[0014] The solution for this problem that has been provided by the present invention, i.e. CD3-binding molecules, in particular anti-CD3 antibodies obtained by genetic immunization of rabbits and screening of affinity matured memory B-cells, and in particular CD3-binding molecules, in particular anti-CD3 antibodies, with specificity for a novel agonistic epitope, has so far not been achieved or suggested by the prior art.

SUMMARY OF THE INVENTION

[0015] The present invention relates to novel isolated CD3-binding molecules, in particular isolated antibodies or functional fragments thereof, each comprising a binding region, particularly an antigen-binding region, wherein said binding molecules, in particular said antibodies or functional fragments thereof, are specific for an epitope of human CD3, particularly for a novel agonistic epitope of CD3ε, wherein said binding molecules, in particular said isolated antibodies or functional fragments thereof, have a higher affinity than the prior art antibodies, particularly OKT-3 and/or TR66, while simultaneously exhibiting a higher potency.

[0015a] In one aspect, there is provided an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding, and wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding, and wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, and a VL domain selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21.

[0015b] In one aspect, there is provided an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, (i) is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M; and (iia), upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; (iic) is resulting in a dose-dependent homogeneous activation state of T-cells; and (iid) is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding, and wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding.

[0015c] In one aspect, there is provided an isolated antibody or functional fragment thereof binding to essentially the same epitope as the isolated antibody or functional fragment thereof as described herein.

[0015d] In one aspect, there is provided a pharmaceutical composition comprising the isolated antibody or functional fragment thereof as described herein, and optionally a pharmaceutically acceptable carrier and/or excipient.

[0015e] In one aspect, there is provided a nucleic acid sequence or a collection of nucleic acid sequences encoding the isolated antibody or functional fragment thereof as described herein.

[0015f] In one aspect, there is provided a vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences as described herein.

[0015g] In one aspect, there is provided 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.

[0015h] In one aspect, there is provided a method for producing the isolated antibody or functional fragment thereof as described 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, or the host cell, particularly an expression host cell, as described herein.

[0016] Thus, in one aspect, the present invention relates to an isolated binding molecule comprising a binding region that is specific for an epitope of human CD3ε, in particular to an isolated antibody or functional fragment thereof comprising an antigen-binding region, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

[0017] In one aspect, the present invention relates to a novel isolated CD3-binding molecule that is specific for an epitope of human CD3, wherein said isolated CD3-binding molecule is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M, in particular to an isolated antibody or functional fragment

CLAIMS

1. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding, and wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding, and wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, and a VL domain selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21.

2. The isolated antibody or functional fragment thereof of claim 1, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain selected from SEQ ID NO: 10 and 22, and a VL domain selected from SEQ ID NO: 9 and 21.

3. The isolated antibody or functional fragment thereof of claim 1, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH/VL domain combination selected from SEQ ID NO: 1/SEQ ID NO: 2; SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 7/SEQ ID NO: 8, SEQ ID NO: 9/SEQ ID NO: 10, SEQ ID NO: 11/SEQ ID NO: 12, SEQ ID NO: 13/SEQ ID NO: 14, SEQ ID NO: 15/SEQ ID NO: 16, SEQ ID NO: 17/SEQ ID NO: 18, SEQ ID NO: 19/SEQ ID NO: 20, and SEQ ID NO: 21/SEQ ID NO: 22; particularly SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 9/SEQ ID NO: 10, and SEQ ID NO: 21/SEQ ID NO: 22.

4. The isolated antibody or functional fragment thereof of claim 3, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH/VL domain combination selected from SEQ ID NO: 9/SEQ ID NO: 10 and SEQ ID NO: 21/SEQ ID NO: 22.

5. The isolated antibody or functional fragment thereof of any one of claims 1 to 4, wherein said isolated antibody or functional fragment thereof comprises an antigen-

binding region which is obtained by humanizing an antigen-binding region of any one of claims 1 to 4.

6. A pharmaceutical composition comprising the isolated antibody or functional fragment thereof of any one of claims 1 to 5, and optionally a pharmaceutically acceptable carrier and/or excipient.

7. A nucleic acid sequence or a collection of nucleic acid sequences encoding the isolated antibody or functional fragment thereof of any one of claims 1 to 5.

8. A vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences of claim 7.

9. A host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences of claim 7, or the vector or collection of vectors of claim 8.

10. A method for producing the isolated antibody or functional fragment thereof of any one of claims 1 to 6, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of claim 7, or the vector or collection of vectors of claim 8, or the host cell, particularly an expression host cell, of claim 9.