

SUMMARY

[0014] The present disclosure addresses the above needs and provides novel antibodies that are specific for human CD3, in particular antibodies specific for the CD3 ϵ domain. The solution provided by the present disclosure, i.e. CD3-binding molecules, in particular anti-CD3 antibodies obtained by peptide immunization of rabbits and screening of affinity matured memory B-cells, and in particular CD3-binding molecules, in particular anti-CD3 antibodies, in particular antibodies specific for the CD3 ϵ domain, with the required cross-reactivity profile, has so far not been achieved or suggested by the prior art. Novel CD3 antibodies of the present disclosure exhibit the desired affinity and potency profile, are cross-reactive with other species, in particular with non-human primates such as cynomolgus monkeys, both *in vitro* and in a cellular context. In addition, the antibodies of the present disclosure have favorable biophysical properties, such as quality, stability or solubility, for example as defined by the percentage of antibody in monomer form and thermal unfolding determined by Differential Scanning Fluorimetry (DSF).

[0015] In a first aspect, the present disclosure refers to an antibody or functional fragment thereof, which is specific for human CD3, comprising:

(a) a variable light chain, wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein said LCDR1 is as set forth in SEQ ID NO: 1; said LCDR2 is as set forth in SEQ ID NO: 2; and said LCDR3 is as set forth in SEQ ID NO: 3; and wherein said variable light chain exhibits at least 95 % sequence identity to the amino acid sequence according to SEQ ID NO: 4;
and

(b) a variable heavy chain, wherein the variable light chain comprises, from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, and wherein said HCDR1 is as set forth in SEQ ID NO: 5; said HCDR2 is as set forth in SEQ ID NO: 6; and said HCDR3 is as set forth in SEQ ID NO: 7, wherein said variable heavy chain exhibits at least 95 % sequence identity to the amino acid sequence according to SEQ ID NO: 8, and wherein said antibody or functional fragment thereof is characterized by one or more of the following parameters: (i) a KD value for the binding to human CD3 of less than 40 nM as measured by surface plasmon resonance; (ii) a KD value for the binding to cynomolgous CD3 of less than 20 nM as measured by surface plasmon resonance; and (iii) an average midpoint of thermal unfolding

temperature (T_m) exceeding at least 60°C, when expressed in the scFv antibody format, determined by differential scanning fluorimetry.

[0016] In a second aspect, the present disclosure refers to a multispecific polypeptide comprising the antibody as disclosed herein or functional fragment thereof.

[0017] In a third aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or functional fragment thereof as disclosed herein, or the multispecific polypeptide as disclosed herein, and a pharmaceutically acceptable carrier and/or excipient.

[0018] In a fourth aspect, the present disclosure refers to the antibody or functional fragment thereof as disclosed herein, or the multispecific polypeptide of the present invention for use as a medicament.

[0019] In a fifth aspect, the present disclosure refers to a nucleic acid sequence or a collection of nucleic acid sequences encoding the antibody or functional fragment thereof as disclosed herein.

[0020] In a sixth aspect, the present disclosure refers to a vector or a collection of vectors comprising the nucleic acid sequence or the collection of nucleic acid sequences as disclosed herein.

[0021] In one example, there is disclosed a host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences as described herein, or the vector or collection of vectors as described herein.

[0022] In a seventh aspect, the present disclosure refers to a method for producing the antibody or functional fragment thereof as disclosed herein, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences as described herein, or the vector or collection of vectors as described herein. In one example, there is described a method for producing the antibody or functional fragment thereof of the present invention, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of the present invention, or the vector or collection of vectors of the present invention, or the host cell, particularly the expression host cell, of the present invention.

[0023] In an eighth aspect, the present disclosure refers to a method of generating a multispecific construct, comprising the step of cloning, in one or more steps, one or more

nucleic acid sequences encoding the antibody or functional fragment thereof as described herein, into a multispecific construct comprising a nucleic acid sequence encoding at least a second binding domain or a fragment thereof, and, optionally, a nucleic acid sequence encoding one or more additional binding domains or fragments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **Figure 1** shows monomer content of each sample determined by SE-HPLC initially (d0) and over a period of 28 days of storage at 4°C and at a concentration of 10 mg/mL.

[0025] **Figure 2** shows the T-cell mediated target cell depletion induced by PRO460 (IL23RxCD3_{12C} Amgen) and PRO389 (IL23RxCD3_{1st gen} Numab) using human PBMCs. The left panel shows cell lysis of target-expressing cells, while the right panel shows cell lysis of target-negative cells. The numerical value of the half maximal effective concentration (EC₅₀) for the molecules in the presence of target-expressing cells is depicted below the graphs.

[0026] **Figure 3** shows the T-cell activation of the molecules PRO460 (IL23RxCD3_{12C} Amgen) and PRO389 (IL23RxCD3_{1st gen} Numab) determined by the FC assay. The left panel shows activation in the presence of target-expressing cells, while the right panel shows activation in the presence of target-negative cells.

[0027] **Figure 4** shows the T-cell mediated target cell depletion induced by PRO624 (IL23RxCD3_{2nd gen} Numab) and PRO389 (IL23RxCD3_{1st gen} Numab) using human PBMCs. The left panel shows cell lysis of target-expressing cells, while the right panel shows cell lysis of target-negative cells. The numerical value of the half maximal effective concentration (EC₅₀) for the molecules in the presence of target-expressing cells is depicted below the graphs.

[0028] **Figure 5** shows the T-cell activation of the molecules PRO624 (IL23RxCD3_{2nd gen} Numab) and PRO389 (IL23RxCD3_{1st gen} Numab) determined by the FC assay. The left panel shows activation in the presence of target-expressing cells, while the right panel shows activation in the presence of target-negative cells.

[0029] **Figure 6** shows the T-cell activation of the molecules PRO624 (IL23RxCD3_{2nd gen} Numab) and PRO389 (IL23RxCD3_{1st gen} Numab) determined by the NFAT reporter gene assay. The left panel shows activation in the presence of target-expressing

CLAIMS

1. An antibody or functional fragment thereof, which is specific for human CD3, comprising:
 - (a) a variable light chain, wherein the variable light chain comprises, from N-terminus to C-terminus, the regions LFW1-LCDR1-LFW2-LCDR2-LFW3-LCDR3-LFW4, wherein each LFW designates a light chain framework region, and each LCDR designates a light chain complementarity-determining region, and wherein said LCDR1 is as set forth in SEQ ID NO: 1; said LCDR2 is as set forth in SEQ ID NO: 2; and said LCDR3 is as set forth in SEQ ID NO: 3, and wherein said variable light chain exhibits at least 95 % sequence identity to the amino acid sequence according to SEQ ID NO: 4;and
 - (b) a variable heavy chain, wherein the variable light chain comprises, from N-terminus to C-terminus, the regions HFW1-HCDR1-HFW2-HCDR2-HFW3-HCDR3-HFW4, wherein each HFW designates a heavy chain framework region, and each HCDR designates a heavy chain complementarity-determining region, and wherein said HCDR1 is as set forth in SEQ ID NO: 5; said HCDR2 is as set forth in SEQ ID NO: 6; and said HCDR3 is as set forth in SEQ ID NO: 7, wherein said variable heavy chain exhibits at least 95 % sequence identity to the amino acid sequence according to SEQ ID NO: 8,and
wherein said antibody or functional fragment thereof is characterized by one or more of the following parameters:
 - (i) a K_D value for the binding to human CD3 of less than 40 nM as measured by surface plasmon resonance;
 - (ii) a K_D value for the binding to cynomolgous CD3 of less than 20 nM as measured by surface plasmon resonance; and
 - (iii) an average midpoint of thermal unfolding temperature (T_m) exceeding at least 60°C, when expressed in the scFv antibody format, determined by differential scanning fluorimetry.
2. The antibody or functional fragment thereof according to claim 1, wherein said variable light chain comprises an Arginine or a Lysine at the light chain amino acid position 54 according to AHo numbering, preferably an Arginine.

3. The antibody or functional fragment thereof according to claim 1 or 2, wherein said variable light chain comprises the amino acid sequence of SEQ ID NO: 4, and said variable heavy chain comprises the amino acid sequence of SEQ ID NO: 8.
4. A multispecific polypeptide comprising the antibody or functional fragment thereof according to any one of claims 1 to 4.
5. A pharmaceutical composition comprising the antibody or functional fragment thereof of any one of claims 1 to 4 or the multispecific polypeptide of claim 5, and a pharmaceutically acceptable carrier and/or excipient.
6. The antibody or functional fragment thereof of any one of claims 1 to 4, or the multispecific polypeptide of claim 5, or the composition of claim 6 for use as a medicament.
7. A nucleic acid sequence or a collection of nucleic acid sequences encoding the antibody or functional fragment thereof according to any one of claims 1 to 4.
8. A vector or a collection of vectors comprising the nucleic acid sequence or the collection of nucleic acid sequences of claim 8.
9. A method for producing the antibody or functional fragment thereof of any one of claims 1 to 4, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of claim 8, or the vector or collection of vectors of claim 9.
10. A method of generating a multispecific construct, comprising the step of

cloning, in one or more steps, one or more nucleic acid sequences encoding the antibody or functional fragment thereof according to any one of claims 1 to 4, into a multispecific construct comprising a nucleic acid sequence encoding at least a second binding domain or a fragment thereof, and, optionally, a nucleic acid sequence encoding one or more additional binding domains or fragments thereof.

11. The method of claim 11, wherein said second binding domain is a second antibody or functional fragment thereof.