CLAIMS

1. A multispecific antibody comprising

(I) a first binding arm that comprises a first antigen-binding region binding to human CD40, wherein said first antigen-binding region comprises heavy chain variable region CDR1, CDR2 and CDR3 having the amino acid sequences set forth in SEQ ID NOs:1, 2 and 3, respectively, and light chain variable region CDR1, CDR2 and CDR3 having the amino acid sequences set forth in SEQ ID NOs:4, YTS and 5, respectively;

and

(II) a second binding arm that comprises a second antigen-binding region binding to human CD137, wherein said second antigen-binding region comprises

a) heavy chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:64, 65 and 66, respectively, and light chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:67, GAS and 68, respectively, or

b) heavy chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:36, 37 and 38, respectively, and light chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:39, SAS and 40, respectively;

said first binding arm comprising a first heavy chain constant sequence and said second binding arm comprises a second heavy chain constant sequence, wherein

(i) the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said first heavy chain constant sequence, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said second heavy chain constant sequence, or

(ii) (ii) the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said first heavy chain constant sequence, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said second heavy chain constant sequence.

2. The multispecific antibody according to claim 1, wherein said first antigen-binding region comprises a first heavy chain variable (VH) sequence and a first light chain variable (VL) sequence, said second antigen-binding region comprises a second VH sequence and a second VL sequence, and said first and second VH and VL sequences

each comprises three CDR sequences, CDR1, CDR2 and CDR3, respectively, and four framework sequences, FR1, FR2, FR3 and FR4, respectively.

3. The multispecific antibody according to claim 2, wherein said VH sequence of the first antigen-binding region comprises an amino acid sequence, which has the amino acid sequences set forth in SEQ ID NOs:1, 2 and 3, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to at least one of SEQ ID NOS:117 and 6.

4. The multispecific antibody according to any one of claims 2-3, wherein said VL sequence of the first antigen-binding region comprises an amino acid sequence, which has the amino acid sequences set forth in SEQ ID NOs:4, YTS and 5, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to at least one of SEQ ID NOS:121 and 7.

5. The multispecific antibody according to any one of claims 2-4, wherein said VH sequence of the first antigen-binding region comprises an amino acid sequence, which has the amino acid sequences set forth in SEQ ID NOs:1, 2 and 3, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to SEQ ID NO:117; and wherein said VL sequence of the first antigen-binding region comprises an amino acid sequence, which has the amino acid sequences set forth in SEQ ID NOs:4, YTS and 5, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to SEQ ID NOS:121.

6. The multispecific antibody according to any one of the preceding claims, wherein the second antigen-binding region binds to cynomolgus CD137.

7. The multispecific antibody according to any one of the preceding claims, wherein said second antigen-binding region binds to human CD137 as defined by SEQ ID NO:92 to a higher degree than it binds to a mutant human CD137 as defined by SEQ ID NO:93.

8. The multispecific antibody according to any one of claims 1-6, wherein said second antigen-binding region binds to human CD137 as defined by SEQ ID NO:92 to a higher degree than it binds to a mutant human CD137 as defined by SEQ ID NO:94.

9. The multispecific antibody according to any one of preceding claims, wherein said second antigen-binding region binds to human CD137 as defined by SEQ ID NO:92 to the same degree that it binds to a mutant human CD137 as defined by SEQ ID NO:95.

10. The multispecific antibody according to any one of claims 7-9, wherein binding to said human CD137 as defined by SEQ ID NO:92 and said mutant human CD137 as defined by SEQ ID NO:93, 94 and 95, respectively is determined by preparing shuffle constructs derived from human CD137 in which protein domains of the human CD137 are replaced by the corresponding domain of CD137 from different species, using human CD137 and the different species of CD137 as reference constructs; transducing cells with plasmids encoding the reference construct or the shuffle constructs, respectively, and measuring binding of the antibody to each these CD137 constructs by flow cytometry.

11. The multispecific antibody according to any one of claims 1-7 and 9-10, wherein said VH sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs:64, 65 and 66, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to SEQ ID NOS:123.

12. The multispecific antibody according to any one of claims 1-7 and 9-11, wherein said VL sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs: 67, GAS and 68, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to at least one of SEQ ID NO:127.

13. The multispecific antibody according to any one of claims 1-7 and 9-12, wherein said VH sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs:64, 65 and 66, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to SEQ ID NO:123 and said VL sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs:67, GAS and 68, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to SEQ ID NO:127, respectively.

14. The multispecific antibody according to any one of claims 1-6 and 8-10 wherein said VH sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs: 36, 37 and 38, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identity to SEQ ID NO:41.

15. The multispecific antibody according to any one of claims 1-6, 8-10, and 14, wherein said VL sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs: 39, SAS and 40, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identity to SEQ ID NO:42.

16. The multispecific antibody according to any one of claims 1-6, 8-10, and 15, wherein said VH sequence of the second antigen-binding region comprises an amino acid sequence, which has the sequences set forth in SEQ ID NOs: 36, 37 and 38, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% identity to SEQ ID NO: 41 and said VL sequence of the second antigen-binding region comprises an amino acid sequence which has the sequences set forth in SEQ ID NOs:39, SAS and 40, and has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identity to SEQ ID NO:42, respectively.

17. The multispecific antibody according to any one of claims 3-16, wherein the respective FR1, FR2, FR3 and FR4 framework sequences of the VH and VL sequences of the first and/or second antigen-binding region have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the respective FR1, FR2, FR3 and FR4 framework sequences of said VH and VL sequences.

18. The multispecific antibody according to claim 18, wherein (I) said first binding arm comprises a first heavy chain comprising a first heavy chain variable (VH) sequence and a first heavy chain constant (CH) sequence, and a first light chain comprising a first light chain variable (VL) sequence, and (II) said second binding arm comprises a second heavy chain comprising a second heavy chain variable (VH) sequence and a second heavy chain constant (CH) sequence, and a second light chain comprising a second light chain variable (VL) sequence.

19. The multispecific antibody according to claim 19, wherein said first light chain further comprises a first light chain constant (CL) sequence, and said second light chain further comprises a second light chain constant (CL) sequence.

20. The multispecific antibody according to any one of the preceding claims, wherein the first antigen-binding region is derived from a mouse antibody.

21. The multispecific antibody according to any one of the preceding claims, wherein the first antigen-binding region is derived from a humanized antibody.

22. The multispecific antibody according to any one of claims 17-21, wherein the first binding arm is derived from a full-length antibody.

23. The multispecific antibody according to any one of claims 17-22, wherein the first binding arm is derived from a full-length IgG1,λ (lambda) or IgG1, κ (kappa) antibody.

24. The multispecific antibody according to any one of the preceding claims, wherein the second antigen-binding region is derived from a rabbit antibody.

25. The multispecific antibody according to any one of the preceding claims, wherein the second antigen-binding region is derived from a humanized antibody.

26. The multispecific antibody according to any one of claims 17-25, wherein the second binding arm is derived from a full-length antibody.

27. The multispecific antibody according to any one of claims 17-26, wherein the second binding arm is derived from a full-length IgG1,λ (lambda) or IgG1, κ (kappa) antibody.

28. The multispecific antibody according to any one of the preceding claims, wherein each of the first and second antigen-binding region is derived from a humanized antibody.

29. The multispecific antibody according to any one of claims 17-28, wherein each of the first and second binding arm is derived from a full-length antibody.

30. The multispecific antibody according to any one of claims 17-29, wherein each of the first and second binding arm is derived from a full-length IgG1,λ (lambda) or IgG1, κ (kappa) antibody.

31. The multispecific antibody according to any one of claims 17-30, wherein said first and said second heavy chains are of an IgG isotype, having a subclass selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

32. The multispecific antibody according to any of claims 18-31, wherein each of said first and second heavy chains comprise at least a hinge region, a CH2 and a CH3 region.

33. The multispecific antibody according to claim 32, wherein the CH3 regions of the first and second heavy chains comprise asymmetrical mutations.

34. The multispecific antibody according to any one of the preceding claims, wherein said antibody comprises a first and a second heavy chain, and wherein said antibody induces Fc-mediated effector function to a lesser extent compared to a multispecific antibody comprising the same first and second antigen-binding regions and two heavy chains comprising human IgG1 hinge, CH2 and CH3 regions.

35. The multispecific antibody according to claim 34, wherein said first and second heavy chains are modified so that the multispecific antibody induces Fc-mediated effector function to a lesser extent compared to a multispecific antibody which is identical except for comprising non-modified first and second heavy chains.

36. The multispecific antibody according to any one of claims 34-35, wherein said Fc-mediated effector function is measured by binding to Fcγ receptors, binding to C1q, or induction of Fc-mediated cross-linking of FcRs.

37. The multispecific antibody according to claim 36, wherein said Fc-mediated effector function is measured by binding to C1q.

38. The multispecific antibody according to any one of the preceding claims, wherein said antibody comprises a first and a second heavy chain, wherein in at least one of said first and second 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.

39. The multispecific antibody according to claim 38, wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering are F and E, respectively, in said first and second heavy chains.

40. The multispecific antibody according to claim 39, wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering are F, E, and A, respectively, in said first and second heavy chains.

41. The multispecific antibody according to claim 40, wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering of both the first heavy chain and the second heavy chain are F, E, and A, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.

42. The multispecific antibody according to claim 39, wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering of both the first heavy chain and the second heavy chain are F and E, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.

43. The multispecific antibody according to any of the preceding claims, wherein said antibody is able to cross-link a first cell expressing human CD40 and a second cell expressing human CD137.

44. The multispecific antibody according to claim 43, wherein said cross-linking is determined by an assay using a first cell line expressing human CD40 and a second cell line expressing human CD137, and wherein either the first or the second cell line

comprises a reporter structure resulting in the production of a measurable reporter upon NF-κB activation.

45. The multispecific antibody according to any one of the preceding claims, wherein said antibody induces and/or enhances proliferation of T cells.

46. The multispecific antibody according to claim 45, wherein said T cells are $CD4^+$ T cells and/or $CD8^+$ T cells.

47. The multispecific antibody according to any one of claims 45-46, wherein said induction or enhancement of proliferation of T cells is determined by sub-optimal activating of T cells in a PBMC pool.

48. The multispecific antibody according to claim 47, wherein sub-optimal activation is determined by titrating the concentration of anti-CD3 antibody added to a PBMC pool, measuring T cell proliferation and choosing the anti-CD3 antibody concentration which results in low T cell proliferation but allows for further enhancement of the T cell proliferation.

49. The multispecific antibody according to any one of claims 45-46, wherein proliferation of T cells is measured by co-culturing T-cells expressing a specific T-cell receptor (TCR) with dendritic cells (DCs) presenting the corresponding antigen on the major histocompatibility complex, which is recognized by the TCR.

50. The multispecific antibody according to any one of claims 45-46, wherein said T cells are tumor-infiltrating lymphocytes (TILs), and said induction or enhancement of proliferation of the TILs is determined by incubating a human tumor sample with interleukin-2 (IL-2) and said antibody, and retrieving and counting TILs after incubation for a period of about 10 to about 14 days.

51. The multispecific antibody of claim 50, wherein said human tumor is a melanoma or a non-small-cell lung cancer (NSCLC) tumor.

52. The multispecific antibody according to any one of claims 45-51, wherein said antibody induces or enhances more proliferation of T cells compared to a bispecific antibody comprising a second antigen-binding region according to any one of claims 1-2, but wherein the first antigen-binding region comprises heavy chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:99, 100

and 101, respectively, and light chain variable region CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:102, GVS and 103, respectively.

53. The antibody according to any one of the preceding claims, wherein said antibody is a bispecific antibody.

54. The multispecific antibody according to claim 53, comprising

(I) a first binding arm comprising a first heavy chain comprising a first heavy chain variable (VH) sequence and a first heavy chain constant (CH) sequence, and a first light chain comprising a first light chain variable (VL) sequence and a first light chain constant (CL) sequence, and wherein said heavy first chain variable sequence comprises CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:1, 2 and 3, respectively, and said first light chain sequence comprises CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs:4, YTS and 5, respectively, and

(II) a second binding arm comprising a second heavy chain comprising a second heavy chain variable (VH) sequence and a second heavy chain constant (CH) sequence, and a second light chain further comprises a second light chain constant (CL) sequence, and a second light chain variable (VL) sequence, wherein said second heavy chain variable sequence comprises CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs: 64, 65 and 66, respectively, and said second light chain sequence comprises CDR1, CDR2 and CDR3 having the sequences set forth in SEQ ID NOs: 67, GAS and 68, respectively;

wherein

the first and second heavy chain are of a human IgG1 isotype,

the first and second light chain is of IgG1,κ,

the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering of both the first and second constant heavy chain are F, E, and A, respectively; and

wherein

(a) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first constant heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second constant heavy chain is R; or

(b) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first constant heavy chain is R, and the position

corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second constant heavy chain is L.

55. The multispecific antibody according to claim 53 or 54, comprising

(I) a first binding arm comprising a first heavy chain comprising a first heavy chain variable (VH) sequence and a first heavy chain constant (CH) sequence, and a first light chain comprising a first light chain variable (VL) sequence and a first light chain constant (CL) sequence, and wherein said first VH sequence comprises SEQ ID NO:117 and said first VL sequence comprises SEQ ID NO:121, and

(II) a second binding arm comprising a second heavy chain comprising a second heavy chain variable (VH) sequence and a second heavy chain constant (CH) sequence, and a second light chain further comprises a second light chain constant (CL) sequence, and a second light chain variable (VL) sequence, wherein said second VH sequence comprises SEQ ID NO:123 and said second VL sequence comprises SEQ ID NO:127;

wherein

the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering of both the first and second heavy chain constant sequence are F, E, and A, respectively; and

wherein

(a) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant sequence is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain constant sequence is R; or

(b) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant sequence is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain constant sequence is L.

56. The multispecific antibody according to claim 54 or 55, wherein the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said second heavy chain.

57. A combination of a first nucleic acid construct encoding a first heavy chain and a first light chain as defined in any one of claims 54-56, and second nucleic acid construct encoding a second heavy chain and a second light chain as defined in any one of claims 54-56.

58. A nucleic acid construct encoding a multispecific antibody as defined in any one of claims 1-56.

59. An expression vector comprising combination of a first and second nucleic acid construct according to claim 57 or a nucleic acid construct according to claim 58.

60. A recombinant eukaryotic or prokaryotic host cell comprising a combination of a fist and second nucleic acid construct according to claim 57, a nucleic acid construct according to claim 58, or an expression vector of claim 59, wherein said host cell produces a multispecific antibody as defined in any one of claims 1-56.

61. The host cell according to claim 60, wherein said host cell is a recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell.

62. A composition comprising a multispecific antibody according to any one of claims 1-56, a nucleic acid construct according to any one of claims 57-58, an expression vector according to claim 59 or a host cell according to any one of claims 60 and 61.

63. The composition according to claim 62, which is a pharmaceutical composition.

64. The pharmaceutical composition according to claim 63, which further comprises a pharmaceutically acceptable carrier.

65. The multispecific antibody according to any one of claims 1-56 for use as a medicament.

66. The multispecific antibody according to any one of claims 1-56 for use in the treatment of a disease.

67. The multispecific antibody for use according to claim 66 wherein the disease is an infectious disease.

68. The multispecific antibody for use according to claim 66, wherein the disease is cancer.

69. The multispecific antibody for use according to claim 68, wherein the cancer is selected from the group consisting of melanoma, lung cancer, breast cancer, colon cancer, colorectal cancer renal cancer, cervical cancer and prostate cancer, such as non-small cell lung cancer (NSCLC).

70. Use of a multispecific antibody according to any one of claims 1-56 for the manufacture of a medicament for the treatment of cancer.

71. The use according to claim 70, wherein the cancer is selected from the group consisting of melanoma, lung cancer, breast cancer, colon cancer, colorectal cancer, renal cancer, cervical cancer and prostate cancer, such as non-small cell lung cancer (NSCLC).

72. The use according to any one of claims 65-71, wherein the use is in combination with one or more further therapeutic agent, such as a chemotherapeutic agent.

73. A method for producing a bispecific antibody as defined in any one of claim 53-46, comprising the steps of

a) providing a first antibody comprising an Fc region, said Fc region comprising a first CH3 region;

b) providing a second antibody comprising a second Fc region, said Fc region comprising a second CH3 region,

wherein the first antibody is a CD40 antibody comprising two first antigen-binding regions according to any of claims 1-56, and the second antibody is a CD137 antibody comprising two second antigen-binding regions according to any of claims 1-59, or vice versa; and wherein the sequences of said first and second CH3 regions are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions;

c) incubating said first antibody together with said second antibody under reducing conditions; and

d) obtaining said bispecific CD40xCD137 antibody.

74. A method for detecting whether cross-linking between CD40- and CD137-expressing cells occurs in a sample obtained from a patient, such as a blood sample, lymph node

sample or bone marrow sample, upon administration of a multispecific antibody according to any one of claims 1-56, comprising the steps of:

(i) contacting the sample with a multispecific antibody according to any one of claims 1-56, under conditions that allow for formation of a complex between said multispecific antibody and the CD40- and CD137-expressing cells; and

(ii) analyzing whether a complex has been formed.

75. An anti-idiotypic antibody which binds to the second or first and second antigen-binding region as defined in any of claims 1-56.