

**Claims**

1. A humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises a mutation in one of the three CDR sequences of a reference antibody having the CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3, which mutation is in one of the positions selected from the group consisting of: T31M, T31P, N57, H101, G105, S110 and Y114, wherein the positions are numbered according to the reference sequence of SEQ ID NO: 4.
2. The antibody according to claim 1, wherein said antibody has a reduced or increased binding affinity to human CD3 compared to the reference antibody having the VH CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3.
3. The antibody according to any one of claims 1 to 2, wherein said antibody comprises a T31M or T31P mutation.
4. The antibody according to any one of claims 1 to 2, wherein said antibody comprises a mutation in position N57.
5. The antibody according to any one of claims 1, 2 and 4, wherein said antibody comprises a N57E mutation.
6. The antibody according to any one of claims 1 to 2, wherein said antibody comprises a mutation in position H101.
7. The antibody according to any one of claims 1, 2 and 6, wherein said antibody comprises an H101G or H101N mutation.
8. The antibody according to any one of claims 1 to 2, wherein said antibody comprises a mutation in position G105.
9. The antibody according to any one of claims 1, 2 and 8, wherein said antibody comprises a G105P mutation.

10. The antibody according to any one of claims 1 to 2, wherein said antibody comprises a mutation in position Y114.
11. The antibody according to any one of claims 1, 2 and 10, wherein said antibody comprises a Y114M, Y114R or Y114V mutation.
12. The antibody according to any one of claims 1 to 2, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences selected from the group consisting of;
- a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
  - b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
  - c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
  - d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
  - e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
  - f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
  - g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
  - h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
  - i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
  - j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
  - k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V]; and
  - l) CDR1, CDR2 and CDR3 sequences having at least 90% or at least 95% amino acid sequence identity, in total across the three CDR sequences, to any one of the three CDR sequences as set forth in a) to k), provided that the CDR1, CDR2 and CDR3 sequences do not have the sequences as set forth in SEQ ID NO: 1, 2, 3.
13. The antibody according to any one of claims 1 to 2, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences selected from the group consisting of;

- a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
- b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
- c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
- d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
- e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
- f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
- g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
- h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
- i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
- j) CDR 1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
- k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V], and
- l) CDR1, CDR2 and CDR3 sequences as specified in a) to k) having at most 5 further mutations or substitutions, at most 4 further mutations or substitutions, at most 3 further mutations or substitutions, at most 2 further mutations or substitutions, or at most 1 further mutation or substitution, in total across the three CDR sequences, and which mutations or substitutions preferably do not modify the binding affinity to human CD3.

14. The antibody according to claim 13, wherein the further mutations or substitutions are conservative, physical or functional amino acids.

15. The antibody according to any one of claims 1 to 2, wherein said VH region has a VH sequence selected from the group consisting of;

- a) a VH sequence as set forth in SEQ ID NO: 55 [T31M],
- b) a VH sequence as set forth in SEQ ID NO: 59 [T31P],
- c) a VH sequence as set forth in SEQ ID NO: 107 [N57E]
- d) a VH sequence as set forth in SEQ ID NO: 177 [H101G],
- e) a VH sequence as set forth in SEQ ID NO: 185 [H101N],
- f) a VH sequence as set forth in SEQ ID NO: 221 [G105P],

- g) a VH sequence as set forth in SEQ ID NO: 237 [S110A],
- h) a VH sequence as set forth in SEQ ID NO: 245 [S110G],
- i) a VH sequence as set forth in SEQ ID NO: 285 [Y114M],
- j) a VH sequence as set forth in SEQ ID NO: 293 [Y114R],
- k) a VH sequence as set forth in SEQ ID NO: 299 [Y114V], and
- l) a VH sequence having at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to any one of the sequences as set forth in a) to k).

16. The humanized or chimeric antibody according to any one of claims 1 to 2, wherein said VH region has a VH sequence selected from the group consisting of;

- a) a VH sequence as set forth in SEQ ID NO: 55[T31M],
- b) a VH sequence as set forth in SEQ ID NO: 59 [T31P],
- c) a VH sequence as set forth in SEQ ID NO: 107 [N57E]
- d) a VH sequence as set forth in SEQ ID NO: 177 [H101G],
- e) a VH sequence as set forth in SEQ ID NO: 185 [H101N],
- f) a VH sequence as set forth in SEQ ID NO: 221 [G105P],
- g) a VH sequence as set forth in SEQ ID NO: 237 [S110A],
- h) a VH sequence as set forth in SEQ ID NO: 245 [S110G],
- i) a VH sequence as set forth in SEQ ID NO: 285 [Y114M],
- j) a VH sequence as set forth in SEQ ID NO: 293 [Y114R], and
- k) a VH sequence as set forth in SEQ ID NO: 299 [Y114V].

17. The antibody according to any one of the preceding claims, wherein said binding region comprises a variable light chain (VL) region, wherein said VL region comprises CDR1, CDR2, and CDR3 regions having the CDR sequences as set forth in SEQ ID NO:6, GTN and 7.

18. The antibody according to any one of the preceding claims, wherein said binding region comprises a variable light chain (VL) region, wherein said VL region is selected from the group consisting of;

- a) a VL sequence as set forth in SEQ ID NO:8;
- b) a VL sequence as set forth in SEQ ID NO:10 or
- c) a VL sequence having at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to any one of the sequences as set forth in a) to b).

19. The antibody according to claim 17, wherein said VL region comprises CDR1, CDR2 and CDR3 regions having the sequences as set forth in SEQ ID NO: 6, GTN and 7, and wherein said VH region comprises CDR1, CDR2, and CDR3 regions having the CDR sequences selected from the group consisting of;
- a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
  - b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
  - c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
  - d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
  - e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
  - f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
  - g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
  - h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
  - i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
  - j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R]; and
  - k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V].
20. The antibody according to any one of the preceding claims, wherein human CD3 is human CD3 epsilon as specified in SEQ ID NO: 399.
21. The antibody according to any one of the preceding claims, wherein the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a  $K_D$  value from  $1.6 \times 10^{-8}$  M to  $9.9 \times 10^{-8}$  M or from  $1.0 \times 10^{-7}$  to  $9.9 \times 10^{-7}$  M as determined by Bio-Layer Interferometry.
22. The antibody according to any one of the preceding claims, wherein the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a  $K_D$  value from  $1.4 \times 10^{-8}$  to  $1.0 \times 10^{-8}$  M or from  $9.9 \times 10^{-9}$  to  $1 \times 10^{-9}$  M as determined by Bio-Layer Interferometry.

23. The antibody according to any one of the preceding claims, wherein the antibody is a humanized antibody.
24. The antibody according to any one of the preceding claims, wherein the antibody is a full-length antibody.
25. The antibody according to any one of the preceding claims, wherein the antibody is of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.
26. The antibody according to any one of the preceding claims, wherein the antibody is monovalent, bivalent or multivalent.
27. The antibody according to any one of the preceding claims, wherein said antibody comprises an Fc region comprising a first and a second immunoglobulin heavy chain.
28. The antibody according to any one of the preceding claims, wherein said antibody comprises a first and a second immunoglobulin heavy chain, wherein in at least one of said first and second immunoglobulin heavy chains one or more amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain according to EU numbering, are not L, L, D, N, and P, respectively.
29. The antibody according to claim 28, wherein in at least one of said first and second immunoglobulin heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain, is not D.
30. The antibody according to claim 28, wherein in at least one of said first and second heavy chains the amino acid in the position corresponding to position N297 in a human IgG1 heavy chain, is not N.
31. The antibody according to claim 28, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively.

32. The antibody according to any of claims 28 and 31, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively.
33. The antibody according to claim 32, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E, respectively.
34. The antibody according to claim 32, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are A and A, respectively.
35. The antibody according to claim 27, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are not L, L, and D, respectively.
36. The antibody according to claim 35, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively.
37. The antibody according to any one of claims 35 to 36, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.
38. The antibody according to any one of claims 35 to 36, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are A, A, and A, respectively.
39. The antibody according to claim 28, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are F, E, A, Q, and S, respectively.

40. A bispecific antibody comprising a first binding region of an antibody according to any one of claims 1 to 39, and a second binding region which binds a different target than said first antigen binding region.
41. The bispecific antibody according to claim 40 wherein said antibody comprises a first and a second heavy chain.
42. The bispecific antibody according to any one of claims 40 to 41, wherein each of said first and second heavy chain comprises at least a hinge region, a CH2 and CH3 region, wherein in said first heavy chain at least one of the amino acids in the positions corresponding to a positions selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and wherein said first and said second heavy chains are not substituted in the same positions.
43. The bispecific antibody according to claim 42, wherein the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said second heavy chain, or vice versa.
44. The bispecific antibody according to any one of claims 40 to 43, wherein said first binding region is according to any one of claims 1 to 27, and said second binding region binds a different target than said first binding region.
45. A method of reducing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in one of the three CDR sequences of the said reference antibody selected from a mutation in one of the positions selected from the group of T31M, T31P, N57, H101, S110 and Y114, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4.

46. The method according to claim 45, wherein said mutation is a T31M or T31P mutation.
47. The method according to claim 45, wherein said method comprises introducing a mutation in the VH CDR2 region corresponding to position N57.
48. The method according to claim 47, wherein said mutation in position N57 is a N57E mutation.
49. The method according to claim 45, wherein said method comprises introducing a mutation in the VH CDR3 region corresponding to a position selected from the group of H101, S110 and Y114.
50. The method according to claim 49, wherein said mutation in the VH CDR3 region is selected from the group consisting of H101G, H101N, S110A, S110G, Y114M, Y114R and Y114V.
51. A method of increasing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in the VH CDR3 corresponding to position G105, wherein the position is numbered according to the reference sequence of the SEQ ID NO: 4.
52. The method according to claim 51, wherein said mutation in position G105 is a G105P mutation.
53. The method according to claim 45-52, wherein said method comprises introducing at most 5 further mutations, at most 4 further mutations, at most 3 further mutations, at most 2 further mutations or at most 1 further mutation into the CDRs of the VH region of the reference antibody as set forth in SEQ ID NO: 1, 2, 3.
54. The method according to any one of claims 45 to 53, wherein the antibody with increased or reduced binding affinity comprises a binding region comprising a heavy

chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences selected from the group consisting of;

- a) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M];
- ;
- b) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
- c) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
- d) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
- e) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
- f) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
- g) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
- h) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
- i) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
- j) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R]; and
- k) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V].

55. The method according to any one of claims 45 to 54, wherein antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a  $K_D$  value from  $1.6 \times 10^{-8}$  M to  $9.9 \times 10^{-8}$  M or from  $1.0 \times 10^{-7}$  to  $9.9 \times 10^{-7}$  M as determined by Bio-Layer Interferometry.

56. The method according to any one of claims 45 to 54, wherein the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a  $K_D$  value from  $1.4 \times 10^{-8}$  to  $1.0 \times 10^{-8}$  M or from  $9.9 \times 10^{-9}$  to  $1 \times 10^{-9}$  M as determined by Bio-Layer Interferometry.

57. The method according to any one of claims 45 to 57, wherein the human CD3 epsilon is expressed on a T cell.
58. The method according to any one of claims 45 to 57, wherein the human CD3 epsilon is in isolated form, e.g. an isolated human CD3 epsilon-peptide.
59. A nucleic acid construct encoding one or more amino acid sequences of any of claims 1-44.
60. An expression vector comprising
- (i) a nucleic acid sequence encoding a heavy chain sequence of a humanized or chimeric antibody according to any one of claims 1 to 16;
  - (ii) a nucleic acid sequence encoding a light chain sequence of a humanized or chimeric antibody according to any one of claims 17 to 18; or
  - (iii) both (i) and (ii).
61. A host cell comprising an expression vector of claim 60.
62. The host cell according to claim 61, wherein said host cell is a recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell.
63. A composition comprising an antibody according to any one of claims 1 to 27 or a bispecific antibody according to claims 28 to 44.
64. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 27, or the bispecific antibody according to any one of claims 28-44 and a pharmaceutical acceptable carrier.
65. The antibody according to any one of claims 1 to 44, the composition according to claim 63, or the pharmaceutical composition according to claim 64 for use as a medicament.
66. The antibody according to any one of claims 1 to 44, the composition according to claim 63, or the pharmaceutical composition according to claim 64 for use as a

medicament in treatment of cancer, an infectious disease, or autoimmune diseases.

67. The antibody according to any one of claims 1 to 27, the bispecific antibody according to claims 28-44, the composition according to claim 63, or the pharmaceutical composition according to claim 64, for use in the treatment of a disease.

68. Use of the antibody according to any one of claims 1 to 27, or the bispecific antibody according to claims 28-44, for the preparation of a medicament for the treatment of a disease.

69. The use according to claim 68, for the treatment of a disease, wherein the disease is cancer, an infectious disease, or autoimmune diseases

70. A method of treatment of a disease comprising administering the antibody according to any one of claims 1 to 27, the bispecific antibody according to claims 28-44, the composition according to claim 63, or the pharmaceutical composition according to claim 64, to a subject in need thereof.

71. The method according to claim 70, wherein the disease is cancer, an infectious disease, or an autoimmune disease.

72. A method of diagnosing a disease characterized by involvement or accumulation of CD3-expressing cells, comprising administering the antibody according to any one of claims 1 to 27, the composition according to claim 63, or the pharmaceutical composition according to claim 64 to a subject, optionally wherein said antibody is labeled with a detectable agent.

73. A method for producing an antibody according to any one of claims 1 to 27 comprising the steps of

- a) culturing a host cell according to any one of claims 61 to 62; and
- b) purifying said antibody from the culture media.

74. A diagnostic composition comprising an antibody according to any one of claims 1 to 27 or a bispecific antibody according to any one of claims 28 to 44.

75. A method for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising the steps of;
- a) contacting the sample with an antibody according to any one of claims 1 to 27 or a bispecific antibody according to any one of claims 28 to 44 , under conditions that allow for formation of a complex between said antibody or bispecific antibody and CD3; and
  - b) analyzing whether a complex has been formed.
76. A kit for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising
- i) an antibody according to any one of claims 1 to 27 or a bispecific antibody according to any one of claims 28 to 44 ; and ii) instructions for use of said kit.
77. An anti-idiotypic antibody which binds to an antibody of any one of claims 1-27 or a bispecific antibody according to any one of claims 28 to 44.