

having CD25 binding features similar to those of 7D4 for mouse CD25 have not been evaluated in detail with respect to the optimized depletion of Treg cells.

[08] As discussed above the infiltration of Treg cells in tumors, and in particular a low ratio of effector 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 (specifically a CD25/CD122/CD132 complex). 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.

[09] Thus, there is a need in the art for improved anti-CD25 antibodies, in particular those that do not block the binding of CD25 to IL-2 or IL-2 signalling, and that deplete Tregs, in particular in tumours, and that can be used in methods for treating cancer.

SUMMARY

[10] The present disclosure provides new anti-CD25 Antibody Agents. In some examples, the provided anti-CD25 Antibody Agents are antibodies or antigen-binding fragments that specifically bind to CD25, particularly to human CD25, without blocking the binding of interleukin 2 (IL-2) to CD25, or signaling of IL-2 via CD25, and which efficiently deplete Tregs, in particular within tumors. The anti-CD25 Antibody Agents allow at least 50% of IL-2 signaling in response to IL-2 binding to CD25 compared to the level of signaling in the absence of the anti-CD25 Antibody Agent. The anti-CD25 Antibody Agents are therefore referred to as non-blocking or non-IL-2 blocking anti-CD25 Antibody Agents.

- [10a] In one aspect, provided herein is an antibody or antigen-binding fragment thereof, comprising:
- a) aCD25-a-686-HCDR3 amino acid sequence (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), GTFSALAI (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), GIIPIFGDASYAQKFQG (SEQ ID NO: 14), GIIPIFGDANYAQKLQG (SEQ ID NO: 15), GIIPLFGRANYAQKFQG (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 sequence (SEQ ID NO: 6) as variable light chain complementarity determining region 1;
 - e) aCD25-a-686-LCDR2 amino acid sequence (SEQ ID NO: 7) as variable light chain complementarity determining region 2; and
 - f) aCD25-a-686-LCDR3 amino acid sequence (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.
- [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
- 30 herein.

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

5 [10h] In one aspect, provided herein is the pharmaceutical composition as described herein, wherein said composition is for use in therapy.

[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.

10 [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.

15 [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.

[11] In some examples, the anti-CD25 Antibody agents or antigen-binding fragments bind CD25 without blocking the binding of IL- 2 to CD25 or signalling of IL-2 via CD25. That is, in some examples, the anti-CD25 antibodies inhibit less than 50% of IL-2 signalling compared to IL-2 signalling in the absence of the antibodies. The anti-CD25 antibodies are characterized by structural elements that allow both binding CD25 without blocking the binding of IL-2 to CD25, or its signalling.

[12] It has surprisingly been found that anti-CD25 antibodies that do not block IL-2 binding or signalling via CD25 and deplete Tregs have strong anti-tumour effects, in particular have a stronger anti-tumour effect than anti-CD25 antibodies that deplete Treg but block IL-2 signalling. The antibodies increased the CD4+Teff/Treg and CD8+Teff/Treg ratios. The effective depletion of tumor-infiltrating Treg cells whilst preserving IL-2 signalling on Teff cells leads to a therapeutic approach for use in treating cancer alone or in combination with other anti-cancer agents.

[13] Those skilled in the art, however, will appreciate that teachings of the present disclosure are not limited by a particular mechanism of action of the provided antibodies or antigen-binding fragments thereof. Relevant structural and/or functional features of the provided antibodies are described herein and speak for themselves.

[14] In some examples, the provided anti-CD25 Antibody Agents are characterized by one or more features that are associated with binding to a specific epitope in human CD25 extracellular domain, and/or that render them particularly amenable to pharmaceutical use and/or manufacturing.

[15] The provided technologies, including the provided anti-CD25 Antibody Agents (e.g., the provided antibodies or antigen-binding fragments thereof), compositions including them, and/or uses for them, are useful in medicine. In some examples, such provided technologies are useful in cancer therapy and/or prophylaxis.

[16] In some examples, the provided anti-CD25 Antibody Agents are exemplified by the antibodies having the sequence of aCD25-a-686, and more in general antibodies or agents that are or comprise one or more antigen-binding fragments or portions thereof, for example that comprise the aCD25-a-686-HCDR3 amino acid sequence as variable heavy chain complementarity determining region 3, and/or, in some examples, comprise one or both of the aCD25-a-686 HCDR1 and HCDR2 sequences. References to anti-CD25 Antibody agents herein, such as aCD25-a-686, include variants thereof, including the

affinity matured variants aCD25-a-686-m1, aCD25-a-686-m2, aCD25-a-686-m3, aCD25-a-686-m4 and aCD25-a-686-m5, unless the context implies otherwise.

[17] In one example, there is provided an anti-CD25 Antibody Agent that comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of aCD25-a-686. In another example
5 there is provided an anti-CD25 Antibody Agent that comprises the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of aCD25-a-686. In another example there is provided an anti-CD25 Antibody Agent that comprises the variable heavy amino acid sequence of aCD25-a-686. In another example there is provided an anti-CD25 Antibody Agent that comprises the variable heavy and variable light amino acid sequences of aCD25-a-
10 686, or of a variant thereof. Equivalent anti-CD25 Antibodies Agents based on the affinity matured variants, aCD25-a-686-m1, aCD25-a-686-m2, aCD25-a-686-m3, aCD25-a-686-m4 and aCD25-a-686-m5, are also specifically contemplated.

[18] In some examples, there is provided an anti-CD25 Antibody Agent that competes with aCD25-a-686 (or an antigen-binding fragment or derivative or variant thereof, including affinity
15 matured variants) for binding to human CD25 extracellular domain.

[19] In some examples, the provided anti-CD25 Antibody Agents (e.g. the provided antibodies or antigen-binding fragments thereof, including variants and derivatives thereof, such as affinity matured variants) bind to human CD25 with a K_d in the range of 10^{-7} M or below (e.g. in the 10^{-8} or 10^{-9} or 10^{-10} or 10^{-11} or 10^{-12} or 10^{-13} range). In some examples the
20 provided anti-CD25 Antibody Agents (e.g. the provided antibodies or antigen-binding fragments thereof, including variants and derivatives thereof, such as affinity matured variants) bind to human CD25 with a K_d value from 10^{-7} to 10^{-10} , or from 10^{-7} to 10^{-9} .

[20] In some examples, the provided anti-CD25 Antibody Agents (e.g., the provided antibodies or antigen-binding fragments thereof, including variants and derivatives thereof)
25 bind to an epitope on human CD25 that is bound by aCD25-a-686 (or an antigen-binding fragment or derivative or variant thereof). In some examples, such provided anti-CD25 Antibody Agents may bind to human CD25 extracellular domain. In some examples, the provided anti-CD25 Antibody Agents may bind to an epitope of CD25 (e.g., when assessed using one or more assays as described herein or otherwise known in the art), in particular the
30 one identified as aCD25ep-a (SEQ ID NO: 23). In some examples the provided anti-CD25 Antibody Agents antibodies, or antigen-binding

fragments, according to the present disclosure specifically bind to the epitope designated aCD25ep-a (SEQ ID NO: 23); or aCD25ep-b (SEQ ID NO: 24); or aCD25ep-c (SEQ ID NO: 25) (see Fig. 2), or combinations thereof such as, for example but without limitation, epitopes designated aCD25ep-b and aCD25ep-c, or all three epitopes. In one example of the disclosure, the provided anti-CD25 Antibody Agents antibodies, or antigen-binding fragments, specifically bind to aCD25ep-b and/or aCD25ep-c.

[21] In some examples, the provided antibodies or antigen-binding fragments thereof may bind to human and non-human primate (for example Cynomolgus Monkey) CD25 (e.g., to an extracellular epitope on human and/or Cynomolgus Monkey CD25) with a K_d value in the 10^{-7} M range or below, for example in the 10^{-8} or 10^{-9} or 10^{-10} or 10^{-11} or 10^{-12} or 10^{-13} range. In some examples the provided anti-CD25 Antibody Agents (e.g. the provided antibodies or antigen-binding fragments thereof, including variants and derivatives thereof, such as affinity matured variants) bind to human CD25 with a K_d value from 10^{-7} to 10^{-10} , or from 10^{-7} to 10^{-9} .

[22] Among other things, the present disclosure provides a procedure that can be utilized to identify and/or characterize particularly useful anti-CD25 Antibody Agents (e.g., anti-CD25 antibodies or antigen-binding fragments thereof) as described herein (e.g., anti-CD25 antibodies or antigen-binding fragments thereof characterized by certain structural and/or functional features, such as specific binding to human CD25 (e.g., to an extracellular epitope thereof), inclusion of one or more CDR sequence elements as described herein (and particularly inclusion of an HCDR3 sequence element, optionally in combination with HCDR1 and/or HCDR2 elements), impact on IL-2 signalling activity as described herein, cytotoxic activity as described herein (e.g., with respect to CD25-positive cells such as immune regulatory cells or CD25 expressing cancer cells), and combinations thereof). In some examples, particularly useful anti-CD25 antibodies as described herein are characterized by a plurality of such features. In some examples, one or more antibodies described herein may be characterized as anti-CD25 Antibody Agents.

[23] Thus, as exemplified herein, certain antibodies and/or antigen-binding fragments comprising aCD25-a686 sequences (in particular aCD25-a-686-HCDR3 and/or aCD25-a-686-LCDR3) are characterized by such desirable structural and/or functional features; such antibodies and/or antigen-binding fragments thereof may be referred to herein as anti-CD25 Antibody Agents. Additionally, in accordance with the present disclosure, antibodies and antigen-binding fragments thereof that compete with aCD25-a-686 may be

particularly useful antibodies; such antibodies and/or antigen-binding fragments thereof may also be referred to herein as anti-CD25 Antibody Agents.

[24] Antibodies (and/or antigen-binding fragments thereof) described herein may be particularly useful in medicine (e.g., in therapy and/or in prophylaxis, for example in the treatment of cancer), and/or for use with respect to methods that require or involve targeting an epitope such as the one identified as aCD25ep-a, aCD25ep-b and/or aCD25ep-c (for example aCD25ep-b and/or aCD25ep-c) within human CD25 extracellular domain. The provided antibodies or antigen-binding fragments thereof may be prepared as presenting the most appropriate isotype, in particular human isotype from the group consisting of IgG1, IgG2, IgG3, and IgG4 isotype antibodies, more particularly human IgG1 or human IgG2, preferably human IgG1.

[25] In one example, the present disclosure provides aCD25-a-686-HCDR3 amino acid sequence and polypeptides that include it, such as, for example, antibodies or antigen-binding fragments comprising the aCD25-a-686-HCDR3 amino acid sequence (SEQ ID NO: 4) as variable heavy chain complementarity determining region 3. In some examples, such antibody or antigen-binding fragment may be further characterized by comprising further aCD25-a-amino acid sequence elements such as:

- a) aCD25-a-686-HCDR1 amino acid sequence (SEQ ID NO: 2) as variable heavy chain complementarity determining region 1; and/or
- b) aCD25-a-686-HCDR2 amino acid sequence (SEQ ID NO: 3) as variable heavy chain complementarity determining region 2.

[26] In some examples, provided antibodies or antigen-binding fragments thereof may comprise variable heavy chain complementarity determining regions defined above (i.e. aCD25-a-686 amino acid sequence elements) further in the correct order, specifically separated by antibody frame sequences, such as the ones included in aCD25-a-686-HCDR123 amino acid sequence (SEQ ID NO: 5), in particular for exerting correctly their binding and functional properties. For example, in some examples, a provided antibody or antigen-binding fragment said thereof can comprise aCD25-a-686-HCDR123 amino acid sequence (SEQ ID NO: 5) and, optionally:

- a) aCD25-a-686-LCDR1 amino acid sequence as variable light chain complementarity determining region 1 (SEQ ID NO:6);

- b) aCD25-a-686-LCDR2 amino acid sequence (SEQ ID NO: 7) as variable light chain complementarity determining region 2; and
- c) aCD25-a-686-LCDR3 amino acid sequence (SEQ ID NO: 8) as variable light chain complementarity determining region 3.

5 [27] Thus, in some examples, the present disclosure provides an isolated antibody or antigen-binding fragments thereof comprising a variable heavy chain comprising aCD25-a-686-HCDR123 amino acid sequence (SEQ ID NO: 5). Preferably, such isolated antibody or antigen-binding fragments thereof further comprises a variable light chain comprising aCD25-a-686-LCDR123 amino acid sequence (SEQ ID NO: 9), as described in the Examples.

10 Moreover, aCD25-a-686 amino acid sequences also refer to antibody sequences that are defined by a number of substitutions with respect to the aCD25-a-686 amino acid sequence elements defined above. For example, such sequence may comprise, as variable heavy chain complementarity determining region 3 (HCDR3) a sequence containing 1, 2, 3, 4, or more amino acid substitutions within aCD25-a-686-HCDR3 (SEQ ID NO:4). In a further example,

15 aCD25-a-686 amino acid sequences also refer to antibody sequences comprising, as variable heavy chain complementarity determining regions 1, 2 and 3 (HCDR1, HCDR2, and HCDR3) a sequence containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions within aCD25-a-686-HCDR1 (SEQ ID NO: 2), aCD25-a-686-HCDR2 (SEQ ID NO: 3), and aCD25-a-686-HCDR3 (SEQ ID NO: 4), and more preferably a sequence containing 1, 2, 3, 4, 5, 6, 7, 8

20, 9, 10 or more amino acid substitutions within aCD25-a-686-HCDR123 (SEQ ID NO: 5). The antibodies presenting such aCD25-a-686 amino acid sequence elements and such substitutions can still present the binding and/or functional properties of aCD25-a-686, and of anti-CD25 Antibody Agents in general.

[28] Accordingly, in one example, the present disclosure provides an anti-CD25 Antibody Agent (i.e. an antibody or antigen-binding fragment thereof) comprising:

- a. the variable heavy chain region sequence of aCD25-a-686 (SEQ ID NO: 5) (or a variant thereof, such as an affinity matured variant thereof) or a variable heavy chain region sequence having up to 5 amino acid substitutions compared to the variable heavy chain region sequence of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof); and/or

5 b. the variable light chain region sequence of aCD25-a-686 (SEQ ID NO: 9) (or a variant thereof, such as an affinity matured variant thereof) or a variable light chain region sequence having up to 5 amino acid substitutions compared to the variable light chain region sequence of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof).

[29] In some examples, the amino acid substitutions do not occur in an CDR sequence.

[30] In some examples, the present disclosure provides an anti-CD25 Antibody Agent (i.e. an antibody or antigen-binding fragment thereof) comprising:

10 a. the variable heavy chain region sequence of aCD25-a-686 (SEQ ID NO: 5) (or a variant thereof, such as an affinity matured variant thereof) or a variable heavy chain region sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the variable heavy chain region sequence of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof); and/or

15 b. the variable light chain region sequence of aCD25-a-686 (SEQ ID NO: 9) (or a variant thereof, such as an affinity matured variant thereof) or a variable light chain region sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the variable light chain region sequence of aCD25-a-686
20 (or a variant thereof, such as an affinity matured variant thereof).

[31] In some examples, the % sequence identity is calculated without the sequences of all 6 CDRs of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof, as disclosed herein). For example, the anti-CD25 Antibody Agent may comprise a variable heavy chain region sequence having at least 95% identity to the variable heavy chain region
25 sequence of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof) and/or a variable light chain region sequence having at least 95% identity to the variable light chain region sequence of aCD25-a-686 (or a variant thereof, such as an affinity matured variant thereof), wherein any amino acid variations occur only in the framework regions of the variable heavy and light chain region

sequences. In such examples, the anti-CD25 Antibody Agents having certain sequence identities comprise the complete heavy and light chain CDR1, CDR2 and CDR3 sequences of the anti-CD25 Antibody Agent from which they are derived.

[32] The disclosure also provides variant antibodies or antigen-binding fragments thereof of the antibodies described herein. The disclosure provides antibodies or antigen-binding fragments thereof wherein any DG motif in the light or heavy chains of the antibodies may be altered, for example to reduce aspartate isomerization and/or wherein any NG motif in the light or heavy chains of the antibodies may be altered, for example to reduce asparagine deamidation and/or wherein any methionine in the light or heavy chains of the antibodies may be altered, for example to reduce methionine oxidation. For example, a DG motif may be altered to substitute one or both of the amino acids in the motif with a different amino acid. An NG motif may be altered to substitute one or both of the amino acids in the motif with a different amino acid. For example, such motifs may be mutated to EG, DQ or DA. A methionine residue may be altered to replaced it with a different amino acid, for example leucine or phenylalanine.

[33] In such examples, the anti-CD25 antibody or antigen-binding fragment thereof may be, or may be derived from, for example, aCD25-a-686. The variant anti-CD25 antibodies provide further antibodies having any, and possibly all, binding and functional properties of aCD25-a-686. For example, the variant anti-CD25 antibodies are non-IL-2 blocking antibodies.

[34] Accordingly, in some examples, the antibodies or fragments thereof provided herein can be mutated to remove or modify DG motifs or NG motifs, in particular DG or NG motifs appearing in the CDR regions, as is standard in the art to reduce susceptibility to chemical modification. Such antibodies that have been modified in this way may need to undergo further modification (for example affinity maturation) before arriving at a final sequence.

[35] In one example of the disclosure, there is provided a variant antibody having CDR1, CDR2 and CDR3 sequences of an antibody as disclosed herein (for example the CDR1, CDR2 and CDR3 sequences of aCD25-a-686), or the variable heavy and variable light chain of any antibody as disclosed herein (for example the variable heavy and variable light chain of any of aCD25-a-686), but differing from the specified sequence in that at least one or at least two DG or NG motifs in the CDRs (if present) have been changed to a different motif. The disclosed variants may be used and formulated as described for aCD25-a-686.

[36] In one example the anti-CD25 antibody or antigen-binding fragment thereof comprises the amino acid sequence of aCD25-a-686, wherein the methionine in the framework 4 region is substituted, preferably with leucine or alanine, i.e. amino acid residue 117M of aCD25-a-686-HCDR123 (as shown in Figure 1) is replaced with leucine or alanine.

5 The disclosure also provides affinity matured antibodies, for example affinity matured variants derived from any of the antibodies disclosed herein. In some examples the antibody or antigen-binding fragments thereof comprise:

- 10 - an HCDR2 region selected from the group consisting of the aCD25-a-686-m1-HCDR2 amino acid sequence (SEQ ID NO: 13), the aCD25-a-686-m2-HCDR2 amino acid sequence (SEQ ID NO: 14), the aCD25-a-686-m3-HCDR2 amino acid sequence (SEQ ID NO: 15), the aCD25-a-686-m4-HCDR2 amino acid sequence (SEQ ID NO: 16) and the aCD25-a-686-m5-HCDR2 amino acid sequence (SEQ ID NO: 17) and/or
- 15 - an HCDR1 region selected from the group consisting of the aCD25-a-686-m2-HCDR1 amino acid sequence (SEQ ID NO: 10), the aCD25-a-686-m3-HCDR1 amino acid sequence (SEQ ID NO: 2), the aCD25-a-686-m4-HCDR1 amino acid sequence (SEQ ID NO: 11) and the aCD25-a-686-m5-HCDR1 amino acid sequence (SEQ ID NO: 12).

In some examples of the disclosure the variant antibodies or antigen-binding fragments thereof have the heavy CDR sequences as provided in Table 2 (e.g. of aCD25-a-686-m1, 20 aCD25-a-686-m2, aCD25-a-686-m3, aCD25-a-686-m4 or aCD25-a-686-m5). In some examples, the variant antibody or antibody binding fragment thereof may have the light chain CDR sequences as provided in Table 3 (e.g. of aCD25-a-686-m1, aCD25-a-686-m2, aCD25-a-686-m3, aCD25-a-686-m4 or aCD25-a-686-m5). The present disclosure specifically provides the affinity matured variants of aCD25-a-686 referred to herein as aCD25-a-686-m1, 25 aCD25-a-686-m2, aCD25-a-686-m3, aCD25-a-686-m4 and aCD25-a-686-m5, as well as fragments thereof. The disclosed affinity matured variants may be used and formulated as described for aCD25-a-686.

[37] In one example, the variant antibody or antibody binding fragment thereof may comprise a variable heavy chain comprising a sequence selected from aCD25-a-686-m1- 30 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). In one example, the variant antibody or antibody binding fragment thereof may comprise a variable light chain comprising a sequence selected from aCD25-a-686-m1-LCDR123 amino acid sequence (SEQ ID NO: 9), aCD25-a-686-m2-LCDR123 amino acid sequence (SEQ ID NO: 9), aCD25-a-686-m3-LCDR123 amino acid sequence (SEQ ID NO: 9), aCD25-a-686-m4-LCDR123 amino acid sequence (SEQ ID NO: 9), and aCD25-a-686-m5-LCDR123 amino acid sequence (SEQ ID NO: 9). Variants having amino acid substitutions as described for aCD25-a-686 are also provided (for example variants having up to 5 amino acid substitutions in each of the heavy and light chain variable regions). In one example, the affinity matured antibodies are affinity matured antibodies having an altered DG motif and/or NG motif and/or altered to remove or mutate any methionine residues.

[38] In some examples the anti-CD25 antibody or antigen-binding fragment thereof comprises the amino acid sequence of aCD25-a-686m1, aCD25-a-686m2, aCD25-a-686m3, aCD25-a-686m4 or aCD25-a-686m5, wherein the methionine in the framework 4 region is substituted, preferably with leucine or alanine, i.e. amino acid residue 117M of aCD25-a-686m1-HCDR123 (as shown in Figure 14 (SEQ ID NO: 18)), amino acid residue 117M of aCD25-a-686m2-HCDR123 (as shown in Figure 15 (SEQ ID NO: 19)), amino acid residue 117M of aCD25-a-686m3-HCDR123 (as shown in Figure 16 (SEQ ID NO: 20)), amino acid residue 117M of aCD25-a-686m4-HCDR123 (as shown in Figure 17 (SEQ ID NO: 21)) or amino acid residue 117M of aCD25-a-686m5-HCDR123 (as shown in Figure 18 (SEQ ID NO: 22)) is replaced with leucine or alanine.

[39] In some examples the disclosure provides a method of preparing an anti-CD25 antibody comprising providing an antibody as herein described (e.g., aCD25-a-686 or an antigen binding fragment or variant thereof), and subjecting the antibody to affinity maturation, wherein the antibody produced binds to CD25 with greater affinity than the parental antibody. Preferably the produced antibody binds to CD25 with at least 20%, at least 30%, at least 40%, more preferably at least 50% greater affinity than the parental antibody binds to CD25, for example as measured by the K_d. Methods for measuring affinity are known in the art and described in the Examples below. The affinity matured antibodies produced by such methods can be formulated and used as described herein for the other anti-CD25 Antibody Agents.

[40] Affinity maturation may be carried out according to any suitable method known to the skilled person. For example, *in vitro* antibody display systems are widely used for the generation of specific antibodies with high affinity. In these systems, the phenotype (i.e., the antibody fragment) is coupled to the genotype (i.e., the antibody gene) allowing the direct determination of the sequence of the antibody. Several systems have been developed to achieve display of antibody repertoires to allow subsequent selection of binders and by increasing the stringency of selection allows for the selection of higher and higher affinity variants. The antibody fragments can be expressed in yeast, ribosomes, phage display particles or by direct coupling to DNA.

[41] Current antibody affinity maturation methods belong to two mutagenesis categories: stochastic and non-stochastic. Error-prone polymerase chain reaction (PCR), mutator bacterial strains, and saturation mutagenesis are typical examples of stochastic mutagenesis methods. Non-stochastic techniques often use alanine-scanning or site-directed mutagenesis to generate limited collections of specific variants. In addition, shuffling approaches to obtain shuffled variants of the parent antibody can also be used to improve antibodies affinity further.

[42] Accordingly, in one example of the disclosure, the method of affinity maturation is selected from the group consisting of stochastic mutagenesis (for example error-prone polymerase chain reaction (PCR), mutator bacterial strains, or saturation mutagenesis), non-stochastic mutagenesis (for example alanine-scanning or site-directed mutagenesis), shuffling (for example DNA shuffling, chain shuffling or CDR shuffling) and the use of the CRISPR-Cas9 system to introduce modifications.

[43] Affinity maturation methods are described in, for example, Rajpal *et al.*, Proc Natl Acad Sci USA, 2005, 102(24):8466-71, Steinwand *et al.*, MAbs, 2014, 6(1):204-18, as well as in Handbook of Therapeutic Antibodies, Wiley, 2014, Chapter 6, Antibody Affinity (pages 115–140).

[44] In some examples there is provided a method of preparing a pharmaceutical composition comprising providing an antibody prepared according to a method above, (i.e. for producing an antibody by affinity maturation) and co-formulating the antibody with at least one or more pharmaceutically acceptable excipients. The antibody used in the preparation of the pharmaceutical composition can be an affinity matured variant of aCD25-a-686. The pharmaceutical compositions produced by such methods can be used in the methods of treatment of the present disclosure as described herein for the other anti-CD25 Antibody Agents.

[45] Antibodies and/or antigen-binding fragments that specifically bind CD25 as described herein (e.g., an anti-CD25 Antibody Agent that may include one or more aCD25-a-686 amino acid sequence elements such as aCD25-a-686-HCDR3 or aCD25-a-686-HCDR123, and/or that may compete with aCD25-a-686 for binding to human CD25 and non-human primate CD25, 5 for example Cynomolgus monkey CD25, etc.) may be provided in any of a variety of formats. For example, in some examples an appropriate format may be or comprise a monoclonal antibody, a domain antibody, a single chain antibody, a Fab fragment, a F(ab')₂ fragment, a single chain variable fragment (scFv), a scFv-Fc fragment, a single chain antibody (scAb), an aptamer, or a nanobody. In some examples, an antibody or antigen-binding fragment thereof 10 (and particularly a monoclonal antibody), may be a rabbit, mouse, chimeric, humanized or fully human antibody or antigen-binding fragment thereof. In some examples, a provided antibody or antigen-binding fragment thereof may be of an IgG, IgA, IgE, or IgM isotype (preferably human ones), as it can be most appropriate for a given use. In some examples, a provided antibody or antigen-binding fragment thereof is an IgG isotype, more particularly an IgG1, 15 IgG2, IgG3, or IgG4 isotype. (In some examples the antibody or antigen-binding fragment thereof is from the human IgG1 subclass. In another example the antibody or antigen-binding fragment thereof is from the human IgG2 subclass). In some examples, a provided antibody or antigen-binding fragment thereof (e.g., is provided as part of a multi-specific binding agent such as, for example, when it is desirable to associate further binding and/or functional 20 moieties to anti-CD25 Antibody Agents such as a aCD25-a-686 amino acid sequence, the isolated antibody or antigen-binding can be comprised in a bispecific antibody, a multispecific antibody, or other multi-specific format that may be available in the art.

[46] In some examples, a provided anti-CD25 Antibody Agent comprises a CD25-binding entity (e.g., an anti-CD25 antibody or antigen-binding fragment thereof) and a conjugated 25 payload such as a therapeutic or diagnostic agent. In many such examples, the agent is considered and/or referred to as an “immunoconjugate”. Examples of technologies and compounds that can be used for generating specific immunoconjugates such as antibody-drug are disclosed in the literature (Beck A et al., 2017) and described as applicable to several known anti-CD25 antibodies (O'Mahony D et al, 2008; Oh et al, 2017; Kreitman RJ et al, 2016; 30 Flynn MJ et al 2016).

[47] In some examples, the present disclosure provides aCD25-a-686 amino acid sequences that identify provided antibodies or antigen-binding fragments thereof (or a variant thereof, including the provided affinity matured variants). In some examples,

such sequences identify provided antibodies or antigen-binding fragments thereof that bind an epitope in human CD25 (such as aCD25ep-a, aCD25ep-b and/or aCD25ep-c [for example aCD25ep-b and/or aCD25ep-c]), and optionally also a corresponding epitope of a non-human primate CD25, e.g. Cynomolgus monkey and/or of a murine CD25, either as isolated proteins
5 or on the surface of cells expressing CD25 (such as immune cells or cell lines, e.g. SU-DHL1 cells).

[48] In some examples, the present disclosure provides anti-CD25 antibodies or antigen-binding fragments that specifically bind to an epitope of human CD25, wherein the epitope comprises one or more amino acid residues comprised in the amino acids 70-84 of SEQ ID
10 NO:1. Preferably the epitope comprises at least 4 amino acids wherein the epitope comprises one or more amino acids comprised in amino acids 70-84 of SEQ ID NO:1. Preferably the epitope comprises at least 5 amino acids, at least 6 amino acids, at least seven amino acids, at least eight amino acids, at least nine amino acids, at least ten amino acids, at least eleven amino acids, at least twelve amino acids, at least thirteen amino acids, or at least fourteen or
15 more amino acids wherein the epitope comprises one or more amino acids comprised in amino acids 70-84 of SEQ ID NO:1. The epitope may be either linear or conformational, i.e. discontinuous. In some examples, the anti-CD25 antibodies or antigen-binding fragments specifically bind to an epitope of human CD25 wherein the epitope comprises at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at
20 least ten, at least eleven, at least twelve, at least thirteen, or at least fourteen or more amino acid residues comprised in amino acids 70-84 of SEQ ID NO:1. In some examples, the anti-CD25 antibodies or antigen-binding fragments bind to an epitope comprising amino acids 70-84 of SEQ ID NO:1.

[49] In some examples, the present disclosure provides procedures for screening and/or
25 characterizing antibodies or antigen-binding fragments that comprise a aCD25-a-686 amino acid sequence and/or that present binding features comparable to antibodies or antigen-binding fragments thereof comprising one or more aCD25-a-686 amino acid sequence elements (e.g. including aCD25-a-686-HCDR3 amino acid sequence and/or competing with aCD25-a-686) that allow binding to human CD25 extracellular domain as isolated protein and
30 on the surface of cells expressing human CD25, competing for the same epitope in particular the one identified in the Examples as aCD25ep-a (protein sequence NSSHSSWDNQCQCTS (SEQ ID NO: 23); amino acids 70-84 in Uniprot sequence P01589), the one identified in the Examples as aCD25ep-b (protein sequence

KAMAYKEGTMNLNCECKRGFR (SEQ ID NO: 24)), and/or the one identified in the Examples as aCD25ep-c (protein sequence ATERIYHF (SEQ ID NO: 25)).

[50] Furthermore, the present disclosure also provides procedures for screening antibodies or antigen-binding fragments thereof that present functional features comparable to antibodies or antigen-binding fragments thereof comprising one or more aCD25-a-686 amino acid sequence elements, such features including lack of inhibition of the interaction between IL-2 and CD25, lack of inhibition of IL-2 signalling, and cytotoxic activities, and acting as anti-CD25 Antibody Agents. At these scopes, the candidate antibodies can be tested in the assays that are described in the Examples or other assays that are known in the art for establishing the presence of any of such features, but possibly all of them when evaluated in in vitro/ex vivo assays, cell-based assays, and/or animal models.

[51] In some examples, the present disclosure provides nucleic acid molecules encoding an isolated antibody or antigen-binding fragment thereof that comprises an anti-CD25 Antibody Agent such as an aCD25-a-686 amino acid sequence (or a variant thereof, including the provided affinity matured variants). In some examples, such provided nucleic acid molecules may contain codon-optimized nucleic acid sequences, and/or may be included in expression cassettes within appropriate nucleic acid vectors for the expression in host cells such as, for example, bacterial, yeast, insect, piscine, murine, simian, or human cells.

[52] In some examples, the present disclosure provides host cells comprising heterologous nucleic acid molecules (e.g. DNA vectors) that express a provided anti-CD25 Antibody Agent (e.g., an antibody or antigen-binding fragment thereof) having one or more properties, e.g., as described herein, of an anti-CD25 Antibody Agent (e.g., comprising a aCD25-a-686 amino acid sequence). In some examples, the present disclosure provides methods of preparing an anti-CD25 Antibody Agent (e.g., an antibody or antigen-binding fragment thereof) having one or more properties, e.g., as described herein, of an anti-CD25 Antibody Agent (e.g. comprising a aCD25-a-686 amino acid sequence). In some examples, such methods may comprise culturing a host cell that comprises nucleic acids (e.g., heterologous nucleic acids that may comprise and/or be delivered to the host cell via vectors). In some examples, such a host cell (and/or the heterologous nucleic acid sequences) is/are arranged and constructed so that the anti-CD25 Antibody Agent (e.g. the antibody or antigen-binding fragment thereof) is secreted from the host cell (e.g., so that it can be isolated from cell culture supernatants), and/or

exposed on the cell surface (for instance, if such aCD25-a-686 amino acid sequences and sequence elements are intended to be used in the context of, or together with, such cells, as in artificial T cell receptors grafting the specificity of a monoclonal antibody onto T cells).

[53] In some examples the antibody or antigen-binding fragment may be afucosylated. It is well known that antibody glycosylation may have impact on the activity, pharmacokinetics and pharmacodynamics of antibodies (e.g., monoclonal antibodies, recombinant antibodies, and/or antibodies that are otherwise engineered or isolated) and Fc-fusion proteins and specific technology may be exploited to obtain an antibody with the desired glycosylation profile (Liu L, 2015). Effector functions supporting the cytotoxicity of an antibody for use in accordance with the present disclosure (e.g., an anti-CD25 antibody as described herein, including for example an antibody which may be or be described as an anti-CD25 Antibody Agent) can be enhanced using methods to decrease antibody fucosylation levels. Antibodies comprising specific aCD25-a-686 sequence elements presenting such properties can be generated, for example, by expressing a aCD25-a-686 sequence using technologies for genetically engineering cell lines with absent or reduced fucosylation capacity, some of them commercially available such as Potelligent (Lonza), GlyMAXX (ProBiogen), those provided by Evitria, or by manipulating the manufacturing process, for example by controlling osmolarity and/or using enzyme inhibitors, see also for example the methods described in EP2480671.

[54] In some examples, the present disclosure provides compositions (e.g. pharmaceutical compositions) comprising a provided antibody or an antigen-binding fragment thereof having desirable properties as described herein (e.g., as described for antibodies that are herein termed anti-CD25 Antibody Agents, specifically including, for example, aCD25-a-686 antibodies or antigen-binding fragments thereof or antibodies or antigen-binding fragments thereof that compete with aCD25-a-686 antibodies for binding with CD25). In some examples, such provided compositions are intended for and/or are used in a medical use, such as a therapeutic, diagnostic, or prophylactic use. In some examples, such a provided composition can further comprise a pharmaceutically acceptable carrier or excipient and/or may be for use in the treatment of cancer. In some examples, a pharmaceutical composition may be formulated with one or more carrier, excipients, salts, buffering agents, etc., as is known in the art. Those of skill in the art will be aware of and readily able to utilize a variety of formulation technologies, including as may be particularly desirable and/or useful for a given method and/or site of administration, for instance for parenteral (e.g. subcutaneous, intramuscular, or

intravenous injection), mucosal, intratumoral, peritumoral, oral, or topical administration. In many examples, provided pharmaceutical compositions, comprising anti-CD25 Antibody Agent as described herein (e.g., an anti-CD25 antibody or antigen binding portion thereof, are formulated for parenteral delivery (e.g., by injection and/or infusion). In some examples, such a provided pharmaceutical composition may be provided, for example, in a pre-loaded syringe or vial format. In some examples, such a provided pharmaceutical composition may be provided and/or utilized, for example, in dry (e.g., lyophilized) form; alternatively, in some examples, such a provided pharmaceutical composition may be provided and/or utilized in a liquid form (e.g., as a solution, suspension, dispersion, emulsion, etc), in a gel form, etc.

[55] In some examples, the present disclosure provides uses of anti-CD25 Antibody Agents (e.g., anti-CD25 antibodies or antigen-binding fragments thereof) as described herein (e.g. comprising a aCD25-a-686 amino acid sequence element), and/or of a composition comprising them, in treatment of and/or in the manufacture of a medicament for treatment of, a cancer, such as a B cell malignancy, a lymphoma, (Hodgkins Lymphoma, non-Hodgkins lymphoma, chronic lymphocytic, leukemia, acute lymphoblastic leukemia, myelomas), a myeloproliferative disorder, a solid tumor (such as a breast carcinoma, a squamous cell carcinoma, a colon cancer, a head and neck cancer, a lung cancer, a genitourinary cancer, a rectal cancer, a gastric cancer, sarcoma, melanoma, an esophageal cancer, liver cancer, testicular cancer, cervical cancer, mastocytoma, hemangioma, eye cancer, laryngeal cancer, mouth cancer, mesothelioma, skin cancer, rectal cancer, throat cancer, bladder cancer, breast cancer, uterine cancer, prostate cancer, lung cancer, pancreatic cancer, renal cancer, stomach cancer, gastric cancer, non-small cell lung cancer, kidney cancer, brain cancer, and ovarian cancer). The cancer can be also defined on the basis of presence of specific tumor-relevant markers and antigens such as CD20, HER2, PD-1, PD-L1, SLAM7F, CD47, CD137, CD134, TIM3, CD25, GITR, CD25, EGFR, etc or a cancer that has been identified as having a biomarker referred to as microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR). Furthermore, such conditions may also be considered when defining pre-cancerous, non-invasive states of the above cancers, such as cancer in-situ, smouldering myeloma, monoclonal gammopathy of undetermined significance, cervical intra-epithelial neoplasia, MALTomas/GALTomes and various lymphoproliferative disorders. Preferably in some examples the subject being treated has a solid tumor.

[56] Thus, in some examples, the present disclosure provides methods of treating cancer in a subject, comprising administering to the subject an effective amount of a

composition comprising a provided anti-CD25 Antibody Agent (e.g., anti-CD25 antibodies or antigen-binding fragments thereof) as described herein (e.g. comprising aCD25-a-686 amino acid sequences) or an antibody or antigen-binding fragment thereof that competes with an antibody comprising aCD25-a-686 amino acid sequences for the binding of CD25. Preferably in some examples the subject has a solid tumor.

[57] Thus, in some examples, the present disclosure provides a method of depleting regulatory T cells in a subject comprising the step of administering to the subject an effective amount of a composition comprising a provided anti-CD25 Antibody Agent (e.g., anti-CD25 antibodies or antigen-binding fragments thereof) as described herein (e.g. comprising aCD25-a-686 amino acid sequences) or an antibody or antigen-binding fragment that competes with an antibody comprising aCD25-a-686 amino acid sequences for the binding of CD25. In one example the subject has a solid tumor. In one example the subject has a haematological cancer.

[58] In some examples, provided methods may further comprise administering, simultaneously or sequentially in any order, at least one additional agent or therapy to the subject (i.e., so that the subject receives a combination therapy). In some examples, such an at least one additional agent or therapy can be or comprise an anticancer drug (e.g., a chemotherapeutic agent), radiotherapy (by applying irradiation externally to the body or by administering radio-conjugated compounds), an anti-tumor antigen or marker antibody (the antigen or marker being for example CD4, CD38, CA125, PSMA, c-MET, VEGF, CD137, VEGFR2, CD20, HER2, HER3, SLAMF7, CD326, CAIX, CD40, CD47, or EGF receptor), a checkpoint inhibitor or an immunomodulating antibody (for example an antibody targeting PD-1, PD-L1, TIM3, CD38, GITR, CD134, CD134L, CD137, CD137L, CD80, CD86, B7-H3, B7-H4, B7RP1, LAG3, ICOS, TIM3, GAL9, CD28, AP2M1, SHP-2, OX-40, VISTA, TIGIT, BTLA, HVEM, CD160, etc.), a vaccine (in particular, a cancer vaccine, for example GVAX), an adjuvant, standard-of-use protocol, one or more other compounds targeting cancer cells or stimulating an immune response against cancer cells, or any combination thereof. Preferably the additional agent is an immune checkpoint inhibitor such as a PD-1 antagonist, for example an anti-PD-1 antibody or an anti-PD-L1 antibody. In certain particular examples, when such at least one additional agent or therapy is or comprises an antibody, the format of and/or the antigen targeted by such antibody can be chosen among those listed in the literature and possibly adapted to a given cancer (Sliwkowski M & Mellman I, 2013; Redman JM et al., 2015; Kijanka M et al., 2015). Such antigens and corresponding antibodies include, without limitation CD22 (Blinatumomab), CD20 (Rituximab, Tositumomab), CD56 (Lorvotuzumab), CD66e/CEA

(Labetuzumab), CD152/CTLA-4 (Ipilimumab), CD221/IGF1R (MK-0646), CD326/Epcam (Edrecolomab), CD340/HER2 (Trastuzumab, Pertuzumab), and EGFR (Cetuximab, Panitumumab). In examples when the at least one additional agent or therapy is a chemotherapeutic agent, the chemotherapeutic agent can be those known in the art for use in cancer therapy. Such chemotherapeutic agents includes, without limitation, alkylating agents, anthracyclines, epothilones, nitrosoureas, ethylenimines/methylmelamine, alkyl sulfonates, alkylating agents, antimetabolites, pyrimidine analogs, epipodophylotoxins, enzymes such as L- asparaginase; biological response modifiers such as IFN α , IFN- γ , IL-2, IL-12, G-CSF and GM-CSF; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin, anthracenediones, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p'-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; non-steroidal antiandrogens such as flutamide; molecules that target TGF β pathways, IDO (indoleamine deoxygenase), Arginase, and/or CSF1R; PARP inhibitors such as olaparib, Veliparib, Iniparib, Rucaparib; and MET inhibitors such as tivantinib, foretinib, golvatinib, cabozantinib and crizotinib.

[59] Still further, the present disclosure provides a variety of kits or articles of manufacture containing a provided anti-CD25 Antibody Agent (e.g., anti-CD25 antibody or antigen-binding fragment thereof) as described herein (e.g. comprising aCD25-a-686 amino acid sequences) or related compositions that allow the administration, storage, or other use of such an isolated antibody or antigen-binding fragment. In some examples, a provided kit comprises a vessel, syringe, a vial, or other container comprising such compositions, optionally together with one or more articles of manufactures, diluents, reagents, solid phases, and/or instructions for the correct use of the kit.

[60] In some examples, identification, characterization, and/or validation of particular anti-CD25 Antibody Agent (e.g., anti-CD25 antibody or antigen-binding fragment thereof) as described herein (e.g. comprising aCD25-a-686 amino acid sequences) for a

particular use, such as a medical use, and in particular for treating cancer, can be performed by using one or more assays or systems as described herein. In some examples, such identification, characterization, and/or validation may involve analysis of activity in one or more cell-based assays, for example using different experimental set-ups and/or a panel of selected (e.g., cancer-derived cell lines). In some examples, particularly given the proposed immunological mechanism associated certain desirable anti-CD25 Antibody Agents as described herein activities, desirable identification, characterization, and/or validation can involve collection of relevant data generated in animal models wherein cancers are induced or wherein cancer cells are implanted as a xenograft or as a syngeneic/alogeneic cancer-derived cells. Alternatively or additionally, in some examples, animal models may be utilized that involve transfer of human cells such as PBMC (i.e. humanized PBMC mouse models) or CD34+ hematopoietic stem cells alone (i.e. CD34+ humanized mice) or CD34+ hematopoietic stem cells together with liver and thymus (e.g. NSG-BLT mice) to allow evaluating activity of the anti-CD25 Antibody Agents on human immune cells within a model system.

[61] In some examples, relevant sequences of anti-CD25 Antibody Agents (e.g., anti-CD25 antibody or antigen-binding fragments thereof) as described herein (e.g. comprising aCD25-a-686 amino acid sequences or otherwise including structural and/or functional characteristics of an agent described herein as anti-CD25 Antibody Agent) can be cloned into and/or expressed in context of an antibody frame that is more appropriate or desirable for pharmaceutical and/or technical reasons. For example, such sequences (possibly as codon-optimized VH and VL coding sequences) can be cloned together with human IgG1 constant regions (hIgG1) and expressed using an appropriate antibody expression vectors and cell line (such as a CHO-derived cell line, e.g. CHO-S). In some particular examples, expression and secretion of provided antibody sequences in human IgG1 format antibodies can be analyzed after transfection in reduced conditions in cell lysates and in non-reduced conditions in supernatants that will be later used to purify the antibody (by affinity chromatography, gel filtration, and/or other appropriate technique). Binding and/or other functional properties of provided anti-CD25 antibody sequences, in human IgG1 format (e.g., anti-CD25 Antibody Agents-hIgG1) can be analysed, for example by using one or more assays described in Examples below. For instance, such hIgG1-format provided antibodies can be evaluated for binding to human and cynomolgus PBMC or purified Treg or in vitro derived Treg or CD25 positive cell lines (such as SU-DHL-1 or Karpas299), e.g., using flow cytometry.

CLAIMS

1. An antibody or antigen-binding fragment thereof, comprising:
- 5 a) aCD25-a-686-HCDR3 amino acid sequence (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), AIIPVFGTASYAQKFQG (SEQ ID NO: 13), GIIPIFGDASYAQKFQG (SEQ ID NO: 14), GIIPIFGDANYAQKLQG (SEQ ID NO: 15), GIIPLFGRANYAQKFQG (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 sequence (SEQ ID NO: 6) as variable light chain complementarity determining region 1;
- e) aCD25-a-686-LCDR2 amino acid sequence (SEQ ID NO: 7) as variable light chain complementarity determining region 2; and
- 20 f) aCD25-a-686-LCDR3 amino acid sequence (SEQ ID NO: 8) as variable light chain complementarity determining region 3.
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
- 25 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).
- 30 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 (SEQ ID NO: 9).
- 35 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

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,
5 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,
10 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
15 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
20 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,
25 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,
30 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
35 thereof of any one of claims 1 to 16.

- 5 14. An antibody or antigen-binding fragment of any of claims 1 to 15, wherein the antibody or antigen-binding fragment thereof comprises:
- a) a variable heavy chain comprising a aCD25-a-686-HCDR123 amino acid sequence (SEQ ID NO: 5) and a variable light chain comprising a aCD25-a-686 - LCDR123 amino acid sequence (SEQ ID NO: 9);
 - 10 b) a variable heavy chain comprising a aCD25-a-686-m1-HCDR123 amino acid sequence (SEQ ID NO: 18) and a variable light chain comprising a aCD25-a-686-m1-LCDR123 amino acid sequence (SEQ ID NO: 9);
 - c) a variable heavy chain comprising a aCD25-a-686-m2-HCDR123 amino acid sequence (SEQ ID NO: 19) and a variable light chain comprising a aCD25-a-686-m2-LCDR123 amino acid sequence (SEQ ID NO: 9);
 - 15 d) a variable heavy chain comprising a aCD25-a-686-m3-HCDR123 amino acid sequence (SEQ ID NO: 20) and a variable light chain comprising a aCD25-a-686-m3-LCDR123 amino acid sequence (SEQ ID NO: 9);
 - e) a variable heavy chain comprising a aCD25-a-686-m4-HCDR123 amino acid sequence (SEQ ID NO: 21) and a variable light chain comprising a aCD25-a-686-m4-LCDR123 amino acid sequence (SEQ ID NO: 9); or
 - 20 f) a variable heavy chain comprising a aCD25-a-686-m5-HCDR123 amino acid sequence (SEQ ID NO: 22) and a variable light chain comprising a aCD25-a-686-m5-LCDR123 amino acid sequence (SEQ ID NO: 9).
- 25 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.
- 30 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.
- 35 19. A 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.
- 5 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.
- 10 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.
- 15 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.
- 20 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 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.
- 30 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.
- 35 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.