 to 25 or the use according to claim 21 to 25 wherein the subject has a solid tumor or a haematological cancer tumour.

27. A method of preparing an anti-CD25 antibody comprising providing an antibody according to any one of claims 1 to 14, and subjecting the antibody to affinity maturation, wherein the anti-CD25 antibody produced has a greater affinity to CD25 than the parental antibody,

28. A method of preparing a pharmaceutical composition comprising providing an antibody prepared according to a method of claim 27 and co-formulating the antibody with at least one of more pharmaceutically acceptable excipient.