

Claims

1. An antibody or antigen-binding fragment thereof that binds to CD40, comprising:
 - (a) 3 complementarity determining regions~~CDRs~~ of a heavy chain variable region (HCDR1, HCDR2 and HCDR3), wherein HCDR1, HCDR2 and HCDR3 comprise or consist of the sequences~~of the heavy chain variable region~~ as set forth in SEQ ID NO:~~13, 58, 60 or 62~~ 1, 3, and 55 respectively; and
 - (b) the 3 CDRs of a light chain variable region (LCDR1, LCDR2 and LCDR3), of the light chain variable region wherein LCDR1, LCDR2 and LCDR3 comprise or consist of the sequences as set forth in SEQ ID NO:~~15, 64 or 66~~ 7, 9 and 56 respectively;

~~3 CDRs HCDR1, HCDR2 and HCDR3 of the heavy chain variable region as set forth in SEQ ID NO: 13 and the 3 CDRs LCDR1, LCDR2 and LCDR3 of the light chain variable region as set forth in SEQ ID NO: 15;~~

 - ~~(i) —~~
 - ~~(ii) — 3 CDRs HCDR1, HCDR2 and HCDR3 of the heavy chain variable region as set forth as SEQ ID NO: 58 and 3 CDRs LCDR1, LCDR2 and LCDR3 of the light chain variable region as set forth as SEQ ID NO: 64;~~
 - ~~(iii) — 3 CDRs HCDR1, HCDR2 and HCDR3 of the heavy chain variable region as set forth as SEQ ID NO: 60 or 62 and the 3 CDRs LCDR1, LCDR2 and LCDR3 of the light chain variable region as set forth as SEQ ID NO: 66;~~
 - ~~(iv) — 3 CDRs HCDR1, HCDR2 and HCDR3 of the heavy chain variable region as set forth in SEQ ID NO: 14 and 3 CDRs LCDR1, LCDR2 and LCDR3 of the light chain variable region as set forth in SEQ ID NO: 16;~~
 - ~~(v) — a CDRs combination of any one of (i) — (v) wherein at least one and no more than 3, 2 or 1 amino acid alternation (preferably amino acid substitution, preferably conservative substitution) is comprised in the sequence as compared to HCDR3 and/or LCDR 3.~~

~~2. An antibody or antigen binding fragment thereof that binds to CD40, comprising~~

 - ~~(i) HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID NO:1, 3 and 5; or~~
 - ~~(ii) HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID NO:1, 3 and 51; or~~
 - ~~(iii) HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence:~~

~~SEQ ID NO:1, 3 and 52-~~

~~(iv) the HCDRs combination of any one of (i) to (iii), wherein at least one and no more than 3, 2 or 1 amino acid alternation (preferably amino acid substitution, preferably conservative substitution) is comprised in said HCDR 3;~~

~~and-~~

~~(i) LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NO:7, 9 and 11 or~~

~~(ii) LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NO:7, 9 and 53; or~~

~~(iii) LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NO:7, 9 and 54; or~~

~~(iv) the LCDRs combination of any one of (i) – (iii), wherein at least one and no more than 3, 2 or 1 amino acid alternation (preferably amino acid substitution, preferably conservative substitution) is comprised in said LCDR 3.~~

32. ~~An~~ The antibody or antigen-binding fragment thereof that binds to CD40 of claim 1, comprising wherein:

~~(ia) __HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence~~ comprise or consist of the sequences set forth in: SEQ ID NOs: 1, 3 and 5 respectively, and LCDR1, LCDR2 and LCDR3 comprise or consist of the sequences set forth in ~~comprising or consisting of the following sequence: SEQ ID NOs: 7, 9 and 11 respectively; or~~

~~(iib) __HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID Nos: 1, 3 and 51 respectively, and LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NOs: 7, 9 and 53 respectively; or~~

~~(iiic) __HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID NOs: 1, 3 and 52 respectively, and LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NOs: 7, 9 and 54 respectively; or~~

~~(iv) HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID NO:2, 4 and 6, and LCDR1, LCDR2 and LCDR3 comprising or consisting~~

- of the following sequence: ~~SEQ ID NO:8, 10 and 12 SEQ ID NO:~~
- (v) ~~HCDR1, HCDR2 and HCDR3 comprising or consisting of the following sequence: SEQ ID NO:1, 3 and 55, and LCDR1, LCDR2 and LCDR3 comprising or consisting of the following sequence: SEQ ID NO:7, 9 and 56;~~
- (vi) ~~the HCDRs and LCDRs combination of any one of (i) to (iv), wherein at least one and no more than 3, 2 or 1 amino acid alternation (preferably amino acid substitution, preferably conservative substitution) is comprised in said HCDR3 and/or LCDR 3.~~

43. The antibody or antigen-binding fragment thereof of ~~any one of claims 1 to 3~~claim 1 or claim 2, comprising a heavy chain variable region (VH) and/or a light chain variable region (VL), wherein;

- (a) ~~the heavy chain variable region VH~~ comprises or consists of:
- (i) ~~comprises or consists of~~ an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with an amino acid sequence selected from SEQ ID NO: 13, 58, 60, 62 or 14; ~~or~~
 - (ii) ~~comprises or consists of~~ an amino acid sequence selected from SEQ ID NO: 13, 58, 60, 62 or 14; or
 - (iii) ~~comprises~~ an amino acid sequence having 1 or more (~~preferably no more than 10, more preferably no more than 5, 4, 3, 2, 1~~) amino acid alternations (~~preferably amino acid substitutions, more preferably amino acid conservative substitutions~~) as compared to an amino acid sequence selected from SEQ ID NOs:13, 58, 60, 62 or 14, ~~preferably the amino acid alternations do not occur in CDR regions, preferably the amino acid alternations occur in FR regions; and/or~~
- (b) ~~the light chain variable region VL~~ comprises or consists of:
- (i) ~~comprises or consists of~~ an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 64, 66 or 16;
 - (ii) ~~comprises or consists of~~ an amino acid sequence selected from SEQ ID NO: 15, 64, 66 or 16; or
 - (iii) ~~comprises~~ an amino acid sequence having 1 or more (~~preferably no more than 10, more preferably no more than 5, 4, 3, 2, 1~~) amino acid alternations (~~preferably amino acid substitutions, more preferably amino acid~~

~~conservative substitutions~~) as compared to an amino acid sequence selected from SEQ ID NO:15, 64, 66 or 16, ~~preferably the amino acid alternations do not occur in the CDR regions, preferably the amino acid alternations occur in the FR regions.~~

4. The antibody or antigen-binding fragment of claim 3, wherein:
 - (a) the VH comprises or consists of an amino acid sequences having no more than 10 amino acid alteration, and preferably no more than 5, 4, 3, 2, or 1 amino acid alternations, as compared to an amino acid sequence selected from SEQ ID NO:13, 58, 60, 62 or 14;
 - (b) the VL comprises or consists of an amino acid sequences having no more than 10 amino acid alteration, and preferably no more than 5, 4, 3, 2, or 1 amino acid alternations, as compared to an amino acid sequence selected from SEQ ID NO:15, 64, 66 or 16;
 - (c) the one or more amino acid alternations are substitutions;
 - (d) the one or more alternations are conservative substitutions;
 - (e) amino acid alternations do not occur in CDR regions; and/or
 - (f) amino acid alterations occur in framework regions.
5. The antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody is a monoclonal antibody, a humanized antibody, a human antibody or a chimeric antibody.
6. An isolated nucleic acid molecule encoding any one or more chains of the antibody or antigen-binding fragment of any one of claims 1 to 5.
7. A vector comprising the nucleic acid molecule of claim 6, preferably wherein said vector is an expression vector.
8. A host cell comprising the nucleic acid molecule of claim 6 or the vector of claim 7, wherein the host cell is suitable for production of an antibody or antigen binding fragment thereof.
9. The host cell of claim 8, wherein the host cell is a yeast cell or a mammalian cell.

10. A method of preparing an antibody or antigen-binding fragment thereof of any one of claim 1 to 5, the method comprising culturing the host cell of claim 8 in culture medium under conditions suitable for expression of the antibody or binding fragment thereof, optionally further comprising recovering the antibody or antigen-binding fragment from the culture medium.
11. An immunoconjugate comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 5 and one or more additional agents.
12. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 5 or the immunoconjugate of claim 11, and optionally a pharmaceutical excipient.
13. The immunoconjugate of claim 11 or the pharmaceutical composition of claim 12, comprising one or more additional agents selected from a therapeutic agent or a label.
14. The immunoconjugate or the pharmaceutical composition of claim 13, wherein the therapeutic agent is selected from the group consisting of a chemotherapeutic agent, another antibody, a cytotoxic agent, a vaccine, an anti-infective active agent, a small molecule drug, and an immunomodulatory agent.
15. An effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-5, the immunoconjugate of any one of claims 11, 13 or 14, or the pharmaceutical composition of any one of claims 12-14 for use in a method of enhancing an immune response in a subject.
16. An effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-5, the immunoconjugate of any one of claims 11, 13 or 14, or the pharmaceutical composition of any one of claims 12-14 for use in a method of preventing or treating a CD40-associated disease.
17. Use of an effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-5, the immunoconjugate of any one of claims 11, 13 or 14, or the pharmaceutical composition of any one of claims 12-14 in the manufacture of a

medicament for the treatment or prevention of a CD40-associated disease.

18. The effective amount of the antibody or antigen-binding fragment, immunoconjugate or pharmaceutical composition for use of claim 16 or the use of claim 17, wherein the CD40-associated disease is a cancer selected from the group consisting of lymphoma, colon cancer, colorectal cancer, rectal cancer, lung cancer, liver cancer, gastric cancer, and metastatic cancers thereof or an infection such as a chronic infection or a viral infection.

19. The effective amount of the antibody or antigen-binding fragment, immunoconjugate or pharmaceutical composition for use of claim 16 or 18 or the use of claim 17 or 18, wherein treating or preventing the CD40-associated disease further comprises surgical treatment and/or radiotherapy, and/or treatment with another therapeutic agent selected from a chemotherapeutic agent, another antibody, a cytotoxic agent, a vaccine, an anti-infective active agent, a small molecule drug, an immunomodulatory agent or combinations thereof.

20. A kit comprising the antibody or antigen-binding fragment thereof of any one of claims 1-5 or the immunoconjugate of any one of claims 11, 13 or 14.

~~5. The antibody or antigen binding fragment thereof of claim 4, wherein-~~

- ~~(i) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO: 13, 58, 60 or 62 and the light chain variable region VL comprises or consists of an amino acid sequence selected from SEQ ID NO: 15, 64 or 66;~~
- ~~(ii) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO: 13 and the light chain variable region VL comprises or consists of the amino acid sequence of SEQ ID NO: 15;~~
- ~~(iii) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO: 58 and the light chain variable region VL comprises or consists of the amino acid sequence of SEQ ID NO: 64;~~
- ~~(iv) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO: 60 and the light chain variable region VL comprises or consists of the amino acid sequence of SEQ ID NO: 66;~~

~~(v) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO:62 and the light chain variable region VL comprises or consists of the amino acid sequence of SEQ ID NO:66;~~

~~(vi) the heavy chain variable region VH comprises or consists of the amino acid sequence of SEQ ID NO: 14 and the light chain variable region VL comprises or consists of the amino acid sequence of SEQ ID NO: 16.~~

~~6. The antibody or antigen binding fragment thereof of any one of claims 1 to 5, comprising a heavy chain and/or a light chain, wherein~~

~~(a) the heavy chain~~

~~(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with an amino acid sequence selected from SEQ ID NO:17, 18, 19, 67, 69, 70 or 20;~~

~~(ii) comprises or consists of an amino acid sequence selected from SEQ ID NO:17, 18, 19, 67, 69, 70 or 20; or~~

~~(iii) comprises an amino acid sequence having 1 or more (preferably no more than 20 or 10, more preferably no more than 5, 4, 3, 2, 1) amino acid alternations (preferably amino acid substitution, more preferably amino acid conservative substitution) as compared to an amino acid sequence selected from SEQ ID NOs:17, 18, 19, 67, 69, 70 or 20, preferably the amino acid alternations do not occur in the CDR regions of the heavy chain, more preferably the amino acid alternations do not occur in the heavy chain variable region, most preferably the amino acid alternations occur in the heavy chain constant region;~~

~~and/or~~

~~(b) the light chain~~

~~(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with an amino acid sequence selected from the group consisting of SEQ ID NO:21, 68, 71 or 22;~~

~~(ii) comprises or consists of an amino acid sequence selected from SEQ ID NO:21, 68, 71 or 22; or~~

~~(iii) comprises an amino acid sequence having 1 or more (preferably no more than 20 or 10, more preferably no more than 5, 4, 3, 2, 1) amino acid alternations (preferably amino acid substitution, more preferably amino acid conservative substitution) as compared to an amino acid sequence selected from SEQ ID NOs: 21, 68, 71 or 22, preferably the amino acid alternations do not occur in the CDR regions of the light chain, more preferably the amino acid~~

~~alternations do not occur in the light chain variable region, most preferably the heavy chain amino acid alternations occur in the light chain constant region.~~

~~7. The antibody or antigen binding fragment thereof of claim 6, wherein~~

- ~~(i) the heavy chain comprises or consists of the amino acid sequence of SEQ ID NO:17, 18, 19, 67, 69 or 70 and the light chain comprises or consists of the amino acid sequence of SEQ ID NO:21, 68 or 71;~~
- ~~(ii) the heavy chain comprises or consists of the amino acid sequence of SEQ ID NO:17, 18 or 19 and the light chain comprises or consists of the amino acid sequence of SEQ ID NO:21;~~
- ~~(iii) the heavy chain comprises or consists of the amino acid sequence of SEQ ID NO:67 and the light chain comprises or consists of the amino acid sequence of SEQ ID NO:68;~~
- ~~(iv) the heavy chain comprises or consists of the amino acid sequence of SEQ ID NO:69 or 70 and the light chain comprises or consists of the amino acid sequence of SEQ ID NO:71;~~
- ~~(v) the heavy chain comprises or consists of the amino acid sequence of SEQ ID NO: 20 and the light chain comprises or consists of the amino acid sequence of SEQ ID NO: 22.~~

~~8. The antibody or antigen binding fragment thereof that binds CD40 of any one of claims 1 to 7, wherein the antibody is a monoclonal antibody.~~

~~9. The antibody or antigen binding fragment thereof that binds CD40 of any one of claims 1 to 8, wherein the antibody is a humanized antibody or a human antibody or a chimeric antibody.~~

~~10. The antibody or antigen binding fragment thereof of any one of claims 1 to 9, wherein the antigen binding fragment is an antibody fragment selected from the following: fab, Fab', Fab' SH, Fv, single chain antibody (e.g.scFv) or (Fab')₂, single domain antibody, diabody (dAb), or linear antibody.~~

~~11. An isolated nucleic acid encoding any one or more chains of an antibody that binds CD40 of any one of claims 1 to 10 or an antigen binding fragment thereof.~~

~~12. A vector comprising the nucleic acid of claim 11, preferably said vector is an expression vector.~~

~~13. A host cell comprising the nucleic acid of claim 11 or the vector of claim 12, preferably the host cell is prokaryotic or eukaryotic, more preferably selected from a yeast cell, a mammalian cell (e.g. the host cell is a CHO cell, such as a CHOS cell a CHOK1SV cell or a CHOK1SV GS KO, or the host cell is a 293 cell, such as HEK293 cell) or other cell suitable for the production of an antibody or antigen binding fragment thereof.~~

~~14. A method of preparing an antibody or antigen binding fragment thereof that binds CD40, the method comprising culturing a host cell comprising a nucleic acid encoding the antibody or antigen binding fragment thereof of any one of claims 1-10, under conditions suitable for expression of the antibody, optionally the method further comprising recovering the antibody or antigen binding fragment thereof from the host cell.~~

~~15. An immunoconjugate comprising the antibody or antigen binding fragment thereof that binds CD40 of any one of claims 1 to 10 and an additional agent, such as a therapeutic agent or a label.~~

~~16. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof that binds CD40 of any one of claims 1 to 10 or the immunoconjugate of claim 13, and optionally one or more additional therapeutic agents, and optionally a pharmaceutical excipient.~~

~~17. A combination product comprising the antibody or antigen binding fragment thereof that binds CD40 of any one of claims 1-10 or the immunoconjugate of claim 15, and one or more additional therapeutic agents.~~

~~18. The immunoconjugate of claim 15, the pharmaceutical composition of claim 16 or the combination product of claim 17, wherein said therapeutic agent is selected from a chemotherapeutic agent, other antibody, cytotoxic agent, vaccine, anti-infective active agent, small molecule drug, or immunomodulatory agent.~~

~~19. A method of enhancing an immune response (e.g. an antigen-specific T cell response)~~

~~in a subject, comprising administering to the subject an effective amount of the antibody or antigen binding fragment thereof of any one of claims 1-10, or the immunoconjugate of claim 16 or 18, or the pharmaceutical composition of claim 16 or 18, or the combination of claim 17 or 18.~~

~~20. A method of preventing or treating in a subject a disease in which modulation (e.g., enhancement) of an immune response in the subject is desired or CD40-associated disease, the method comprising administering to the subject an effective amount of the antibody or antigen binding fragment thereof of any one of claims 1-10, or the immunoconjugate of claim 15 or 18, or the pharmaceutical composition of claim 16 or 18, or the combination product of claim 17 or 18, preferably the disease is a tumor, such as lymphoma, colon cancer, colorectal cancer, rectal cancer, lung cancer (e.g. non-small cell lung cancer), liver cancer, gastric cancer, and metastatic cancers thereof; or an infection, such as a chronic infection, or a viral infection.~~

~~21. The method of claim 20, further comprising co-administering to the subject one or more therapies, such as a therapeutic modality and/or other therapeutic agent, preferably the therapeutic modality comprising surgical treatment and/or radiotherapy, and/or other therapeutic agent selected from a chemotherapeutic agent, other antibody, cytotoxic agent, vaccine, anti-infective active agent, small molecule drug, or immunomodulatory agent.~~

~~22. A detecting kit comprising the antibody or antigen binding fragment thereof of any one of claims 1-10 or the immunoconjugate of claim 15.~~