CLAIMS

~~It is claimed:~~

1. An isolated monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises:

a. a variable heavy chain region (VH);

b. a variable light chain region (VL);

wherein the antibody or antigen-binding fragment thereof binds to a target antigen, preferably a human target antigen;

wherein an amino acid residue in the VH, VL, or within a twenty (20)-amino acid distance of the VH or VL on one or both arms is substituted with an amino acid residue that is conjugated to a fatty acid (FA);

and wherein upon conjugation with the FA at the substituted amino acid residue, the monoclonal antibody or antigen-binding fragment thereof still binds to the target antigen.

2. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the substituted amino acid residue thereof is within a five (5)-amino acid distance of the VH or VL on one or both arms.

~~3.~~ The isolated monoclonal antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the substituted amino acid residue thereof is a cysteine residue, a lysine residue, or a modified amino acid that is suitable for chemical conjugation~~.~~

~~4.~~3. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of claim 3,~~ optionally, wherein the substituted amino acid residue occurs at an amino acid residue corresponding to:

(1) residue 25, 27, 62, 64, 73, 76, 101, 112, or 113 of SEQ ID NO:1;

(2) residue 26, 27, 52, 53, 56, or 67 of SEQ ID NO:2;

(3) residue 119 or 120 of SEQ ID NO:9, 10, 11, or 12; or

(4) residue 121 or 124 of SEQ ID NO:13 or 14~~.~~;

~~The isolated monoclonal antibody or antigen-binding fragment thereof of claim 4~~ further optionally, wherein the substituted amino acid residue occurs at an amino acid residue corresponding to:

(1) a K64C substitution of SEQ ID NO:1;

(2) a S26C substitution of SEQ ID NO:2; or

(3) a T120C substitution of SEQ ID NO:9, 10, 11, or 12.

~~5.~~ The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to ~~5~~3, wherein the monoclonal antibody or antigen-binding fragment thereof is an anti-immune cell modulator (ICM) antibody or antigen-binding fragment thereof and capable of specific binding to the ICM, preferably a human ICM~~.~~

~~6.~~4. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of claim 6,~~ optionally, wherein the ICM is selected from the group consisting of CD3, CD27, CD28, CD40, CD122, OX40, CD16, 4-1BB, GITR, ICOS, CTLA-4, PD-1, LAG-3, TIM-3, TIGIT, VISTA, SIGLEC7, NKG2D, SIGLEC9, KIR, CD91, BTLA, NKp46, B7-H3, SIPRα, and other cell surface immune regulatory antigens.

~~7.~~ The isolated monoclonal antibody or antigen-binding fragment thereof of claim ~~7~~4 , wherein the ICM is CD3, and wherein the monoclonal antibody or antigen-binding fragment thereof comprise a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 having the polypeptide sequences of SEQ ID NOs:3, 4, 5, 6, 7, and 8, respectively; or SEQ ID NOs:33, 34, 35, 36, 37, and 38, respectively~~.~~

~~8.~~5. ~~The isolated monoclonal antibody or antigen-binding fragment thereof of claim 7 or 8,~~ optionally, wherein the substituted amino acid residue occurs at an amino acid residue selected from:

(1) residue 25, 27, 62, 64, 73, 76, 101, 112, or 113 of SEQ ID NO:1 or 27;

(2) residue 26, 27, 52, 53, 56, or 67 of SEQ ID NO:2 or 28;

(3) residue 119 or 120 of SEQ ID NO: 9, 10, 11, or 12; or

(4) residue 121 or 124 of SEQ ID NO: 13 or 14.

~~9.~~6. The isolated monoclonal antibody or antigen-binding fragment thereof of ~~any one or claims 5 to 9~~claim 5, comprising:

1) a CH1 region having a polypeptide sequence selected from SEQ ID NO: 9, 10, 11 or 12 with an amino acid substitution of T120C and a CL region having a polypeptide sequence selected from SEQ ID NO:13 or 14;

~~1)~~2) a VH region having a polypeptide sequence of SEQ ID NO:1 with an amino acid substitution of K64C and a VL region having a polypeptide sequence of SEQ ID NO:2;

~~2)~~3) a VH region having a polypeptide sequence of SEQ ID NO:27 with an amino acid substitution of K64C and a VL region having a polypeptide sequence of SEQ ID NO:28;

~~3)~~4) a VH region having a polypeptide sequence of SEQ ID NO:1 and a VL region having a polypeptide sequence of SEQ ID NO:2 with an amino acid substitution of S26C;

~~4)~~5) a VH region having a polypeptide sequence of SEQ ID NO:27 and a VL region having a polypeptide sequence of SEQ ID NO:28 with an amino acid substitution of S26C;

~~5) a CH1 region having a polypeptide sequence selected from SEQ ID NO: 9, 10, 11 or 12 with an amino acid substitution of T120C and a CL region having a polypeptide sequence selected from SEQ ID NO:13 or 14;~~

6) a VH region having a polypeptide sequence of SEQ ID NO:1, a VL region having a polypeptide sequence of SEQ ID NO:2, a CH1 region having a polypeptide sequence selected from SEQ ID NO: 9, 10, 11 or 12 with an amino acid substitution of T120C, and a CL region having a polypeptide sequence selected from SEQ ID NO:13 or 14;

7) a VH region having a polypeptide sequence of SEQ ID NO:27, a VL region having a polypeptide sequence of SEQ ID NO:28, a CH1 region having a polypeptide sequence selected from SEQ ID NO: 9, 10, 11 or 12 with an amino acid substitution of T120C, and a CL region having a polypeptide sequence selected from SEQ ID NO:13 or 14.

~~10.~~7. An isolated multi-specific antibody or antigen-binding fragment thereof, wherein the multi-specific antibody or antigen-binding fragment thereof comprises the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to ~~10~~6, and wherein the multi-specific antibody or antigen-binding fragment thereof comprises one or more antigen-binding arm(s) comprising a substituted amino acid residue that is conjugated to a FA.

~~11.~~8. The isolated multi-specific antibody or antigen-binding fragment thereof of claim ~~11~~7, wherein the multi-specific antibody or antigen-binding fragment thereof is a bispecific antibody or antigen-binding fragment comprising a first antigen-binding arm (Ab1) and a second antigen-binding arm (Ab2), wherein Ab1 and/or Ab2 comprise a substituted amino acid that is conjugated to a FA.

~~12.~~ The isolated bispecific antibody or antigen-binding fragment thereof of claim ~~12~~8, wherein Ab1 binds an immune cell modulator (ICM), preferably a human ICM~~.~~

~~13.~~9. ~~The isolated bispecific antibody or antigen-binding fragment thereof of claim 13,~~ optionally, wherein the ICM is selected from the group consisting of CD3, CD27, CD28, CD40, CD122, OX40, CD16, 4-1BB, GITR, ICOS, CTLA-4, PD-1, LAG-3, TIM-3, TIGIT, VISTA, SIGLEC7, NKG2D, SIGLEC9, KIR, CD91, BTLA, NKp46, B7-H3, SIPRα, and other cell surface immune regulatory molecules.

~~14.~~10. The isolated bispecific antibody or antigen-binding fragment thereof of ~~any one of claims 12 to 14~~claim 8 or 9, wherein Ab2 binds a tumor-associated antigen (TAA), preferably a human tumor-associated antigen (human TAA~~).~~), optionally, wherein the TAA is DLL3.

~~15.~~ The isolated bispecific antibody or antigen-binding fragment thereof of ~~claim 15, wherein the TAA is DLL3.~~

~~16.~~11. ~~The isolated bispecific antibody or antigen-binding fragment thereof~~ any one of claims ~~12~~8 to ~~16~~10, wherein the first antigen-binding arm (Ab1) comprises H1 and L1 and a second antigen-binding arm (Ab2) comprises H2 and L2, wherein

(a) H1 and H2 each comprises a CH1 region of human IgG1, IgG2, IgG3, or IgG4; and

(b) L1 and L2 each comprises a CL region of a human kappa light chain or a human lambda light chain;

wherein H1L1 and H2L2 each comprise a charge pair selected from the group consisting of the following amino acid substitutions:

(1) G166D/E in CH1 of H1 and S114K/R in CL of L1, respectively, and G166K/R in CH1 of H2 and S114D/E in CL of L2, respectively;

(2) T187D/E in CH1 of H1 and D/N170K/R in CL of L1, respectively, and T187K/R in CH1 of H2 and D/N170D/E in CL of L2, respectively;

(3) S131D/E in CH1 of H1 and P119K/R in CL of L1, respectively, and S131K/R in CH1 of H2 and P119D/E in CL of L2, respectively;

(4) A129D/E in CH1 of H1 and S121K/R in CL of L1, respectively, and A129K/R in CH1 of H2 and S121D/E in CL of L2, respectively;

(5) K/R133D/E in CH1 of H1 and K207K/R in CL of L1, respectively, and K/R133K/R in CH1 of H2 and K207D/E in CL of L2, respectively;

(6) K/R133D/E in CH1 of H1 and I/L117K/R in CL of L1, respectively, and K/R133K/R in CH1 of H2 and I/L117D/E in CL of L2, respectively;

(7) K/R133D/E in CH1 of H1 and F/V209K/R in CL of L1, respectively, and K/R133K/R in CH1 of H2 and F/V209D/E in CL of L2, respectively;

(8) G166D/E in CH1 of H2 and S114K/R in CL of L2, respectively, and G166K/R in CH1 of H1 and S114D/E in CL of L1, respectively;

(9) T187D/E in CH1 of H2 and D/N170K/R in CL of L2, respectively, and T187K/R in CH1 of H1 and D/N170D/E in CL of L1, respectively;

(10) S131D/E in CH1 of H2 and P119K/R in CL of L2, respectively, and S131K/R in CH1 of H1 and P119D/E in CL of L1, respectively;

(11) A129D/E in CH1 of H2 and S121K/R in CL of L2, respectively, and A129K/R in CH1 of H1 and S121D/E in CL of L1, respectively;

(12) K/R133D/E in CH1 of H2 and K207K/R in CL of L2, respectively, and K/R133K/R in CH1 of H1 and K207D/E in CL of L1, respectively;

(13) K/R133D/E in CH1 of H2 and I/L117K/R in CL of L2, respectively, and K/R133K/R in CH1 of H1 and I/L117D/E in CL of L1, respectively; or

(14) K/R133D/E in CH1 of H2 and F/V209K/R in CL of L2, respectively, and K/R133K/R in CH1 of H1 and F/V209D/E in CL of L1, respectively.

~~17.~~12. The isolated bispecific antibody or antigen-binding fragment thereof of any one of claims ~~12~~8 to ~~17~~11, comprising:

1) a first antigen-binding arm (Ab1) comprising a VH region having a polypeptide sequence of SEQ ID NO:19, a VL region having a polypeptide sequence of SEQ ID NO:21, a CH1 region having a polypeptide sequence of SEQ ID NO:20, and a CL region having a polypeptide sequence of SEQ ID NO:22;

~~1)~~2) a first antigen-binding arm (Ab1) comprising a VH region having a polypeptide sequence of SEQ ID NO:15, a VL region having a polypeptide sequence of SEQ ID NO:17, a CH1 region having a polypeptide sequence of SEQ ID NO:16, and a CL region having a polypeptide sequence of SEQ ID NO:18;

~~2)~~1) ~~a first antigen-binding arm (Ab1) comprising a VH region having a polypeptide sequence of SEQ ID NO:19, a VL region having a polypeptide sequence of SEQ ID NO:21, a CH1 region having a polypeptide sequence of SEQ ID NO:20, and a CL region having a polypeptide sequence of SEQ ID NO:22;~~

3) a first antigen-binding arm (Ab1) comprising a VH region having a polypeptide sequence of SEQ ID NO:29, a VL region having a polypeptide sequence of SEQ ID NO:30, a CH1 region having a polypeptide sequence of SEQ ID NO:16, and a CL region having a polypeptide sequence of SEQ ID NO:18; or

4) a first antigen-binding arm (Ab1) comprising a VH region having a polypeptide sequence of SEQ ID NO:31, a VL region having a polypeptide sequence of SEQ ID NO:32, a CH1 region having a polypeptide sequence of SEQ ID NO:20, and a CL region having a polypeptide sequence of SEQ ID NO:22.

~~18.~~13. The isolated bispecific antibody or antigen-binding fragment thereof of claim ~~18~~12, wherein the second antigen-binding arm (Ab2) comprises a VH region having a polypeptide sequence of SEQ ID NO:23, a VL region having a polypeptide sequence of SEQ ID NO:25, a CH1 region having a polypeptide sequence of SEQ ID NO:24, and a CL region having a polypeptide sequence of SEQ ID NO:26.

~~19.~~14. The isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~19~~13, wherein the FA is selected from a FA with 6 carbons, 8 carbons, 10 carbons, 12 carbons, 14 carbons, 16 carbons, or 18 carbons, or any number of carbons in between.

~~20. The isolated antibody or antigen-binding fragment thereof of claim 20, wherein the FA is selected from a FA with 14 carbons or 18 carbons or any number of carbons in between.~~

~~21.~~ The isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~21~~14, wherein the FA comprises a linker for conjugation to the substituted amino acid residue~~.~~

~~22. The isolated antibody or antigen-binding fragment thereof of claim 22,~~, optionally wherein the linker is selected from a peptide linker or a polyethylene glycol linker~~.~~

~~23.~~15. ~~The isolated antibody or antigen-binding fragment thereof of claim 23~~, further, optionally, wherein the peptide linker is less than 50 amino acids.

~~24.~~ The isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~24~~15, wherein the FA conjugated to the antibody or antigen-binding fragment thereof is capable of binding albumin, wherein the binding of albumin to the FA results in (a) a partial or a complete blocking of the binding between the target antigen and the antibody or antigen-binding fragment thereof~~.~~

~~25.~~16. ~~The isolated antibody or antigen-binding fragment thereof of any one of claims 1 to 25, wherein the isolated antibody or antigen-binding fragment thereof has~~; and/or (b) reduced ability to activate T cells upon binding to albumin as compared to the isolated antibody or antigen-binding fragment thereof not binding to albumin.

~~26.~~17. An isolated nucleic acid encoding the isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~26~~16.

~~27.~~18. A vector comprising the isolated nucleic acid of claim ~~27~~17.

~~28.~~19. An isolated host cell comprising the vector of claim ~~28~~18.

~~29.~~20. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~26~~16, and a pharmaceutical acceptable carrier.

~~30. A method of treating a~~Use of the pharmaceutical composition of claim 20, in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, ~~the method comprising administering~~wherein the pharmaceutical composition is to be administered to the subject ~~the pharmaceutical composition of claim 30.~~

~~31.~~21. ~~The method of claim 31~~, optionally, wherein the cancer is selected from the group consisting of a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

22. The pharmaceutical composition of claim 20 for use in therapy.

~~32.~~ ~~A method of producing the isolated antibody or antigen-binding fragment thereof of any one of claims 1 to 26~~16, the method comprising culturing a cell comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof under conditions to produce the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture~~.~~

~~33.~~23. ~~A method of producing the isolated antibody or antigen-binding fragment thereof of claim 33,~~, optionally further comprising conjugating the FA to the antibody or antigen-binding fragment thereof at the substituted amino acid residue.

~~34.~~24. A method of producing a pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~26~~16, the method comprising combining the antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

~~35. A method, comprising contacting albumin with~~Use of the isolated antibody or antigen-binding fragment thereof of any one of claims 1 to ~~26~~16 in the manufacture of a medicament for the treatment of a tumor, wherein the antibody or antigen-binding fragment thereof is capable of specific binding to a target antigen, the FA is capable of binding to albumin, and the binding of albumin to the FA results in a partial or a complete blocking of the binding between the target antigen and the antibody or antigen-binding fragment thereof~~.~~

~~36. The method of claim 36~~ , optionally, wherein ~~the contacting step comprises administering~~ a pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof is to be administered to a subject in need of a treatment of a tumor, and wherein the tumor comprises the target antigen~~.~~

~~37.~~25. ~~The method of claim 36 or 37~~, further optionally, wherein albumin has a higher turnover rate in the tumor microenvironment compared with the circulating blood, and/or is present in the tumor microenvironment at a level lower than the albumin level in circulating blood of the subject, preferably, the lower level of albumin in the tumor microenvironment is due to high albumin catabolism in the tumor microenvironment and/or a high level of proteases in the tumor microenvironment.

26. The isolated antibody or antigen-binding fragment thereof of any one of claims 1 to 16 for use in therapy.