ANTI-CD137 MOLECULES AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of International Patent Application Serial No. PCT/CN2017/098332, filed August 21, 2017, which is hereby incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 695402000341seqlist.txt, date recorded: August 20, 2018, size: 542 KB).

FIELD OF THE INVENTION

The present disclosure relates to antibodies that bind to human CD137 or antigen binding fragments thereof, nucleic acid encoding the same, therapeutic compositions thereof, and their anti-tumor use.

BACKGROUND

CD137 (also referred to as CD137 receptor, 4-1BB, TNFRSF9, etc.) is a transmembrane protein of the Tumor Necrosis Factor Receptor Superfamily (TNFRS). Current understanding of CD137 indicates that its expression is generally activation dependent and is present in a broad subset of immune cells including activated NK and NKT cells, regulatory T cells, dendritic cells (DC), stimulated mast cells, differentiating myeloid cells, monocytes, neutrophils, and eosinophils (Wang, 2009, Immunological Reviews 229: 192-215). CD137 expression has also been demonstrated on tumor vasculature (Broll, 2001, Amer. J. Clin. Pathol. 115(4):543-549; Seaman, 2007, Cancer Cell 11: 539-554) and at sites of inflamed or atherosclerotic endothelium (Drenkard, 2007 FASEB J. 21: 456-463; Olofsson, 2008, Circulation 117: 1292-1301). The ligand that stimulates CD137, i.e., CD137 Ligand (CD137L), is expressed on activated antigen-presenting cells (APCs), myeloid progenitor cells, and hematopoietic stem cells.

Human CD137 is a 255 amino acid protein (GenBank Accession No. NM_001561; NP_001552; SEQ ID NO.: 1). The protein comprises a signal sequence (amino acid residues 1-17), followed by an extracellular domain (169 amino acids), a transmembrane region (27 amino acids), and an intracellular domain (42 amino acids) (Cheuk ATC et al. 2004 Cancer Gene Therapy 11: 215-226). The receptor is expressed on the cell surface in monomer and dimer forms and likely trimerizes with CD137 ligand to signal.

Numerous studies of murine and human T cells indicate that CD137 promotes enhanced cellular proliferation, survival, and cytokine production (Croft, 2009, Nat Rev Immunol 9:271-285). Studies have indicated that some CD137 agonist mAbs increase costimulatory molecule expression and markedly enhance cytolytic T lymphocyte responses, resulting in anti-tumor efficacy in various models. CD137 agonist mAbs have demonstrated efficacy in prophylactic and therapeutic settings. Further, CD137 monotherapy and combination therapy tumor models have established durable anti-tumor protective T cell memory responses (Lynch, 2008, Immunol Rev. 22: 277-286). CD137 agonists also have been shown to inhibit autoimmune reactions in a variety of art-recognized autoimmunity models (Vinay, 2006, J Mol Med 84:726-736). This dual activity of CD137 offers the potential to provide anti-tumor activity while dampening autoimmune side effects that can be associated with immunotherapy approaches that break immune tolerance.

There is a long-felt unmet need for antibodies that bind human CD137, increase a CD137-mediated response, and thereby provide a potential therapeutics for treatment of various diseases and conditions, including cancer and autoimmune diseases. Furthermore, a need exists for anti-CD137 antibody that is cross-reactive among different species such as human and experimental animals (mouse, monkey, dog, etc.) to enable animal model studies and provide therapeutic candidates at the same time.

SUMMARY

It is an object of the disclosure to provide an isolated binding molecule that binds to human CD137, such as an antibody or a binding fragment thereof, or derivative thereof. It is another object of the disclosure to provide a composition comprising a binding molecule that binds to CD137. It is also an object of the present disclosure to provide methods for treating a disease and/or condition associated with or mediated by CD137 signaling by using one or more binding molecules of the disclosure. These and other objects of the disclosure are more fully described herein.

Accordingly, in one aspect, provided herein is one or more antibodies (*e.g.,* isolated antibodies), or one or more antigen-binding fragments thereof, that binds to an extracellular domain of human CD137, and which comprise one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or all 10) of the following functional characteristics: (a) binds one or more amino acid residues within amino acid residues 34-108 of SEQ ID NO:1; (b) does not bind to one or more amino acid residues within amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1; (c) binds to human CD137 with a K_D of 100 nM or less; (d) has agonist activity on human CD137; (e) does not bind to human OX40, CD40, GITR and/or CD27 receptor at concentration up to 1000 nM; (f) is cross-reactive with monkey, mouse, rat, and/or dog CD137; (g) does not induce ADCC effect; (h) is

capable of inhibiting tumor cell growth; (i) has therapeutic effect on a cancer; and/or (j)blocks binding between CD137 and CD137L.

Accordingly, in one aspect, provided herein is an antibody (*e.g.*, an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (I): $X_1TFX_2X_3YX_4IHWV$ (SEQ ID NO:2), wherein X1 is F or Y, X2 is S or T, X3 is G, N, or S, and X4 is A, G, or W; Formula (II): $YSIX_1SGX_2X_3WX_4WI$ (SEQ ID NO:3), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, G, N, S, or T; and Formula (III): $FSLSTX_1GVX_2VX_3WI$ (SEQ ID NO:4), wherein X1 is G or S, X2 is A or G, and X3 is A, G, S, or T; wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV): LALIDWX1X2DKX3YSX4SLKSRL (SEQ ID NO:5), wherein X1 is A, D, or Y, X2 is D or G, X3 is R, S, or Y, and X4 is P or T; Formula (V): IGX1IYHSGX2TYYX3PSLKSRV (SEQ ID NO:6), wherein X1 is D or E, X2 is N or S, and X3 is N or S; and Formula (VI): VSX1ISGX2GX3X4TYYADSVKGRF (SEQ ID NO:7), wherein X1 is A, G, S, V, or Y, X2 is A, D, S, or Y, X3 is D, G, or S, and X4 is S or T; and wherein the HVR-H3 comprises an amino acid sequence according to Formula (VII): ARX1GX2X3X4VX5GDWFX6Y (SEQ ID NO:8), wherein X1 is E or G, X2 is E or S, X3 is D or T, X4 is A, T, or V, X5 is A, I, L, T, or V, and X6 is A, D, or G.

In some embodiments, provided herein is an antibody (*e.g.*, an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137 comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (XII): X1TFSX2YWIHWV (SEQ ID NO:853), wherein X1 is F or Y, and X2 is N, or S; Formula (XIII): YSIX1SGX2X3WX4WI (SEQ ID NO:854), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, G, N, or S; and Formula (XIV): FSLSTX1GVX2VX3WI (SEQ ID NO:855), wherein X1 is G or S, X2 is A or G, and X3 is A, G, or S; wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV): LALIDWX1X2DKX3YSX4SLKSRL (SEQ ID NO:5), wherein X1 is A, D, or Y, X2 is D or G, X3 is R, S, or Y, and X4 is P or T; and Formula (XV): VSX1ISGX2GX3X4TYYADSVKGRF (SEQ ID NO:856), wherein X1 is G, S, V, or Y, X2 is A, D, S, or Y, X3 is D, G, or S, and X4 is S or T; and wherein the HVR-H3 comprises an amino acid

sequence according to Formula (VII): ARX1GX2X3X4VX5GDWFX6Y (SEQ ID NO:8), wherein X1 is E or G, X2 is E or S, X3 is D or T, X4 is A, T, or V, X5 is A, I, L, T, or V, and X6 is A, D, or G.

In another aspect, provided herein is an antibody (*e.g.*, an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to Formula (VIII): X1ASQX2X3X4X5X6X7X8 (SEQ ID NO:9), wherein X1 is Q or R, X2 is D, G, or S, X3 is I or V, X4 is G, R, S, or T, X5 is P, R, S, or T, X6 is A, D, F, S, V, or Y, X7 is L or V, and X8 is A, G, or N; wherein the HVR-L2 comprises an amino acid sequence according to Formula (IX): X1ASX2X3X4X5GX6 (SEQ ID NO:10), wherein X1 is A or D, X2 is N, S, or T, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and wherein the HVR-L3 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (X): YCQQX1YX2X3X4T (SEQ ID NO:11), wherein X1 is A, G, S, or Y, X2 is Q, S, or Y, X3 is I, L, T, or Y, and X4 is I, S, V, or W; and Formula (XI): YCX1QX2X3X4X5PX6T (SEQ ID NO:12), wherein X1 is E or Q, X2 is P, S, or Y, X3 is D, L, S, T, or Y, X4 is D, E, H, S, or T, X5 is D, L T, or W, and X6 is L, P, R, or V.

In some embodiments, provided herein is an antibody (*e.g.*, an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137 comprising a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to Formula (XVI): X1ASQX2X3X4X5X6X7X8 (SEQ ID NO:857), wherein X1 is Q or R, X2 is D, G, or S, X3 is I or V, X4 is G, R, S, or T, X5 is P, R, S, or T, X6 is A, F, S, V, or Y, X7 is L or V, and X8 is A or G; wherein the HVR-L2 comprises an amino acid sequence according to Formula (XVII): $X_1ASX_2X_3X_4X_5GX_6$ (SEQ ID NO:858), wherein X1 is A or D, X2 is N or S, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and wherein the HVR-L3 comprises an amino acid sequence according to Formula (XVIII): $YCQQX_1YX_2X_3WT$ (SEQ ID NO:859), wherein X1 is A or G, X2 is S or Y, and X3 is I, L, or T.

In another aspect, provided herein is an antibody (*e.g.*, an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the HVR-H1, HVR-H2, and HVR-H3 of: VH1, VH2, VH3, VH4, VH5, VH6, VH7, VH8, VH9, VH10, VH11,

VH12, VH13, VH14, VH15, VH16, VH17, VH18, VH19, VH20, VH21, VH22, VH23, VH24, VH25, VH26, VH27, VH28, VH29, VH30, VH31, VH32, VH33, VH34, VH35, VH36, VH37, VH38, VH39, VH40, VH41, VH42, VH43, VH44, VH45, VH46, VH47, VH48, VH49, VH50, VH51, VH52, VH53, VH54, VH55, VH56, VH57, VH58, VH59, or VH60; and/or the light chain variable region comprises the HVR-L1, HVR-L2, and HVR-L3 of: VL1, VL2, VH3, VL4, VH5, VL6, VL7, VL8, VL9, VL10, VL11, VL12, VL13, VL14, VL15, VL16, VL17, VL18, VL19, VL20, VL21, VL22, VL23, VL24, VL25, VL26, VL27, VL28, VL29, VL30, VL31, VL32, VL33, VL34, VL35, VL36, VL37, VL38, VL39, VL40, VL41, VL42, VL43, VL44, VL45, VL46, VL47, VL48, VL49, VL50, VL51, VL52, VL53, VL54, VL55, VL56, VL57, VL58, VL59, or VL60 (as shown in Table 1c). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and light chain variable region comprise the HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of: VH1 and VL1, VH2 and VL2, VH3 and VL3, VH4 and VL4, VH5 and VL5, VH6 and VL6, VH7 and VL7, VH8 and VL8, VH9 and VL9, VH10 and VL10, VH11 and VL11, VH12 and VL12, VH13 and VL13, VH14 and VL14, VH15 and VL15, VH16 and VL16,VH17 and VL17, VH18 and VL18, VH19 and VL19, VH20 and VL20, VH21 and VL21, VH22 and VL22, VH23 and VL23, VH24 and VL24, VH25 and VL25, VH26 and VL26, VH27 and VL27, VH28 and VL28, VH29 and VL29, VH30 and VL30, VH31 and VL31, VH32 and VL32, VH33 and VL33, VH34 and VL34, VH35 and VL35, VH36 and VL36, VH37 and VL37, VH38 and VL38, VH39 and VL39, VH40 and VL40, VH41 and VL41, VH42 and VL42, VH43 and VL43, VH44 and VL44, VH45 and VL45, VH46 and VL46, VH47 and VL47, VH48 and VL48, VH49 and VL49, VH50 and VL50, VH51 and VL51, VH52 and VL52, VH53 and VL53, VH54 and VL54, VH55 and VL55, VH56 and VL56, VH57 and VL57, VH58 and VL58, VH59 and VL59, or VH60 and VL60 (as shown in Table 1c).

In another aspect, provided herein is an antibody (*e.g.,* an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the heavy chain variable region of: VH1, VH2, VH3, VH4, VH5, VH6, VH7, VH8, VH9, VH10, VH11, VH12, VH13, VH14, VH15, VH16, VH17, VH18, VH19, VH20, VH21, VH22, VH23, VH24, VH25, VH26, VH27, VH28, VH29, VH30, VH31, VH32, VH33, VH34, VH35, VH36, VH37, VH38, VH39, VH40, VH41, VH42, VH43, VH44, VH45, VH46, VH47, VH48, VH49, VH50, VH51, VH52, VH53, VH54, VH55, VH56, VH57, VH58, VH59, or VH60; and/or the light chain variable region comprises the light chain variable region of: VL1, VL2, VH3, VL4, VH5, VL6, VL7, VL8, VL9, VL10, VL11, VL12, VL13, VL14, VL15, VL16, VL17, VL18, VL19, VL20,

VL21, VL22, VL23, VL24, VL25, VL26, VL27, VL28, VL29, VL30, VL31, VL32, VL33, VL34, VL35, VL36, VL37, VL38, VL39, VL40, VL41, VL42, VL43, VL44, VL45, VL46, VL47, VL48, VL49, VL50, VL51, VL52, VL53, VL54, VL55, VL56, VL57, VL58, VL59, or VL60 (as shown in Table 1c). In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and light chain variable region comprise the heavy chain variable region and light chain variable region of: VH1 and VL1, VH2 and VL2, VH3 and VL3, VH4 and VL4, VH5 and VL5, VH6 and VL6, VH7 and VL7, VH8 and VL8, VH9 and VL9, VH10 and VL10, VH11 and VL11, VH12 and VL12, VH13 and VL13, VH14 and VL14, VH15 and VL15, VH16 and VL16,VH17 and VL17, VH18 and VL18, VH19 and VL19, VH20 and VL20, VH21 and VL21, VH22 and VL22, VH23 and VL23, VH24 and VL24, VH25 and VL25, VH26 and VL26, VH27 and VL27, VH28 and VL28, VH29 and VL29, VH30 and VL30, VH31 and VL31, VH32 and VL32, VH33 and VL33, VH34 and VL34, VH35 and VL35, VH36 and VL36, VH37 and VL37, VH38 and VL38, VH39 and VL39, VH40 and VL40, VH41 and VL41, VH42 and VL42, VH43 and VL43, VH44 and VL44, VH45 and VL45, VH46 and VL46, VH47 and VL47, VH48 and VL48, VH49 and VL49, VH50 and VL50, VH51 and VL51, VH52 and VL52, VH53 and VL53, VH54 and VL54, VH55 and VL55, VH56 and VL56, VH57 and VL57, VH58 and VL58, VH59 and VL59, or VH60 and VL60 (as shown in Table 1c).

In another aspect, provided herein is an antibody (*e.g.,* an isolated antibody), or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137. In some embodiments, the antibody or the antigen-binding fragment thereof binds to one or more amino acid residues within amino acid residues 34-108 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment binds to one or more amino acid residues within amino acid residues 34-93 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment binds to one or more amino acid residues selected from the group consisting of amino acid residues 34-36, 53-55, and 92-93 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment binds to one or more of amino acid residues 34-36, one or more of amino acid residues 53-55, and one or more or amino acid residues 92-93 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment does not bind to one or more of amino acid residues selected from the group consisting of amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment does not bind to amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1. In some embodiments, the antibody or antigen-binding fragment is cross-reactive with a CD137 polypeptide from at least one non-human species selected from cynomolgus monkey, mouse, rat and/or dog. In some embodiments, the antibody or antigen-binding fragment binds to cynomolgus monkey CD137.

In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:711, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:735, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:759; and/or wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:783, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:807, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:831. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:41, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:42. In some embodiments, the antibody comprises a heavy chain and a light chain, and wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:617, and/or the light chain comprises the amino acid sequence of SEQ ID NO:618.

In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:712, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:736, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:760; and/or wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:784, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:808, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:832. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:61, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:62. In some embodiments, the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:619, and/or the light chain comprises the amino acid sequence of SEQ ID NO:620.

In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:731, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:755, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:779; and/or wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:803, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:827, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:851. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:71, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:72. In some embodiments, the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:657, and/or the light chain comprises the

amino acid sequence of SEQ ID NO:658.

In another aspect, provided herein is an antibody (*e.g.,* an isolated antibody), that binds to an extracellular domain of human CD137, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (I), Formula (II), and Formula (III); the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV), Formula (V), and Formula (VI); and the HVR-H3 comprises an amino acid sequence according to Formula (VII); and/or the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to Formula (VIII); the HVR-L2 comprises an amino acid sequence according to Formula (IX); and the HVR-L3 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (X) and Formula (XI). In some embodiments, provided herein is an antibody (*e.g.,* an isolated antibody), that binds to an extracellular domain of human CD137, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (XIII) and Formula (XVI); the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV) and Formula (XV); and the HVR-H3 comprises an amino acid sequence according to Formula (VII); and/or the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to Formula (XVI); the HVR-L2 comprises an amino acid sequence according to Formula (XVII); and the HVR-L3 comprises an amino acid sequence according to Formula (XVIII).

In some embodiments, the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 253-312, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 313-372, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 373-432, the HVR-L1 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 433-492, the HVR-L2 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 493-552, and/or the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 553-612. In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, and 131, and/or the light chain variable region comprises an amino acid sequence selected from the

group consisting of SEQ ID NOS:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, and 132.

In some embodiments, the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of Formula (XII), Formula (XIII), and Formula (XIV); the HVR-H2 comprises an amino acid sequence according to Formula (IV) or Formula (XV); and the HVR-H3 comprises an amino acid sequence according to Formula (VII); and/or wherein the HVR-L1 comprises an amino acid sequence according to Formula (XVI); the HVR-L2 comprises an amino acid sequence according to Formula (XVII); and the HVR-L3 comprises an amino acid sequence according to Formula (XVIII). In some embodiments, the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:709-732, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:733-756, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:757-780, the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:781-804, the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:805-828, and the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:829-852. In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:15, 17, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 53, 61, 63, 65, 67, 71, 73, 75, 79, 83, 85, and 87, and/or the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:16, 18, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 54, 62, 64, 66, 68, 72, 74, 76, 80, 84, 86, and 88. In some embodiments, the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, and 659, and/or the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, and 660.

In some embodiments, the HVR-H1 comprises an amino acid sequence of SEQ ID NO:711 or 731, the HVR-H2 comprises an amino acid sequence of SEQ ID NO:735 or 755, the HVR-H3 comprises an amino acid sequence of SEQ ID NO:759 or 779, the HVR-L1 comprises an amino acid sequence of SEQ ID NO:783 or 803, the HVR-L2 comprises an amino acid sequence or SEQ ID NO:807 or 827, and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:831 or 851. In some embodiments, the heavy chain variable region comprises an amino acid sequence of SEQ ID NO:41 or 71, and the light chain variable region comprises an amino acid sequence of SEQ ID NO:42 or 72. In some embodiments, the antibody comprises a heavy chain and a light chain,

wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 617 or 657, and the light chain comprises an amino acid sequence of SEQ ID NO: 618 or 658.

In some embodiments, the HVR-H1 comprises an amino acid sequence of SEQ ID NO:712, the HVR-H2 comprises an amino acid sequence of SEQ ID NO:736, the HVR-H3 comprises an amino acid sequence of SEQ ID NO:760, the HVR-L1 comprises an amino acid sequence of SEQ ID NO:784, the HVR-L2 comprises an amino acid sequence or SEQ ID NO:808, and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:832. In some embodiments, the heavy chain variable region comprises an amino acid sequence of SEQ ID NO:61, and the light chain variable region comprises an amino acid sequence of SEQ ID NO:62. In some embodiments, the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 619, and the light chain comprises an amino acid sequence of SEQ ID NO:620.

In some embodiments that may be combined with any of the preceding embodiments, the antibody or antigen-binding fragment binds human CD137 with a K_D of 100 nM or less (*e.g.*, as measured by surface plasmon resonance). In some embodiments, the antibody or antigen-binding fragment binds human CD137 with a K_D of 50 nM or less (*e.g.*, as measured by surface plasmon resonance).

In some embodiments that may be combined with any of the preceding embodiments, the antibody or antigen-binding fragment is cross-reactive with a CD137 polypeptide from at least one non-human species selected from cynomolgus monkey (*e.g.*, GenBank Gene ID 102127961), mouse (*e.g.*, GenBank Gene ID 21942), rat (*e.g.*, GenBank Gene ID 500590) and/or dog (*e.g.*, GenBank Gene ID 608274). In some embodiments, the antibody or antigen-binding fragment binds to cynomolgus monkey CD137.

In some embodiments that may be combined with any of the preceding embodiments, an activity of human CD137 (*e.g.*, when expressed on a cell such as a human cell) is decreased when contacted with the antibody or antigen-binding fragment.

In some embodiments that may be combined with any of the preceding embodiments, the antibody or antigen-binding fragment has a half maximal inhibitory concentration (IC_{50}) of about 100 nM or less for blocking binding of human CD137 to human CD137L *in vitro*. In some embodiments, the antibody or antigen-binding fragment completely blocks binding of human CD137 to human CD137L in vitro when the antibody or antigen-binding fragment is provided at a concentration of about 1 μM or greater. In some embodiments that may be combined with any of the preceding embodiments, an activity of human CD137 (*e.g.*, when expressed on a cell such as a human cell) is increased when contacted with the antibody or antigen-binding fragment. In some embodiments, contacting CD137 (*e.g.*, expressed on a human cell) with the antibody or antigen-

binding fragment results in increased NF-κB-dependent transcription. In some embodiments of any of the above embodiments, the antibody or antigen-binding fragment blocks one or more aspects of CD137 signaling stimulated by CD137L, *e.g.*, CD137L-stimulated NF-κB-dependent transcription, in a cell that expresses CD137.

In some embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG2 Fc region. In some embodiments that may be combined with any of the preceding embodiments, the antibody comprises a human IgG4 Fc region. In some embodiments, the human IgG4 Fc region comprises an S241P mutation, wherein numbering is according to Kabat. In some embodiments, the antibody or antigen-binding fragment does not induce ADCC effects.

In another aspect, provided herein are antibody heavy chain variable regions encoded by a polynucleotide comprising a sequence selected from SEQ ID NOs: 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, and 251, and/or antibody light chain variable regions encoded by a polynucleotide comprising a sequence selected from SEQ ID NOs: 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, and 252. In some embodiments, provided herein are antibody heavy chains encoded by a polynucleotide comprising a sequence selected from SEQ ID NOs:661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, and 707, and/or antibody light chains encoded by a polynucleotide comprising a sequence selected from SEQ ID NOs:662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, and 708.

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the sequence GFSLSTSGVGVG (SEQ ID NO:866), an HVR-H2 comprising the sequence LIDWDDDKYYSPSLKS (SEQ ID NO:867), and an HVR-H3 comprising the sequence GGSDTVLGDWFAY (SEQ ID NO:868); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQSVSPYLA (SEQ ID NO:869), an HVR-L2 comprising the sequence DASSLES (SEQ ID NO:870), and an HVR-L3 comprising the sequence QQGYSLWT (SEQ ID NO:871).

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the

sequence GYSITSGHYWA (SEQ ID NO:872), an HVR-H2 comprising the sequence SISGYGSTTYYADSVKG (SEQ ID NO:873), and an HVR-H3 comprising the sequence GGSDAVLGDWFAY (SEQ ID NO:874); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQGIGSFLA (SEQ ID NO:875), an HVR-L2 comprising the sequence DASNLET (SEQ ID NO:876), and an HVR-L3 comprising the sequence QQGYYLWT (SEQ ID NO:877).

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the sequence GFSLSTGGVGVG (SEQ ID NO:878), an HVR-H2 comprising the sequence LIDWADDKYYSPSLKS (SEQ ID NO:879), and an HVR-H3 comprising the sequence GGSDTVIGDWFAY (SEQ ID NO:880); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQSIGSYLA (SEQ ID NO:881), an HVR-L2 comprising the sequence DASNLET (SEQ ID NO:882), and an HVR-L3 comprising the sequence QQGYYLWT (SEQ ID NO:883).

In some embodiments of any of the above embodiments, an antibody of the present disclosure is a multimeric antibody (*e.g.*, a bispecific antibody). In some embodiments of any of the above embodiments, an antibody of the present disclosure is an IgM antibody, *e.g.*, comprises an IgM Fc region (*e.g.,* a human IgM Fc region).

In another aspect, provided herein is a polynucleotide encoding any of the antibodies or antigen-binding fragments described herein. In some embodiments, provided herein is a polynucleotide comprising a sequence selected from SEQ ID NOs:133-252.

In another aspect, provided herein is a vector comprising any of the polynucleotides described above. In some embodiments, the vector is an expression vector.

In another aspect, provided herein is a host cell (*e.g.,* a bacterial cell, a yeast cell, an insect cell, a mammalian cell (such as a CHO cell or a 293T cell), *etc.*) comprising any of the polynucleotides or vectors described herein. In some embodiments, provided herein is a method of making an antibody or antigen-binding fragment comprising culturing the host cell under conditions suitable for producing the antibody or antigen-binding fragment. In some embodiments, the method further comprises recovering the antibody or antigen-binding fragment produced by the host cell.

In another aspect, provided herein is a pharmaceutical composition comprising any of the antibodies or antigen-binding fragments described herein (or any derivatives thereof) and a pharmaceutically acceptable carrier.

In another aspect, provided herein are methods of treating abnormal cell growth (*e.g.,* a cancer) in a subject in need thereof, comprising administering to the subject a therapeutically

effective amount of any of the antibodies, antigen-binding fragments, and/or pharmaceutical compositions described herein. In some embodiments, provided herein are methods of reducing tumor cell metastasis in a subject, comprising administering to said subject a therapeutically effective amount of any of the antibodies, antigen-binding fragments, and/or pharmaceutical compositions described herein. In some embodiments, the methods further comprise administering to the subject a therapeutically effective amount of at least one (*e.g.*, at least one, at least two, at least three, at least four, at least five, at least 10, *etc.*) additional therapeutic agent. In some embodiments, the at least one additional therapeutic agent is selected from the group consisting of viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, and chemotherapies. In some embodiments, the at least one additional therapeutic agent is selected from the group consisting of pomalyst, revlimid, lenalidomide, pomalidomide, thalidomide, a DNA-alkylating platinum-containing derivative, cisplatin, 5-fluorouracil, cyclophosphamide, an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-DR5 antibody, an anti-CD1d antibody, an anti-TIM3 antibody, an anti-SLAMF7 antibody, an anti-KIR receptor antibody, an anti-OX40 antibody, an anti-HER2 antibody, an anti-ErbB-2 antibody, an anti-EGFR antibody, cetuximab, rituximab, trastuzumab, pembrolizumab, radiotherapy, single dose radiation, fractionated radiation, focal radiation, whole organ radiation, IL-12, IFNα, GM-CSF, a chimeric antigen receptor, adoptively transferred T cells, an anti-cancer vaccine, and an oncolytic virus. Also provided herein is the use of any of the pharmaceutical compositions, antibodies, and/or antigen-binding fragments described herein (or any derivatives thereof) for the treatment of abnormal cell growth (*e.g.*, a cancer) and/or the reduction of tumor cell metastasis in a subject in need thereof. Also provided herein is the use of any of the antibodies or antigen-binding fragments described herein (or any derivatives thereof) for the manufacture of a medicament for the treatment of abnormal cell growth (*e.g.*, a cancer) and/or the reduction of tumor cell metastasis in a subject in need thereof.

It is to be understood that one, some, or all of the properties of the various embodiments described above and herein may be combined to form other embodiments of the present disclosure. These and other aspects of the present disclosure will become apparent to one of skill in the art. These and other embodiments of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows hyper-variable region (HVR) definition as compared to the Kabat CDR definition for an exemplary heavy chain variable region (VH) (SEQ ID NO:13) and an exemplary light chain variable region (VL) (SEQ ID NO:14).

FIG. 1B shows selection of Fab hits that are cross-reactive with mouse CD137.

FIG. 2 shows ELISA binding assays to human, monkey and mouse CD137 of exemplary antibodies. Each panel is for a different antibody as indicated at top of the panel.

FIG. 3A shows FACS-based binding assays to human, monkey, mouse and rat CD137 of exemplary antibodies. Each panel is for a different antigen as indicated at top of the panel.

FIG. 3B shows a comparison of species cross-reactivity among exemplary antibodies and reference antibodies.

FIG. 4A shows exemplary antibodies binding to activated human and monkey T cells, but not naïve human T cells.

FIG. 4B shows binding of AG10131 to activated human, monkey, mouse and rat T cells.

FIG. 5 shows binding specificity of exemplary antibodies to CD137, but to other TNFR family members.

FIGS. 6A and **FIG. 6B** show that exemplary antibodies block the binding of CD137 and its cognate ligand CD137L by both ELISA (FIG. 6A) and flow cytometry assay (FIG. 6B).

FIG. 7A shows epitope mapping results by flow cytometry.

FIG. 7B shows a multiple sequence alignment of a portion of human (SEQ ID NO:1), cynomolgus monkey (SEQ ID NO:860), and mouse (SEQ ID NO:861) CD137, with CD137 sequences/regions of interest (annotated) that were identified from the epitope mapping experiments.

FIG. 8 shows agonist activity of exemplary antibodies in NFκB reporter assay.

FIG. 9 shows agonist activity of exemplary antibodies in CD8+ T cell proliferation (top panel) and INF-γ secretion (bottom panel).

FIG. 10 shows anti-tumor efficacy of exemplary antibodies, as well as infiltration of $CD4^+$ and $CD8^+$ T cells into tumors, in H22 mouse liver cancer model.

FIG. 11 shows anti-tumor efficacy of exemplary antibodies in CT26 mouse colon cancer model.

FIG. 12 shows anti-tumor efficacy of exemplary antibodies in EMT6 mouse breast cancer model.

FIGS. 13 shows CT26 mice treated with exemplary antibodies maintained tumor free after re-challenging with the same tumor cells.

FIG. 14 shows tumor cell killing with splenocytes from tumor-rejecting re-challenged mice.

FIG. 15 shows that AG10131 does not show ADCC effects.

FIG. 16 shows exemplary antibodies displayed little aggregation at high concentration.

FIG. 17 shows stability of exemplary antibodies under accelerated stress conditions.

FIG. 18 shows thermostability.

FIG. 19 shows that AG10131 has no hematological toxicity in normal mice up to 100 mg/kg bi-weekly (BIW) x 2.

FIG. 20 shows that AG10131 has no histological liver abnormality in normal mice up to 100 mg/kg bi-weekly (BIW) x 2.

FIG. 21 shows that AG10131 has no hematological toxicity in cynomolgus monkeys at 10 mg/kg/week x 4.

FIG. 22 shows that AG10131 has no liver toxicity in monkey at 10 mg/kg/week x 4.

FIG. 23 shows the pharmacokinetics profiles of AG10131 in monkey.

FIG. 24 shows the pharmacokinetics profiles of AG10131 in rat.

FIG. 25 shows the pharmacokinetics profiles of various antibodies in mouse.

FIG. 26 shows a crystal structure of the human CD137-CD137L complex with the CD137 cysteine rich domains (CRDs) indicated.

FIG. 27 shows the epitope mapping of the indicated anti-CD137 antibody binding to the indicated human CD137 CRDs by flow cytometry.

FIG. 28 shows that the anti-CD137 antibody AG10131 blocks CD137L-stimulated CD137 signaling. Results are from a cellular NFκB luciferase reporter assay in which 293T cells stably expressing an NFκB luciferase reporter were transfected with a DNA construct expressing human CD137 were co-cultivated with the human B-cell lymphoma cells Daudi (top row) or Raji (bottom row) at the indicated ratios, then incubated with serial dilutions (as indicated) of isotype control (left column) or ligand-blocking anti-CD137 antibodies (right column) overnight, followed by measurement of luciferase activity.

FIG. 29 shows the activation of human CD137-mediated NFκB signaling by anti-CD137 antibodies AG10131, AC1121, or AC1097 in the presence or absence of crosslinking antibody. The EC50 (nM) for each antibody against NFκB signaling activation (in the presence or absence of crosslinking) is indicated.

FIG. 30 shows AG10131 and its human IgG4 isotype control antibody lack the ability to bind to human complement C1q component in the concentration range tested, whereas a human IgG1 isotype control antibody is able to bind to C1q.

FIG. 31 shows the enhancement of tumor-infiltrating T lymphocytes by AG10131 treatment in various tumor models. Top left: representative IHC staining images of mouse CD4+ (upper panels) and CD8+ (lower panels) T cells in H22 tumors after treatment. Top right: representative IHC staining images of mouse CD4+ (upper panels) and CD8+ (lower panels) T cells in EMT6 tumors after treatment. Bottom center: representative IHC staining images of mouse CD4+ (upper panels) and CD8+ (lower panels) T cells in CT26 tumors after treatment. CD4+ or

CD8+ T cells were stained in black in the background of nuclear counterstain by Hematoxylin. CD4+ and CD8+ T cells are indicated by black arrows.

FIG. 32 quantifies numbers of tumor infiltrating T lymphocytes from the experiments shown in **FIG. 31**. The % $CD4^+$ (top row) and $CD8^+$ T cells (bottom row) in tumors from H22, EMT6, and CT26 tumor samples were compared between vehicle and AG10131 treated groups. **, $p < 0.01$; ***, $p < 0.001$.

FIG. 33 shows the anti-tumor effects of AG10131 and anti-PD-1 antibody, alone and in combination, in the established mouse CT26 colon cancer syngeneic model. Top panel: plot with average tumor growth for each treatment group. Bottom panels: spider plots with individual tumor growth for each group.

DETAILED DESCRIPTION

A. Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, antibody engineering, immunotherapy, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry described herein are those well-known and commonly used in the art.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles “a” and “an” refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. The term “amino acid analogs” refers to compounds that have the same basic chemical structure as a naturally occurring amino acid but the C-terminal carboxy group, the N-terminal amino group, or side chain functional group has been chemically modified to another functional group. The term “amino acid mimetics” refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

The term "antibody" is used herein in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments (*e.g.*, a single-chain variable fragment or scFv) so long as they exhibit the desired biological activity.

The term "antibody" is an art-recognized term and may refer to an antigen-binding protein (i.e, immunoglobulin) having a basic four-polypeptide chain structure consisting of two identical heavy (H) chains and two identical light (L) chains. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each heavy chain has, at the N-terminus, a variable region (abbreviated herein as V_H) followed by a constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain has, at the N-terminus, a variable region (abbreviated herein as V_I) followed by a constant region at its other end. The light chain constant region is comprised of one domain, C_L. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (CH1). The pairing of a V_H and V_L together forms a single antigen-binding site. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called J chain, and therefore contains 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain.

The V_H and V_L regions can be further subdivided into regions of hypervariability, termed hyper-variable regions (HVR) based on the structural and sequence analysis. HVRs are interspersed with regions that are more conserved, termed framework regions (FW). For comparison, the Kabat CDR definition by Yvonne Chen, et al. (Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen, J. Mol. Biol. (1999) 293, 865-881) is listed below (see also FIG. 1a). Each V_H and V_L is composed of three HVRs and four FWs, arranged from amino-terminus to carboxy-terminus in the following order: FW1, HVR1, FW2, HVR2, FW3, HVR3, FW4. Throughout the present disclosure, the three HVRs of the heavy chain are referred to as HVR_H1, HVR_H2, and HVR_H3. Similarly, the three HVRs of the light chain are referred to as HVR_L1, HVR_L2, and HVR_L3.

The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)).

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), antibodies can be assigned to different classes or isotypes. There are five classes of antibodies: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated α (alpha), δ (delta), ε (epsilon), γ (gamma), and μ (mu), respectively. The IgG class of antibody can be further classified into four subclasses IgG1, IgG2, IgG3, and IgG4 by the gamma heavy chains, Y1-Y4, respectively.

The term "antibody derivative" or "derivative" of an antibody refers to a molecule that is capable of binding to the same antigen (e.g., CD137) that the antibody binds to and comprises an amino acid sequence of the antibody linked to an additional molecular entity. The amino acid sequence of the antibody that is contained in the antibody derivative may be a full-length heavy chain, a full-length light chain, any portion or portions of a full-length heavy chain, any portion or portions of the full-length light chain of the antibody, any other fragment(s) of an antibody, or the complete antibody. The additional molecular entity may be a chemical or biological molecule. Examples of additional molecular entities include chemical groups, amino acids, peptides, proteins (such as enzymes, antibodies), and chemical compounds. The additional molecular entity may have any utility, such as for use as a detection agent, label, marker, pharmaceutical or therapeutic agent. The amino acid sequence of an antibody may be attached or linked to the additional molecular entity by chemical coupling, genetic fusion, noncovalent association, or otherwise. The term "antibody derivative" also encompasses chimeric antibodies, humanized antibodies, and molecules that are derived from modifications of the amino acid sequences of a CD137 antibody, such as conservation amino acid substitutions, additions, and insertions.

The term "antigen-binding fragment" or "antigen binding portion" of an antibody refers to one or more portions of an antibody that retain the ability to bind to the antigen that the antibody bonds to (e.g., CD137). Examples of "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., *Nature* 341:544-546 (1989)), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR).

The term "binding molecule" encompasses (1) antibody, (2) antigen-binding fragment of an antibody, and (3) derivative of an antibody, each as defined herein.

The term "binding CD137," "binds CD137," "binding to CD137," or "binds to CD137" refers to the binding of a binding molecule, as defined herein, to the human CD137 in an in vitro

assay, such as a Biacore assay as described in Example 4, with an affinity (K_D) of 100 nM or less.

The terms "CD137" and "CD137 receptor" are used interchangeably in the present application, and include the human CD137 receptor, as well as variants, isoforms, and species homologs thereof. Accordingly, a binding molecule, as defined and disclosed herein, may also bind CD137 from species other than human. In other cases, a binding molecule may be completely specific for the human CD137 and may not exhibit species or other types of cross-reactivity.

The term "CD137 antibody" refers to an antibody, as defined herein, capable of binding to human CD137 receptor.

The term "chimeric antibody" refers to an antibody that comprises amino acid sequences derived from different animal species, such as those having a variable region derived from a human antibody and a murine immunoglobulin constant region.

The term "compete for binding" refers to the interaction of two antibodies in their binding to a binding target. A first antibody competes for binding with a second antibody if binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not, be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s).

The term "epitope" refers to a part of an antigen to which an antibody (or antigen-binding fragment thereof) binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope can include various numbers of amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography, 2-dimensional nuclear magnetic resonance, deuterium and hydrogen exchange in combination with mass spectrometry, or site-directed mutagenesis, or all methods used in combination with computational modeling of antigen and its complex structure with its binding antibody and its variants. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996). Once a desired epitope of an antigen is determined, antibodies to that epitope can be generated, e.g., using the techniques described herein. The generation and characterization of antibodies may also elucidate information about desirable epitopes. From this information, it is then possible to

competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, i.e., the antibodies compete for binding to the antigen. A high throughput process for "binning" antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

The term "germline" refers to the nucleotide sequences of the antibody genes and gene segments as they are passed from parents to offspring via the germ cells. The germline sequence is distinguished from the nucleotide sequences encoding antibodies in mature B cells which have been altered by recombination and hypermutation events during the course of B cell maturation.

The term "glycosylation sites" refers to amino acid residues which are recognized by a eukaryotic cell as locations for the attachment of sugar residues. The amino acids where carbohydrate, such as oligosaccharide, is attached are typically asparagine (N-linkage), serine (O-linkage), and threonine (O-linkage) residues. The specific site of attachment is typically signaled by a sequence of amino acids, referred to herein as a "glycosylation site sequence". The glycosylation site sequence for N-linked glycosylation is: -Asn-X-Ser- or -Asn-X-Thr-, where X may be any of the conventional amino acids, other than proline. The terms "N-linked" and "O-linked" refer to the chemical group that serves as the attachment site between the sugar molecule and the amino acid residue. N-linked sugars are attached through an amino group; O-linked sugars are attached through a hydroxyl group. The term "glycan occupancy" refers to the existence of a carbohydrate moiety linked to a glycosylation site (i.e., the glycan site is occupied). Where there are at least two potential glycosylation sites on a polypeptide, either none (0-glycan site occupancy), one (1-glycan site occupancy) or both (2-glycan site occupancy) sites can be occupied by a carbohydrate moiety.

The term "host cell" refers to a cellular system which can be engineered to generate proteins, protein fragments, or peptides of interest. Host cells include, without limitation, cultured cells, e.g., mammalian cultured cells derived from rodents (rats, mice, guinea pigs, or hamsters) such as CHO, BHK, NSO, SP2/0, YB2/0; or human tissues or hybridoma cells, yeast cells, and insect cells, and cells comprised within a transgenic animal or cultured tissue. The term encompasses not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the scope of the term "host cell."

The term "human antibody" refers to an antibody in which the entire amino acid sequences of the light chains and heavy chains are from the human immunoglobulin genes. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell or in a hybridoma derived from a mouse cell. Human antibodies may be prepared in a variety of ways known in the art.

The term "humanized antibody" refers to a chimeric antibody that contains amino acid

residues derived from human antibody sequences. A humanized antibody may contain some or all of the CDRs or HVRs from a non-human animal or synthetic antibody while the framework and constant regions of the antibody contain amino acid residues derived from human antibody sequences.

The term "illustrative antibody" refers to any one of the antibodies described in the disclosure and designated as those listed in Tables 1a and 1b. These antibodies may be in any class (e.g., IgA, IgD, IgE, IgG, and IgM). Thus, each antibody identified above encompasses antibodies in all five classes that have the same amino acid sequences for the V_L and V_H regions. Further, the antibodies in the IgG class may be in any subclass (e.g., IgG1 IgG2, IgG3, and IgG4). Thus, each antibody identified above in the IgG subclass encompasses antibodies in all four subclasses that have the same amino acid sequences for the V_L and V_H regions. The amino acid sequences of the heavy chain constant regions of human antibodies in the five classes, as well as in the four IgG subclasses, are known in the art. The amino acid sequence of the full length heavy chain and light chain for the IgG4 subclass of each of the illustrative antibodies shown in in Table 1b is provided in the disclosure.

The term "isolated antibody" or "isolated binding molecule" refers to an antibody or a binding molecule, as defined herein, that: (1) is not associated with naturally associated components that accompany it in its native state; (2) is free of other proteins from the same species; (3) is expressed by a cell from a different species; or (4) does not occur in nature. Examples of isolated antibodies include a CD137 antibody that has been affinity purified using CD137, a CD137 antibody that has been generated by hybridomas or other cell line in vitro, and a CD137 antibody derived from a transgenic animal.

The term "isolated nucleic acid" refers to a nucleic acid molecule of genomic, cDNA, or synthetic origin, or a combination thereof, which is separated from other nucleic acid molecules present in the natural source of the nucleic acid. For example, with regard to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5′ and 3′ ends of the nucleic acid of interest.

The term "k_a" refers to the association rate constant of a particular antibody -antigen interaction, whereas the term "k_d" refers to the dissociation rate constant of a particular antibody - antigen interaction.

The term "K_D" refers to the equilibrium dissociation constant of a particular antibody - antigen interaction. It is obtained from the ratio of k_d to k_a (i.e., k_d/k_a) and is expressed as a molar concentration (M). K_D is used as a measure for the affinity of an antibody's binding to its binding

partner. The smaller the K_D, the more tightly bound the antibody is, or the higher the affinity between antibody and the antigen. For example, an antibody with a nanomolar (nM) dissociation constant binds more tightly to a particular antigen than an antibody with a micromolar (μM) dissociation constant. K_D values for antibodies can be determined using methods well established in the art. One method for determining the K_D of an antibody is by using surface plasmon resonance, typically using a biosensor system such as a Biacore® system. An assay procedure using the BIACORE™ system (BIAcore assay) is described in the Examples section of this disclosure.

The term "mammal" refers to any animal species of the Mammalia class. Examples of mammals include: humans; laboratory animals such as rats, mice, simians and guinea pigs; domestic animals such as cats, dogs, rabbits, cattle, sheep, goats, horses, and pigs; and captive wild animals such as lions, tigers, elephants, and the like.

The term "prevent" or "preventing," with reference to a certain disease condition in a mammal, refers to preventing or delaying the onset of the disease, or preventing the manifestation of clinical or subclinical symptoms thereof.

As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences. The amino acid sequence identity of polypeptides can be determined conventionally using known computer programs such as Bestfit, FASTA, or BLAST (see, e.g. Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); Altschul et al., *Nucelic Acids Res.* 25:3389-3402 (1997)). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference amino acid sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed. This aforementioned method in determining the percentage of identity between polypeptides is applicable to all proteins, fragments, or variants thereof disclosed herein.

The term "specifically binds" or "specifically binds to," in reference to the interaction of a binding molecule, as defined herein, (e.g., an antibody) with its binding partner (e.g., an antigen), refers to the ability of the binding molecule to discriminate between an antigen of interest from an animal species and the antigen orthologue from a different animal species under a given set of conditions. A CD137 binding molecule is said to specifically bind to human CD137 if it binds to human CD137 at an EC50 that is below 50 percent of the EC50 at which it binds CD137 of rat or mouse as determined in an in vitro assay. Binding specificity of an antibody can be determined using methods known in the art. Examples of such methods include FACS using PHA stimulated primary cells, Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans.

The term "selectively binds" or "selectively binds to," in reference to the interaction of a binding molecule, as defined herein, (e.g., an antibody) with its binding partner (e.g., an antigen), refers to the ability of the binding molecule to discriminate between an antigen of interest from an animal species (such as human CD137) and a different antigen from the same animal species (such as human CD40) under a given set of conditions. A CD137 binding molecule is said to selectively bind to human CD137 if it binds to human CD137 at an EC50 that is below 10 percent of the EC50 at which it binds to human CD40 or human CD134 as determined in an in vitro assay.

The term "treat", "treating", or "treatment", with reference to a certain disease condition in a mammal, refers causing a desirable or beneficial effect in the mammal having the disease condition. The desirable or beneficial effect may include reduced frequency or severity of one or more symptoms of the disease (i.e., tumor growth and/or metastasis, or other effect mediated by the numbers and/or activity of immune cells, and the like), or arrest or inhibition of further development of the disease, condition, or disorder. In the context of treating cancer in a mammal, the desirable or beneficial effect may include inhibition of further growth or spread of cancer cells, death of cancer cells, inhibition of reoccurrence of cancer, reduction of pain associated with the cancer, or improved survival of the mammal. The effect can be either subjective or objective. For example, if the mammal is human, the human may note improved vigor or vitality or decreased pain as subjective symptoms of improvement or response to therapy. Alternatively, the clinician may notice a decrease in tumor size or tumor burden based on physical exam, laboratory parameters, tumor markers or radiographic findings. Some laboratory signs that the clinician may observe for response to treatment include normalization of tests, such as white blood cell count, red blood cell count, platelet count, erythrocyte sedimentation rate, and various enzyme levels. Additionally, the clinician may observe a decrease in a detectable tumor marker. Alternatively, other tests can be used to evaluate objective improvement, such as sonograms, nuclear magnetic resonance testing and positron emissions testing.

The term "vector" refers to a nucleic acid molecule capable of transporting a foreign nucleic acid molecule. The foreign nucleic acid molecule is linked to the vector nucleic acid molecule by a recombinant technique, such as ligation or recombination. This allows the foreign nucleic acid molecule to be multiplied, selected, further manipulated or expressed in a host cell or organism. A vector can be a plasmid, phage, transposon, cosmid, chromosome, virus, or virion. One type of vectors can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome (e.g., non-episomal mammalian vectors). Another type of vector is capable of autonomous replication in a host cell into which it is introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Another specific type of vector capable of directing the expression of expressible foreign

nucleic acids to which they are operatively linked is commonly referred to as "expression vectors." Expression vectors generally have control sequences that drive expression of the expressible foreign nucleic acids. Simpler vectors, known as "transcription vectors," are only capable of being transcribed but not translated: they can be replicated in a target cell but not expressed. The term "vector" encompasses all types of vectors regardless of their function. Vectors capable of directing the expression of expressible nucleic acids to which they are operatively linked are commonly referred to "expression vectors."

The methods and techniques of the present disclosure are generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Such references include, e.g., Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology—A Synthesis* (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)).

B. Binding Molecules that Bind to Human CD137

The present disclosure provides isolated binding molecules that bind to human CD137, including CD137 antibodies, antigen-binding fragments of the CD137 antibodies, and derivatives of the CD137 antibodies. In some embodiments, the binding molecules are any of the antibodies described herein, including antibodies described with reference to epitope binding and antibodies described with reference to specific amino acid sequences of HVRs, variable regions (VL, VH), and IgG (e.g., IgG4) light and heavy chains. In some embodiments, the present disclosure relates to binding molecules that bind to human CD137, and have at least one (e.g., at least one, at least two, at least three, at least four, at least five, at least six, at least seven, eight, or all nine) of the following functional properties: (a) bind to human CD137 with a KD of 500 nM or less; (b) have agonist

activity on human CD137; (c) do not bind to human OX40, CD40, GITR and/or CD27 receptor at concentration up to 1000 nM; (d) is cross-reactive with monkey, mouse, rat, or dog CD137; (e) do not induce ADCC effects; (f) are capable of inhibiting tumor cell growth; (g) have therapeutic effect on a cancer; (h) blocks binding between CD137 and CD137L; and (i) blocks CD137 signaling stimulated by CD137L (*e.g.*, CD137L-stimulated NF-κB-dependent transcription) in a cell that expresses CD137. In some embodiments, the antibodies disclosed herein can also block, e.g., completely block, the binding between CD137 and its ligand CD137L. Also provided herein are one or more anti-CD137 antibodies or antigen-binding fragments that cross-compete for binding to human CD137 with one or more of the antibodies or antigen-binding fragments as described herein.

In some embodiments, the antibodies or the antigen-binding fragments thereof bind to one or more amino acid residues within amino acid residues 34-108 of SEQ ID NO:1. In some embodiments, the antibodies or antigen-binding fragments bind to one or more amino acid residues within amino acid residues 34-93 of SEQ ID NO:1. In some embodiments, the antibodies or antigen-binding fragments bind to one or more amino acid residues selected from the group consisting of amino acid residues 34-36, 53-55, and 92-93 of SEQ ID NO:1. In some embodiments, the antibodies or antigen-binding fragments bind to one or more of amino acid residues 34-36, one or more of amino acid residues 53-55, and one or more or amino acid residues 92-93 of SEQ ID NO:1. In some embodiments, the antibodies or antigen-binding fragments do not bind to one or more of amino acid residues selected from the group consisting of amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1. In some embodiments, the antibodies or antigen-binding fragments do not bind to amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1. Methods of measuring an antibody or antigen-binding fragment's ability to bind a target antigen may be carried out using any method known in the art, including for example, by surface plasmon resonance, an ELISA, isothermal titration calorimetry, a filter binding assay, an EMSA, *etc.* In some embodiments, the ability of the antibody or antigen-binding fragment to bind a target antigen is measured by surface plasmon resonance (*See e.g.,* Example 1 below).

In some embodiments, the antibodies or antigen-binding fragments bind to human CD137 with a KD of about 500 nM or less (e.g., about 500 nM or less, about 400 nM or less, about 300 nM or less, about 200 nM or less, about 150 nM or less, about 100 nM or less, about 90 nM or less, about 80 nM or less, about 75 nM or less, about 70 nM or less, about 60 nM or less, about 50 nM or less, about 40 nM or less, about 30 nM or less, about 25 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, about 0.1 nM or less, etc.) In some embodiments, the antibodies or antigen-binding fragments bind to human CD137 with a KD of about 100 nM or less. In some embodiments, the antibodies or antigen-binding fragments bind to human CD137 with a KD of

about 50 nM or less. Methods of measuring the KD of an antibody or antigen-binding fragment may be carried out using any method known in the art, including for example, by surface plasmon resonance, an ELISA, isothermal titration calorimetry, a filter binding assay, an EMSA, etc. In some embodiments, the KD is measured by surface plasmon resonance (See e.g., Example 1 below).

Anti-CD137 antibodies need to be cross-linked to become agonistic. For example, cross-linking is achieved *in vivo* through Fcgamma receptors, while typically polyclonal anti-Fc antibodies are used in cell-based experiments *in vitro*. In some embodiments, the antibodies or antigen-binding fragments described herein have agonist activity on human CD137. In some embodiments, the antibodies or antigen-binding fragments induce one or more (e.g., one or more, two or more, three or more, etc.) activities of human CD137 when a cell (e.g., a human cell) expressing human CD137 is contacted by the antibody or antigen binding fragment. Various CD137 activities are known in the art and may include, without limitation, induction of NF-κB-dependent transcription, induction of T cell proliferation, prolonging T cell survival, co-stimulation of activated T cells, induction of cytokine secretion (such as IL-2), and induction of monocyte activation . In some embodiments, the one or more CD137 activities is not CD137 binding to its ligand. Methods of measuring CD137 activity (e.g., the induction of NF-κB-dependent transcription and/or T cell proliferation, etc.) are known in the art, including, for example, via the methods described in Examples 8 and 9 below. In some embodiments, the antibodies or antigen-binding fragments increase NF-κB dependent transcription in cells (e.g., human cells) expressing human CD137. In some embodiments, NF-κB dependent transcription is increased by about 10% or more, about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, or about 99% or more in cells (e.g., human cells) expressing CD137 contacted with the antibody or antigen-binding fragment, relative to a corresponding cell not contacted with the antibody or antigen-binding fragment (e.g., a corresponding cell not contacted with an antibody, or contacted with an isotype control antibody). In some embodiments, NF-κB dependent transcription is increased by about 2-fold, 3-fold, 4-folr, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 100-fold, 1000-fold or more in cells (e.g., human cells) expressing CD137 contacted with the antibody or antigen-binding fragment, relative to a corresponding cell not contacted with the antibody or antigen-binding fragment (e.g., a corresponding cell not contacted with an antibody, or contacted with an isotype control antibody).

In some embodiments, the antibodies or antigen-binding fragments are cross-reactive with monkey (e.g., cynomolgus monkey), mouse, rat, and/or dog CD137. In some embodiments, the antibodies or antigen-binding fragments are cross-reactive with monkey CD137. In some embodiments, the antibodies or antigen-binding fragments are cross-reactive with mouse CD137. In some embodiments, the antibodies or antigen-binding fragments are cross-reactive with rat CD137.

In some embodiments, the antibodies or antigen-binding fragments are cross-reactive with dog CD137. In some embodiments, the antibodies or antigen binding fragments are cross reactive with monkey and mouse CD137; monkey and rat CD137; monkey and dog CD137; mouse and rat CD137; mouse and dog CD137; rat and dog CD137; monkey, mouse, and rat CD137; monkey, mouse, and dog CD137; monkey, rat, and dog CD137; mouse, rat, and dog CD137; or monkey, mouse, rat, and dog CD137. In some embodiments, the antibodies or antigen-binding fragments are cross-reactive at about 100 nM (e.g., at about 1nM, at about 10nM, at about 25nM, at about 50nM, at about 75nM, at about 100nM). Methods of measuring antibody cross-reactivity are known in the art, including, without limitation, surface plasmon resonance, an ELISA, isothermal titration calorimetry, a filter binding assay, an EMSA, etc. In some embodiments, the cross-reactivity is measured by ELISA (See e.g., Example 2 below).

In some embodiments, the antibodies do not induce ADCC effects. Methods of measuring ADCC effects (e.g., in vivo methods) are known in the art, including, without limitation, via the methods described in Example 11 below. In some embodiments, the antibodies do not ADCC effects by more than about 10% (do not induce ADCC by more than about 10%, more than about 5%, more than about 1%, more than about 0.1%, more than about 0.01%) relative to a control.

In some embodiments, the antibodies or antigen-binding fragments are capable of inhibiting tumor cell growth/proliferation. In some embodiments, the tumor cell growth/proliferation is inhibited by at least about 5% (e.g., at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) when contacted with the antibodies or antigen-binding fragments relative to corresponding tumor cells not contacted with the antibodies or antigen-binding fragments. In some embodiments, the antibodies or antigen-binding fragments are capable of reducing tumor volume in a subject when the subject is administered the antibodies or antigen-binding fragments. In some embodiments, the antibodies or antigen-binding fragments are capable of reducing tumor volume in a subject by at least about 5% (e.g., at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) relative to the initial tumor volume in the subject (e.g., prior to administration of the antibodies or antigen-binding fragments). Methods of monitoring tumor cell growth/proliferation, tumor volume, and/or tumor inhibition are known in the art, including, for example, via the methods described in Example 10 below.

In some embodiments, the antibodies or antigen-binding fragments have therapeutic effect on a cancer. In some embodiments, the antibodies or antigen-binding fragments reduce one or more signs or symptoms of a cancer. In some embodiments, a subject suffering from a cancer goes into

partial or complete remission when administered the antibodies or antigen-binding fragments.

In another aspect, the disclosure provides isolated antibodies that compete or cross-compete for binding to human CD137 with any of the illustrative antibodies of the disclosure, such as AG10058, AG10059, and/or AG10131. In a particular embodiment, the disclosure provides isolated antibodies that compete or cross-compete for binding to the same epitope on the human CD137 with any of the illustrative antibodies of the disclosure. The ability of an antibody to compete or cross-compete for binding with another antibody can be determined using standard binding assays known in the art, such as BIAcore analysis, ELISA assays, or flow cytometry. For example, one can allow an illustrative antibody of the disclosure to bind to human CD137 under saturating conditions and then measure the ability of the test antibody to bind to the CD137. If the test antibody is able to bind to the CD137 at the same time as the illustrative antibody, then the test antibody binds to a different epitope as the illustrative antibody. However, if the test antibody is not able to bind to the CD137 at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the illustrative antibody. This experiment can be performed using various methods, such as ELISA, RIA, FACS or surface plasmon resonance.

In some embodiments, the antibodies or antigen-binding fragments block the binding between CD137 and its ligand (e.g., human CD137 and human CD137L). In some embodiments, the antibodies or antigen-binding fragments block the binding between CD137 and its ligand in vitro. In some embodiments, the antibody or antigen-binding fragment has a half maximal inhibitory concentration (IC50) of about 500 nM or less (e.g., about 500 nM or less, about 400nM or less, about 300nM or less, about 200nM or less, about 100nM or less, about 50nM or less, about 25nM or less, about 10nM or less, about 1nM or less, etc.) for blocking binding of CD137 its ligand. In some embodiments, the antibody or antigen-binding fragment has a half maximal inhibitory concentration (IC50) of about 100 nM or less for blocking binding of CD137 its ligand. In some embodiments, the antibody or antigen-binding fragment completely blocks binding of human CD137 to its ligand when provided at a concentration of about 100 nM or greater (e.g., about 100nM or greater, about 500nM or greater, about 1μM or greater, about 10μM or greater, etc.). As used herein, the term "complete blocking" or "completely blocks" refers to the antibody or antigen-binding fragment's ability to reduce binding between a first protein and a second protein by at least about 80% (e.g., at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, etc.). Methods of measuring the ability of an antibody or antigen-binding fragment to block binding of a first protein (e.g., a CD137) and a second protein (e.g., CD137L) are known in the art, including, without limitation, via BIAcore analysis, ELISA assays, and flow cytometry (See e.g., Example 6 below).

B-1. CD137 Antibodies

In some aspects, the present disclosure provides an isolated antibody that binds to human CD137 at an epitope within amino acid residues 34-108 or 34-93 of SEQ ID NO.: 1. The antibody, in some embodiments, binds human CD137 with a K_D of 50 nM or less as measured by surface plasmon resonance. In certain embodiments, the antibody can be cross-reactive with at least one non-human species selected from the list consisting of cynomolgus monkey, mouse, rat and dog.

In one aspect, the present disclosure provides an isolated antibody comprising a heavy chain variable region and a light chain variable region, a) wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (I): X1TFX2X3YX4IHWV (SEQ ID NO:2), wherein X1 is F or Y, X2 is S or T, X3 is G, N, or S, and X4 is A, G, or W; Formula (II): YSIX1SGX2X3WX4WI (SEQ ID NO:3), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, G, N, S, or T; and Formula (III): FSLSTX1GVX2VX3WI (SEQ ID NO:4), wherein X1 is G or S, X2 is A or G, and X3 is A, G, S, or T; wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV): LALIDWX1X2DKX3YSX4SLKSRL (SEQ ID NO:5), wherein X1 is A, D, or Y, X2 is D or G, X3 is R, S, or Y, and X4 is P or T; Formula (V): IGX1IYHSGX2TYYX3PSLKSRV (SEQ ID NO:6), wherein X1 is D or E, X2 is N or S, and X3 is N or S; and Formula (VI): VSX1ISGX2GX3X4TYYADSVKGRF (SEQ ID NO:7), wherein X1 is A, G, S, V, or Y, X2 is A, D, S, or Y, X3 is D, G, or S, and X4 is S or T; and wherein the HVR-H3 comprises an amino acid sequence according to Formula (VII): ARX1GX2X3X4VX5GDWFX6Y (SEQ ID NO:8), wherein X1 is E or G, X2 is E or S, X3 is D or T, X4 is A, T, or V, X5 is A, I, L, T, or V, and X6 is A, D, or G; and/or b) wherein the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to Formula (VIII): X1ASQX2X3X4X5X6X7X8 (SEQ ID NO:9), wherein X1 is Q or R, X2 is D, G, or S, X3 is I or V, X4 is G, R, S, or T, X5 is P, R, S, or T, X6 is A, D, F, S, V, or Y, X7 is L or V, and X8 is A, G, or N; wherein the HVR-L2 comprises an amino acid sequence according to Formula (IX): X1ASX2X3X4X5GX6 (SEQ ID NO:10), wherein X1 is A or D, X2 is N, S, or T, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and wherein the HVR-L3 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (X): YCQQX1YX2X3X4T (SEQ ID NO:11), wherein X1 is A, G, S, or Y, X2 is Q, S, or Y, X3 is I, L, T, or Y, and X4 is I, S, V, or W; and Formula (XI): YCX1QX2X3X4X5PX6T (SEQ ID NO:12), wherein X1 is E or Q, X2 is P, S, or Y, X3 is D, L, S, T, or Y, X4 is D, E, H, S, or T, X5 is D, L T, or W, and X6 is L, P, R, or V.

In some embodiments, the antibody can comprise an HVR_H1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 253-312, an HVR_H2 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 313-372, an HVR_H3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 373-432, an HVR_L1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 433-492, an HVR_L2 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 493-552, and/or an HVR_L3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 553-612.

In certain embodiments, the antibody can comprise a VL and/or VH having the amino acid sequence selected from the group consisting of SEQ ID NOs:13-132, which can preferably be encoded by the DNA sequence selected from the group consisting of SEQ ID NOs: 133-252, respectively.

In some embodiments, the antibody can comprise an HVR_H1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 709-732, an HVR_H2 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 733-756, an HVR_H3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 757-780, an HVR_L1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 781-804, an HVR_L2 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 805-828, and/or an HVR_L3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 829-852.

In certain embodiments, the antibody can comprise a light chain and/or heavy chain (e.g., those of IgG such as IgG4) having the amino acid sequences selected from the group consisting of SEQ ID NOs: 613-660, which can be preferably encoded by the DNA sequence selected from the group consisting of SEQ ID NOs: 661-708, respectively.

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the sequence GFSLSTSGVGVG (SEQ ID NO:866), an HVR-H2 comprising the sequence LIDWDDDKYYSPSLKS (SEQ ID NO:867), and an HVR-H3 comprising the sequence GGSDTVLGDWFAY (SEQ ID NO:868); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQSVSPYLA (SEQ ID NO:869), an HVR-L2 comprising the sequence DASSLES (SEQ ID NO:870), and an HVR-L3 comprising the sequence QQGYSLWT (SEQ ID NO:871).

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the sequence GYSITSGHYWA (SEQ ID NO:872), an HVR-H2 comprising the sequence

SISGYGSTTYYADSVKG (SEQ ID NO:873), and an HVR-H3 comprising the sequence GGSDAVLGDWFAY (SEQ ID NO:874); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQGIGSFLA (SEQ ID NO:875), an HVR-L2 comprising the sequence DASNLET (SEQ ID NO:876), and an HVR-L3 comprising the sequence QQGYYLWT (SEQ ID NO:877).

In some embodiments, the HVRs are according to Kabat. In some embodiments, the antibody comprises a heavy chain variable (VH) domain comprising an HVR-H1 comprising the sequence GFSLSTGGVGVG (SEQ ID NO:878), an HVR-H2 comprising the sequence LIDWADDKYYSPSLKS (SEQ ID NO:879), and an HVR-H3 comprising the sequence GGSDTVIGDWFAY (SEQ ID NO:880); and/or a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence RASQSIGSYLA (SEQ ID NO:881), an HVR-L2 comprising the sequence DASNLET (SEQ ID NO:882), and an HVR-L3 comprising the sequence QQGYYLWT (SEQ ID NO:883).

The CD137 antibodies described herein can be in any class, such as IgG, IgM, IgE, IgA, or IgD. It is preferred that the CD137 antibodies are in the IgG class, such as IgG1, IgG2, IgG3, or IgG4 subclass. A CD137 antibody can be converted from one class or subclass to another class or subclass using methods known in the art. An exemplary method for producing an antibody in a desired class or subclass comprises the steps of isolating a nucleic acid encoding a heavy chain of an CD137 antibody and a nucleic acid encoding a light chain of a CD137 antibody, isolating the sequence encoding the V_H region, ligating the V_H sequence to a sequence encoding a heavy chain constant region of the desired class or subclass, expressing the light chain gene and the heavy chain construct in a cell, and collecting the CD137 antibody.

Further, the antibodies provided by the present disclosure can be monoclonal or polyclonal, but preferably monoclonal.

Examples of specific isolated antibodies provided by the present disclosure include those listed in Tables 1a and 1b. The nucleotide and amino acid sequences of the heavy chain variable region, full length heavy chain for the IgG2 and IgG4 subclass, light chain variable region, and full length light chain of these antibodies are also provided hereunder.

Antibodies of the present disclosure can be produced by techniques known in the art, including conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique (See e.g., Kohler and Milstein, *Nature* 256:495 (1975), viral or oncogenic transformation of B lymphocytes, or recombinant antibody technologies as described in detail herein below.

Hybridoma production is a very well-established procedure. The common animal system for preparing hybridomas is the murine system. Immunization protocols and techniques for isolation of

immunized splenocytes for fusion are known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known. One well-known method that may be used for making human CD137 antibodies provided by the present disclosure involves the use of a XenoMouse™ animal system. XenoMouse™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green et al., Nature Genetics 7:13-21 (1994) and WO2003/040170. The animal is immunized with a CD137 antigen. The CD137 antigen is isolated and/or purified CD137, preferably CD137. It may be a fragment of CD137, such as the extracellular domain of CD137, particularly a CD137 extracellular domain fragment comprising amino acid resides 34-108 or 34-93 of SEQ ID NO: 1. Immunization of animals can be carried out by any method known in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane, supra, and U.S. Pat. No. 5,994,619. The CD137 antigen may be administered with an adjuvant to stimulate the immune response. Exemplary adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). After immunization of an animal with a CD137 antigen, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized. Methods of immortalizing cells include, but are not limited to, transferring them with oncogenes, inflecting them with the oncogenic virus cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, supra. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells are screened using CD137, a portion thereof, or a cell expressing CD137. CD137 antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded in vivo in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture in vitro. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

Antibodies of the disclosure can also be prepared using phage display or yeast display methods. Such display methods for isolating human antibodies are established in the art, such as Achim Knappik, et al., “Fully Synthetic Human Combinatorial Antibody Libraries (HuCAL) Based on Modular Consensus Frameworks and CDRs Randomized with Trinucleotides.” J. Mol. Biol. (2000) 296, 57-86; and Michael J. Feldhaus, et al, “Flow-cytometric isolation of human antibodies

from a non-immune Saccharomyces cerevisiae surface display library" Nat Biotechnol (2003) 21:163-170.

B-2. Antigen Binding Fragments

In some other aspects, the present disclosure provides antigen-binding fragments of any of the CD137 antibodies provided by the present disclosure.

The antigen-binding fragment may comprise any sequences of the antibody. In some embodiments, the antigen-binding fragment comprises the amino acid sequence of: (1) a light chain of a CD137 antibody; (2) a heavy chain of a CD137 antibody; (3) a variable region from the light chain of a CD137 antibody; (4) a variable region from the heavy chain of a CD137 antibody; (5) one or more HVRs (two, three, four, five, or six HRVs) of a CD137 antibody; or (6) three HVRs from the light chain and three HVRs from the heavy chain of a CD137 antibody.

In some particular embodiments, the disclosure provides an antigen-binding fragment of an antibody selected from those listed in Tables 1a and 1b.

In some other particular embodiments, the antigen-binding fragments of an CD137 antibody include: (i) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a V_H domain; (vi) an isolated CDR, and (vii) single chain antibody (scFv), which is a polypeptide comprising a V_L region of an antibody linked to a V_H region of an antibody. Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883.

In some particular embodiments, the antigen-binding fragment is a Fab fragment selected from those listed in Table 1a.

B-3. Antibody Derivatives

In some further aspects, the present disclosure provides derivatives of any of the CD137 antibodies provided by the present disclosure.

In one aspect, the antibody derivative is derived from modifications of the amino acid sequences of an illustrative antibody ("parent antibody") of the disclosure while conserving the overall molecular structure of the parent antibody amino acid sequence. Amino acid sequences of any regions of the parent antibody chains may be modified, such as framework regions, HVR regions, or constant regions. Types of modifications include substitutions, insertions, deletions, or combinations thereof, of one or more amino acids of the parent antibody.

In some embodiments, the antibody derivative comprises a V_L or V_H region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 13-132. In some embodiments, the antibody derivative comprises an HVR_H1 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 253-312. In some embodiments, the antibody derivative comprises an HVR_H2 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 313-372. In some embodiments, the antibody derivative comprises an HVR_H3 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 373-432. In some embodiments, the antibody derivative comprises an HVR_L1 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 433-492. In some embodiments, the antibody derivative comprises an HVR_L2 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 493-552.

In some embodiments, the antibody derivative comprises an HVR_L3 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 553-612. In some particular embodiments, the derivative comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 conservative or non-conservative substitutions, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additions and/or deletions to an amino acid sequence as set forth in any of SEQ ID NOs: 13-132 and 253-612.

In some embodiments, the antibody derivative comprises a light chain or heavy chain that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 613-660.

In some embodiments, the antibody derivative comprises an HVR_H1 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 709-732. In some embodiments, the antibody derivative comprises an HVR_H2 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 733-756. In some embodiments, the antibody derivative comprises an HVR_H3 amino acid sequence region that is at least 65%, at least 75%, at least 85%,

at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 757-780. In some embodiments, the antibody derivative comprises an HVR_L1 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 781-804. In some embodiments, the antibody derivative comprises an HVR_L2 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 805-828. In some embodiments, the antibody derivative comprises an HVR_L3 amino acid sequence region that is at least 65%, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence as set forth in any of SEQ ID NOs: 829-852. In some particular embodiments, the derivative comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 conservative or non-conservative substitutions, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additions and/or deletions to an amino acid sequence as set forth in any of SEQ ID NOs: 613-660 and 709-852.

Amino acid substitutions encompass both conservative substitutions and non-conservative substitutions. The term "conservative amino acid substitution" means a replacement of one amino acid with another amino acid where the two amino acids have similarity in certain physico-chemical properties such as polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, substitutions typically may be made within each of the following groups: (a) nonpolar (hydrophobic) amino acids, such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids, such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids, such as arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids, such as aspartic acid and glutamic acid.

The modifications may be made in any positions of the amino acid sequences of the antibody, including the HVRs, framework regions, or constant regions. In one embodiment, the present disclosure provides an antibody derivative that contains the V_H and V_L HVR sequences of an illustrative antibody of this disclosure, yet contains framework sequences different from those of the illustrative antibody. Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the Genbank database or in the "VBase" human germline sequence database (Kabat, E. A., et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991); Tomlinson, I. M., et al., *J. Mol. Biol.* 227:776-798 (1992);

and Cox, J. P. L. et al., *Eur. J. Immunol.* 24:827-836 (1994)). Framework sequences that may be used in constructing an antibody derivative include those that are structurally similar to the framework sequences used by illustrative antibodies of the disclosure, e.g., similar to the V_H 3-23 framework sequences and/or the V_L λ3 or λ1-13 framework sequences used by illustrative antibodies of the disclosure. For example, the HVR_H1, HVR_H2, and HVR_H3 sequences, and the HVR_L1, HVR_L2, and HVR_L3 sequences of an illustrative antibody can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the HVR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences.

In a particular embodiment, the antibody derivative is a chimeric antibody which comprises an amino acid sequence of an illustrative antibody of the disclosure. In one example, one or more HVRs from one or more illustrative human antibodies are combined with HVRs from an antibody from a non-human animal, such as mouse or rat. In another example, all of the HVRs of the chimeric antibody are derived from one or more illustrative antibodies. In some particular embodiments, the chimeric antibody comprises one, two, or three HVRs from the heavy chain variable region or from the light chain variable region of an illustrative antibody. Chimeric antibodies can be generated using conventional methods known in the art.

Another type of modification is to mutate amino acid residues within the HRV regions of the V_H and/or V_L chain. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in in vitro or in vivo assays known in the art. Typically, conservative substitutions are introduced. The mutations may be amino acid additions and/or deletions. Moreover, typically no more than one, two, three, four or five residues within a HVR region are altered. In some embodiments, the antibody derivative comprises 1, 2, 3, or 4 amino acid substitutions in the heavy chain HVRs and/or in the light chain HVRs. In another embodiment, the amino acid substitution is to change one or more cysteines in an antibody to another residue, such as, without limitation, alanine or serine. The cysteine may be a canonical or non-canonical cysteine. In one embodiment, the antibody derivative has 1, 2, 3, or 4 conservative amino acid substitutions in the heavy chain HVR regions relative to the amino acid sequences of an illustrative antibody.

Modifications may also be made to the framework residues within the V_H and/or V_L regions. Typically, such framework variants are made to decrease the immunogenicity of the antibody. One approach is to "back mutate" one or more framework residues to the corresponding germline sequence. An antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which

the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "back mutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis.

In addition, modifications may also be made within the Fc region of an illustrative antibody, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. In one example, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Pat. No. 5,677,425. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody. In another case, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody.

Furthermore, an antibody of the disclosure may be modified to alter its potential glycosylation site or pattern in accordance with routine experimentation known in the art. In another aspect, the present disclosure provide an derivative of an CD137 antibody of the disclosure that contains at least one mutation in an variable region of a light chain or heavy chain that changes the pattern of glycosylation in the variable region. Such an antibody derivative may have an increased affinity and/or a modified specificity for binding an antigen. The mutations may add a novel glycosylation site in the V region, change the location of one or more V region glycosylation site(s), or remove a pre-existing V region glycosylation site. In one embodiment, the present disclosure provides a derivative of a CD137 antibody having a potential N-linked glycosylation site at asparagine in the heavy chain variable region, wherein the potential N-linked glycosylation site in one heavy chain variable region is removed. In another embodiment, the present disclosure provides a derivative of a CD137 antibody having a potential N-linked glycosylation site at asparagine in the heavy chain variable region, wherein the potential N-linked glycosylation site in both heavy chain variable regions is removed. Method of altering the glycosylation pattern of an antibody is known in the art, such as those described in U.S. Pat. No. 6,933,368, the disclosure of which incorporated herein by reference.

In another aspect, the present disclosure provides an antibody derivative that comprises a CD137 antibody, or antigen-binding fragment thereof, as described herein, linked to an additional molecular entity. Examples of additional molecular entities include pharmaceutical agents, peptides or proteins, detection agent or labels, and antibodies.

In some embodiments, the antibody derivative comprises an antibody of the disclosure linked to a pharmaceutical agent. Examples of pharmaceutical agents include cytotoxic agents or other cancer therapeutic agents, and radioactive isotopes. Specific examples of cytotoxic agents

include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine). Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90} and lutetium^{177}. Methods for linking an antibody to a pharmaceutical agent are known in the art, such as using various linker technologies. Examples of linker types include hydrazones, thioethers, esters, disulfides and peptide-containing linkers. For further discussion of linkers and methods for linking therapeutic agents to antibodies, see also Saito et al., *Adv. Drug Deliv. Rev.* 55:199-215 (2003); Trail, et al., *Cancer Immunol. Immunother.* 52:328-337 (2003); Payne, *Cancer Cell* 3:207-212 (2003); Allen, *Nat. Rev. Cancer* 2:750-763 (2002); Pastan, I. and Kreitman, *Curr. Opin. Investig. Drugs* 3:1089-1091 (2002); Senter, P. D. and Springer, C. J. (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

In a particular embodiment, the antibody derivative is a CD137 antibody multimer, which is a multimeric form of a CD137 antibody, such as antibody dimers, trimers, or higher-order multimers of monomeric antibodies. Individual monomers within an antibody multimer may be identical or different. In addition, individual antibodies within a multimer may have the same or different binding specificities. Multimerization of antibodies may be accomplished through natural aggregation of antibodies. For example, some percentage of purified antibody preparations (e.g., purified IgG4 molecules) spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art, such as through using crosslinking agents. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester, succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate, and N-succinimidyl S-acethylthio-acetate) or homobifunctional (such as disuccinimidyl suberate). Such linkers are commercially available from, for example, Pierce Chemical Company, Rockford, IL. Antibodies can also be made to multimerize through recombinant DNA techniques known in the art.

In some embodiments, an antibody of the present disclosure is a multimeric antibody (*e.g.*, a bispecific antibody). In some embodiments, an antibody of the present disclosure is an IgM antibody, *e.g.*, comprises an IgM Fc region (*e.g.*, a human IgM Fc region).

Examples of other antibody derivatives provided by the present disclosure include single chain antibodies, diabodies, domain antibodies, nanobodies, and unibodies. A "single-chain antibody" (scFv) consists of a single polypeptide chain comprising a V_L domain linked to a V_H domain wherein V_L domain and V_H domain are paired to form a monovalent molecule. Single chain antibody can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). A "diabody" consists of two chains, each chain comprising a heavy chain variable region connected to a light chain variable region on the same polypeptide chain connected by a short peptide linker, wherein the two regions on the same chain do not pair with each other but with complementary domains on the other chain to form a bispecific molecule. Methods of preparing diabodies are known in the art (See, e.g., Holliger P. et al., (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448, and Poljak R. J. et al., (1994) Structure 2:1121-1123). Domain antibodies (dAbs) are small functional binding units of antibodies, corresponding to the variable regions of either the heavy or light chains of antibodies. Domain antibodies are well expressed in bacterial, yeast, and mammalian cell systems. Further details of domain antibodies and methods of production thereof are known in the art (see, for example, U.S. Pat. Nos. 6,291,158; 6,582,915; 6,593,081; 6,172,197; 6,696,245; European Patents 0368684 & 0616640; WO05/035572, WO04/101790, WO04/081026, WO04/058821, WO04/003019 and WO03/002609). Nanobodies are derived from the heavy chains of an antibody. A nanobody typically comprises a single variable domain and two constant domains (CH2 and CH3) and retains antigen-binding capacity of the original antibody. Nanobodies can be prepared by methods known in the art (See e.g., U.S. Pat. No. 6,765,087, U.S. Pat. No. 6,838,254, WO 06/079372). Unibodies consist of one light chain and one heavy chain of an IgG4 antibody. Unibodies may be made by the removal of the hinge region of IgG4 antibodies. Further details of unibodies and methods of preparing them may be found in WO2007/059782.

C. Nucleic Acids, Vectors, Host Cells, and Recombinant Methods of Producing CD137 Antibodies

Another aspect of the disclosure provides an isolated nucleic acid molecule that comprises a nucleotide sequence encoding an amino acid sequence of a binding molecule provided by the present disclosure. The amino acid sequence encoded by the nucleotide sequence may be any portion of an antibody, such as a HVR, a sequence comprising one, two, or three HVRs, a variable region of a heavy chain, variable region of a light chain, or may be a full-length heavy chain or full

length light chain. A nucleic acid of the disclosure can be, for example, DNA or RNA, and may or may not contain intronic sequences. Typically, the nucleic acid is a cDNA molecule.

In some embodiments, the disclosure provides an isolated nucleic acid molecule that comprises or consists of a nucleotide sequence encoding an amino acid sequence selected from the group consisting of: (1) amino acid sequence of a HVR_H3 or HVR_L3 of an illustrative antibody; (2) a variable region of a heavy chain or variable region of a light chain of an illustrative antibody; or (3) a full length heavy chain or full length light chain of an illustrative antibody.

In other embodiments, the nucleic acid molecule comprises or consists of a nucleotide sequence that encodes an amino acid sequence as set forth in any one of SEQ ID NOs: 13-132, 253-612, 613-660 and 709-852.

In still other embodiments, the nucleic acid molecule comprises or consists of nucleotide sequence selected from the group consisting of SEQ ID NOs: 133-252 and 661-708.

Nucleic acids of the disclosure can be obtained using any suitable molecular biology techniques. For antibodies expressed by hybridomas, cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), the nucleic acid encoding the antibody can be recovered from the library.

The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see e.g., Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG4 or IgG2 constant region without ADCC effect. The IgG4 constant region sequence can be any of the various alleles or allotypes known to occur among different individuals. These allotypes represent naturally occurring amino acid substitution in the IgG4 constant regions. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see e.g., Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by

standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

To create a scFv gene, the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H regions joined by the flexible linker (see e.g., Bird et al., *Science* 242:423-426 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and McCafferty et al., *Nature* 348:552-554 (1990)).

The present disclosure further provides a vector that comprises a nucleic acid molecule provided by the present disclosure. The nucleic acid molecule may encode a portion of a light chain or heavy chain (such as a CDR or a HVR), a full-length light or heavy chain, polypeptide that comprises a portion or full-length of a heavy or light chain, or an amino acid sequence of an antibody derivative or antigen-binding fragment. In some embodiments, the vector is an expression vector useful for the expression of a binding molecule, such as an antibody or an antigen binding fragment thereof. In some embodiments, provided herein are vectors, wherein a first vector comprises a polynucleotide sequence encoding a heavy chain variable region as described herein, and a second vector comprises a polynucleotide sequence encoding a light chain variable region as described herein. In some embodiments, a single vector comprises polynucleotides encoding a heavy chain variable region as described herein and a light chain variable region as described herein.

To express a binding molecule of the disclosure, DNAs encoding partial or full-length light and heavy chains are inserted into expression vectors such that the DNA molecules are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" means that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the DNA molecule. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by any suitable methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or homologous recombination-based DNA ligation). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype and subclass by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype and subclass such that the V_H segment is operatively linked to the C_H segment(s) within the vector and

the V_L segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the expression vectors of the disclosure typically carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Examples of regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SR promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. et al. (1988) *Mol. Cell. Biol.* 8:466-472).

In addition to the antibody chain genes and regulatory sequences, the expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by any suitable techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the

introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the disclosure in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and typically mammalian host cells, is most typical.

The present disclosure further provides a host cell containing a nucleic acid molecule provided by the present disclosure. The host cell can be virtually any cell for which expression vectors are available. It may be, for example, a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, and may be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant nucleic acid construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, electroporation or phage infection.

Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas, Streptomyces*, and *Staphylococcus.*

Mammalian host cells for expressing a binding molecule of the disclosure include, for example, Chinese Hamster Ovary (CHO) cells (including dhfr-CHO cells, described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220 (1980), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, *J. Mol. Biol.* 159:601-621 (1982), NS0 myeloma cells, COS cells and Sp2 cells. In particular, for use with NS0 myeloma or CHO cells, another expression system is the GS (glutamine synthetase) gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using any suitable protein purification methods.

D. Compositions

In other aspects, the present disclosure provides a composition containing a binding molecule provided by the disclosure. In one aspect, the composition is a pharmaceutical composition comprising a binding molecule and a pharmaceutically acceptable carrier. The compositions can be prepared by conventional methods known in the art.

In some embodiments, present disclosure provides a composition comprising an antibody, or an antigen-binding fragment thereof, provided by the present disclosure and a pharmaceutically acceptable carrier, wherein said antibody comprises a variable domain comprising the HVR amino acid sequence disclosed herein, and wherein said composition comprises not more than about 11%,

10%, 8%, 5%, 3%, or 2% of said antibody, or antigen-binding portion, that is glycosylated at the asparagine of said amino acid sequence compared with the total amount of antibody, or antigen-binding portion thereof, present in said composition. In another embodiment, the composition comprises at least about 2% of said antibody, or antigen-binding portion, that is glycosylated at the asparagine of said amino acid sequence compared with the total amount of antibody, or antigen-binding portion thereof, present in said composition.

The term "pharmaceutically acceptable carrier" refers to any inactive substance that is suitable for use in a formulation for the delivery of a binding molecule. A carrier may be an antiadherent, binder, coating, disintegrant, filler or diluent, preservative (such as antioxidant, antibacterial, or antifungal agent), sweetener, absorption delaying agent, wetting agent, emulsifying agent, buffer, and the like. Examples of suitable pharmaceutically acceptable carriers include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like) dextrose, vegetable oils (such as olive oil), saline, buffer, buffered saline, and isotonic agents such as sugars, polyalcohols, sorbitol, and sodium chloride.

The compositions may be in any suitable forms, such as liquid, semi-solid, and solid dosage forms. Examples of liquid dosage forms include solution (e.g., injectable and infusible solutions), microemulsion, liposome, dispersion, or suspension. Examples of solid dosage forms include tablet, pill, capsule, microcapsule, and powder. A particular form of the composition suitable for delivering a binding molecule is a sterile liquid, such as a solution, suspension, or dispersion, for injection or infusion. Sterile solutions can be prepared by incorporating the antibody in the required amount in an appropriate carrier, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the antibody into a sterile vehicle that contains a basic dispersion medium and other carriers. In the case of sterile powders for the preparation of sterile liquid, methods of preparation include vacuum drying and freeze-drying (lyophilization) to yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The various dosage forms of the compositions can be prepared by conventional techniques known in the art.

The relative amount of a binding molecule included in the composition will vary depending upon a number of factors, such as the specific binding molecule and carriers used, dosage form, and desired release and pharmacodynamic characteristics. The amount of a binding molecule in a single dosage form will generally be that amount which produces a therapeutic effect, but may also be a lesser amount. Generally, this amount will range from about 0.01 percent to about 99 percent, from about 0.1 percent to about 70 percent, or from about 1 percent to about 30 percent relative to the total weight of the dosage form.

In addition to the binding molecule, one or more additional therapeutic agents may be

included in the composition. Examples of additional therapeutic agents are described herein below. The suitable amount of the additional therapeutic agent to be included in the composition can be readily selected by a person skilled in the art, and will vary depending on a number of factors, such as the particular agent and carriers used, dosage form, and desired release and pharmacodynamic characteristics. The amount of the additional therapeutic agent included in a single dosage form will generally be that amount of the agent which produces a therapeutic effect, but may be a lesser amount as well.

E. Use of the Binding Molecules and Pharmaceutical Compositions

Binding molecules and pharmaceutical compositions provided by the present disclosure are useful for therapeutic, diagnostic, or other purposes, such as modulating an immune response, treating cancer, enhancing efficacy of other cancer therapy, enhancing vaccine efficacy, or treating autoimmune diseases. Thus, in other aspects, the present disclosure provides methods of using the binding molecules or pharmaceutical compositions. In one aspect, the present disclosure provides a method of treating a disorder in a mammal, which comprises administering to the mammal in need of treatment a therapeutically effective amount of a binding molecule provided by the disclosure. The binding molecule may be a CD137 agonist or antagonist. In some embodiments, the binding molecule is a CD137 agonist. In some embodiments, the mammal is a human.

In some embodiments, the disorder is a cancer. A variety of cancers where CD137 is implicated, whether malignant or benign and whether primary or secondary, may be treated or prevented with a method provided by the disclosure. Examples of such cancers include lung cancers such as bronchogenic carcinoma (e.g., squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and adenocarcinoma), alveolar cell carcinoma, bronchial adenoma, chondromatous hamartoma (noncancerous), and sarcoma (cancerous); heart cancer such as myxoma, fibromas, and rhabdomyomas; bone cancers such as osteochondromas, condromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, chondrosarcoma, multiple myeloma, osteosarcoma, fibrosarcomas, malignant fibrous histiocytomas, Ewing's tumor (Ewing's sarcoma), and reticulum cell sarcoma; brain cancer such as gliomas (e.g., glioblastoma multiforme), anaplastic astrocytomas, astrocytomas, oligodendrogliomas, medulloblastomas, chordoma, Schwannomas, ependymomas, meningiomas, pituitary adenoma, pinealoma, osteomas, hemangioblastomas, craniopharyngiomas, chordomas, germinomas, teratomas, dermoid cysts, and angiomas; cancers in digestive system such as leiomyoma, epidermoid carcinoma, adenocarcinoma, leiomyosarcoma, stomach adenocarcinomas, intestinal lipomas, intestinal neurofibromas, intestinal fibromas, polyps in large intestine, and colorectal cancers; liver cancers such as hepatocellular adenomas, hemangioma, hepatocellular carcinoma, fibrolamellar carcinoma, cholangiocarcinoma,

hepatoblastoma, and angiosarcoma; kidney cancers such as kidney adenocarcinoma, renal cell carcinoma, hypernephroma, and transitional cell carcinoma of the renal pelvis; bladder cancers; hematological cancers such as acute lymphocytic (lymphoblastic) leukemia, acute myeloid (myelocytic, myelogenous, myeloblastic, myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., Sezary syndrome and hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, granulocytic) leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, B cell lymphoma, mycosis fungoides, and myeloproliferative disorders (including myeloproliferative disorders such as polycythemia vera, myelofibrosis, thrombocythemia, and chronic myelocytic leukemia); skin cancers such as basal cell carcinoma, squamous cell carcinoma, melanoma, Kaposi's sarcoma, and Paget's disease; head and neck cancers; eye-related cancers such as retinoblastoma and intraoccular melanocarcinoma; male reproductive system cancers such as benign prostatic hyperplasia, prostate cancer, and testicular cancers (e.g., seminoma, teratoma, embryonal carcinoma, and choriocarcinoma); breast cancer; female reproductive system cancers such as uterine cancer (endometrial carcinoma), cervical cancer (cervical carcinoma), cancer of the ovaries (ovarian carcinoma), vulvar carcinoma, vaginal carcinoma, fallopian tube cancer, and hydatidiform mole; thyroid cancer (including papillary, follicular, anaplastic, or medullary cancer); pheochromocytomas (adrenal gland); noncancerous growths of the parathyroid glands; pancreatic cancers; and hematological cancers such as leukemias, myelomas, non-Hodgkin's lymphomas, and Hodgkin's lymphomas.

In some other embodiments, the disorder is an autoimmune disease. Examples of autoimmune diseases that may be treated with the binding molecules include autoimmune encephalomyelitis, lupus erythematosus, and rheumatoid arthritis. The binding molecule may also be used to treat inflammation (such as allergic asthma) and chronic graft-versus-host disease,

In another aspect, the present disclosure provides a method of enhancing an immune response in a mammal, which comprises administering to the mammal a therapeutically effective amount of a binding molecule provided by the disclosure. In some embodiments, the binding molecule is a CD137 antibody or antigen-binding fragment thereof and the mammal is a human. In a further embodiment, the binding molecule is CD137 agonist antibody or an antigen-binding fragment thereof. The term “enhancing immune response” or its grammatical variations, means stimulating, evoking, increasing, improving, or augmenting any response of a mammal's immune system. The immune response may be a cellular response (i.e. cell-mediated, such as cytotoxic T lymphocyte mediated) or a humoral response (i.e. antibody mediated response), and may be a primary or secondary immune response. Examples of enhancement of immune response include increased CD4+ helper T cell activity and generation of cytolytic T cells. The enhancement of immune response can be assessed using a number of in vitro or in vivo measurements known to

those skilled in the art, including, but not limited to, cytotoxic T lymphocyte assays, release of cytokines (for example IL-2 production), regression of tumors, survival of tumor bearing animals, antibody production, immune cell proliferation, expression of cell surface markers, and cytotoxicity. Typically, methods of the disclosure enhance the immune response by a mammal when compared to the immune response by an untreated mammal or a mammal not treated using the claimed methods. In one embodiment, the binding molecule is used to enhance the immune response of a human to a microbial pathogen (such as a virus). In another embodiment, the binding molecule is used to enhance the immune response of a human to a vaccine. The binding molecule may be a CD137 agonist or antagonist. In some embodiments, the binding molecule is a CD137 agonist. In one embodiment, the method enhances a cellular immune response, particularly a cytotoxic T cell response. In another embodiment, the cellular immune response is a T helper cell response. In still another embodiment, the immune response is a cytokine production, particularly IL-2 production. The binding molecule may be used to enhance the immune response of a human to a microbial pathogen (such as a virus) or to a vaccine. The binding molecule may be a CD137 agonist or antagonist. In some embodiments, the binding molecule is a CD137 agonist.

In practicing the therapeutic methods, the binding molecules may be administered alone as monotherapy, or administered in combination with one or more additional therapeutic agents or therapies. Thus, in another aspect, the present disclosure provides a combination therapy, which comprises a binding molecule in combination with one or more additional therapies or therapeutic agents for separate, sequential or simultaneous administration. The term "additional therapy" refers to a therapy which does not employ a binding molecule provided by the disclosure as a therapeutic agent. The term "additional therapeutic agent" refers to any therapeutic agent other than a binding molecule provided by the disclosure. In one particular aspect, the present disclosure provides a combination therapy for treating cancer in a mammal, which comprises administering to the mammal a therapeutically effective amount of a binding molecule provided by the disclosure in combination with one or more additional therapeutic agents. In a further embodiment, the mammal is a human.

A wide variety of cancer therapeutic agents may be used in combination with a binding molecule provided by the present disclosure. One of ordinary skill in the art will recognize the presence and development of other cancer therapies which can be used in combination with the methods and binding molecules of the present disclosure, and will not be restricted to those forms of therapy set forth herein. Examples of categories of additional therapeutic agents that may be used in the combination therapy for treating cancer include (1) chemotherapeutic agents, (2) immunotherapeutic agents, and (3) hormone therapeutic agents.

The term "chemotherapeutic agent" refers to a chemical or biological substance that can

cause death of cancer cells, or interfere with growth, division, repair, and/or function of cancer cells. Examples of chemotherapeutic agents include those that are disclosed in WO 2006/129163, and US 20060153808, the disclosures of which are incorporated herein by reference. Examples of particular chemotherapeutic agents include: (1) alkylating agents, such as chlorambucil (LEUKERAN), mcyclophosphamide (CYTOXAN), ifosfamide (IFEX), mechlorethamine hydrochloride (MUSTARGEN), thiotepa (THIOPLEX), streptozotocin (ZANOSAR), carmustine (BICNU, GLIADEL WAFER), lomustine (CEENU), and dacarbazine (DTIC-DOME); (2) alkaloids or plant vinca alkaloids, including cytotoxic antibiotics, such as doxorubicin (ADRIAMYCIN), epirubicin (ELLENCE, PHARMORUBICIN), daunorubicin (CERUBIDINE, DAUNOXOME), nemorubicin, idarubicin (IDAMYCIN PFS, ZAVEDOS), mitoxantrone (DHAD, NOVANTRONE). dactinomycin (actinomycin D, COSMEGEN), plicamycin (MITHRACIN), mitomycin (MUTAMYCIN), and bleomycin (BLENOXANE), vinorelbine tartrate (NAVELBINE)), vinblastine (VELBAN), vincristine (ONCOVIN), and vindesine (ELDISINE); (3) antimetabolites, such as capecitabine (XELODA), cytarabine (CYTOSAR-U), fludarabine (FLUDARA), gemcitabine (GEMZAR), hydroxyurea (HYDRA), methotrexate (FOLEX, MEXATE, TREXALL), nelarabine (ARRANON), trimetrexate (NEUTREXIN), and pemetrexed (ALIMTA); (4) Pyrimidine antagonists, such as 5-fluorouracil (5-FU); capecitabine (XELODA), raltitrexed (TOMUDEX), tegafur-uracil (UFTORAL), and gemcitabine (GEMZAR); (5) taxanes, such as docetaxel (TAXOTERE), paclitaxel (TAXOL); (6) platinum drugs, such as cisplatin (PLATINOL) and carboplatin (PARAPLATIN), and oxaliplatin (ELOXATIN); (7) topoisomerase inhibitors, such as irinotecan (CAMPTOSAR), topotecan (HYCAMTIN), etoposide (ETOPOPHOS, VEPESSID, TOPOSAR), and teniposide (VUMON); (8) epipodophyllotoxins (podophyllotoxin derivatives), such as etoposide (ETOPOPHOS, VEPESSID, TOPOSAR); (9) folic acid derivatives, such as leucovorin (WELLCOVORIN); (10) nitrosoureas, such as carmustine (BiCNU), lomustine (CeeNU); (11) inhibitors of receptor tyrosine kinase, including epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), insulin receptor, insulin-like growth factor receptor (IGFR), hepatocyte growth factor receptor (HGFR), and platelet-derived growth factor receptor (PDGFR), such as gefitinib (IRESSA), erlotinib (TARCEVA), bortezomib (VELCADE), imatinib mesylate (GLEEVEC), genefitinib, lapatinib, sorafenib, thalidomide, sunitinib (SUTENT), axitinib, rituximab (RITUXAN, MABTHERA), trastuzumab (HERCEPTIN), cetuximab (ERBITUX), bevacizumab (AVASTIN), and ranibizumab (LUCENTIS), lym-1 (ONCOLYM), antibodies to insulin-like growth factor-1 receptor (IGF-1R) that are disclosed in WO2002/053596); (12) angiogenesis inhibitors, such as bevacizumab (AVASTIN), suramin (GERMANIN), angiostatin, SU5416, thalidomide, and matrix metalloproteinase inhibitors (such as batimastat and marimastat), and those that are disclosed in WO2002055106; and (13) proteasome inhibitors, such

as bortezomib (VELCADE).

The term "immunotherapeutic agents" refers to a chemical or biological substance that can enhance an immune response of a mammal. Examples of immunotherapeutic agents include: *bacillus* Calmette-Guerin (BCG); cytokines such as interferons; vaccines such as MyVax personalized immunotherapy, Onyvax-P, Oncophage, GRNVAC1, Favld, Provenge, GVAX, Lovaxin C, BiovaxID, GMXX, and NeuVax; and antibodies such as alemtuzumab (CAMPATH), bevacizumab (AVASTIN), cetuximab (ERBITUX), gemtuzunab ozogamicin (MYLOTARG), ibritumomab tiuxetan (ZEVALIN), panitumumab (VECTIBIX), rituximab (RITUXAN, MABTHERA), trastuzumab (HERCEPTIN), tositumomab (BEXXAR), ipilimumab (YERVOY) tremelimumab, CAT-3888, agonist antibodies to OX40 receptor (such as those disclosed in WO2009/079335), agonist antibodies to CD40 receptor (such as those disclosed in WO2003/040170, and TLR-9 agonists (such as those disclosed in WO2003/015711, WO2004/016805, and WO2009/022215).

The term "hormone therapeutic agent" refers to a chemical or biological substance that inhibits or eliminates the production of a hormone, or inhibits or counteracts the effect of a hormone on the growth and/or survival of cancerous cells. Examples of such agents suitable for the methods herein include those that are disclosed in US20070117809. Examples of particular hormone therapeutic agents include tamoxifen (NOLVADEX), toremifene (Fareston), fulvestrant (FASLODEX), anastrozole (ARIMIDEX), exemestane (AROMASIN), letrozole (FEMARA), megestrol acetate (MEGACE), goserelin (ZOLADEX), and leuprolide (LUPRON). The binding molecules of this disclosure may also be used in combination with non-drug hormone therapies such as (1) surgical methods that remove all or part of the organs or glands which participate in the production of the hormone, such as the ovaries, the testicles, the adrenal gland, and the pituitary gland, and (2) radiation treatment, in which the organs or glands of the patient are subjected to radiation in an amount sufficient to inhibit or eliminate the production of the targeted hormone.

The combination therapy for treating cancer also encompasses the combination of a binding molecule with surgery to remove a tumor. The binding molecule may be administered to the mammal before, during, or after the surgery.

The combination therapy for treating cancer also encompasses combination of a binding molecule with radiation therapy, such as ionizing (electromagnetic) radiotherapy (e.g., X-rays or gamma rays) and particle beam radiation therapy (e.g., high linear energy radiation). The source of radiation can be external or internal to the mammal. The binding molecule may be administered to the mammal before, during, or after the radiation therapy.

The binding molecules and compositions provided by the present disclosure can be administered via any suitable enteral route or parenteral route of administration. The term "enteral

route" of administration refers to the administration via any part of the gastrointestinal tract. Examples of enteral routes include oral, mucosal, buccal, and rectal route, or intragastric route. "Parenteral route" of administration refers to a route of administration other than enteral route. Examples of parenteral routes of administration include intravenous, intramuscular, intradermal, intraperitoneal, intratumor, intravesical, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, transtracheal, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal, subcutaneous, or topical administration. The antibodies and compositions of the disclosure can be administered using any suitable method, such as by oral ingestion, nasogastric tube, gastrostomy tube, injection, infusion, implantable infusion pump, and osmotic pump. The suitable route and method of administration may vary depending on a number of factors such as the specific antibody being used, the rate of absorption desired, specific formulation or dosage form used, type or severity of the disorder being treated, the specific site of action, and conditions of the patient, and can be readily selected by a person skilled in the art

The term "therapeutically effective amount" of a binding molecule refers to an amount that is effective for an intended therapeutic purpose. For example, in the context of enhancing an immune response, a "therapeutically effective amount" is any amount that is effective in stimulating, evoking, increasing, improving, or augmenting any response of a mammal's immune system. In the context of treating a disease, a "therapeutically effective amount" is any amount that is sufficient to cause any desirable or beneficial effect in the mammal being treated. Specifically, in the treatment of cancer, examples of desirable or beneficial effects include inhibition of further growth or spread of cancer cells, death of cancer cells, inhibition of reoccurrence of cancer, reduction of pain associated with the cancer, or improved survival of the mammal. The therapeutically effective amount of a CD137 antibody usually ranges from about 0.001 to about 500 mg/kg, and more usually about 0.01 to about 100 mg/kg, of the body weight of the mammal. For example, the amount can be about 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, 50 mg/kg, or 100 mg/kg of body weight of the mammal. In some embodiments, the therapeutically effective amount of a CD137 antibody is in the range of about 0.01-30 mg/kg of body weight of the mammal. In some other embodiments, the therapeutically effective amount of a CD137 antibody is in the range of about 0.05-15 mg/kg of body weight of the mammal. The precise dosage level to be administered can be readily determined by a person skilled in the art and will depend on a number of factors, such as the type, and severity of the disorder to be treated, the particular binding molecule employed, the route of administration, the time of administration, the duration of the treatment, the particular additional therapy employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A binding molecule or composition is usually administered on multiple occasions. Intervals between single doses can be, for example, weekly, monthly, every three months or yearly. An exemplary treatment regimen entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every three months or once every three to six months. Typical dosage regimens for a CD137 antibody include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

The present disclosure will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. The contents of all figures and all references, patents and published patent applications cited throughout this disclosure are expressly incorporated herein by reference in their entirety.

EXAMPLES

Example 1

Generation of primary Fabs that specifically binds to human CD137

Proprietary phagemid libraries (*See* PCT International Application titled "Dynamic Human Antibody Light Chain Libraries" filed concurrently herewith under Attorney Docket No. 69540-2000140, incorporated herein by reference in its entirety; *See also* PCT International Application titled "Dynamic Human Heavy Chain Antibody Libraries" filed concurrently herewith under Attorney Docket No. 69540-2000240, incorporated herein by reference in its entirety) were employed to pan against human CD137 antigens. A total of three or four rounds of panning were conducted. After the final round of panning, single-colony supernatant ELISA was performed to identify the primary hits that specifically recognize human CD137. The primary hits were defined as those whose ELISA signals were at least twice that of background. They were sequenced, the unique clones were expressed and purified for affinity measurement by ForteBio and Biacore. The list was refined to 124 in Fab with both ELISA positive hits and unique sequences. Following the criteria of K_D response signal R>0.1, R^2 >0.9 and affinity K_D < 100 nM, the list was further refined to 60 hits (Table 1a). 24 of them were then converted into IgG (Table 1b) for detailed biophysical and functional characterization.

The Fabs corresponding to the unique hits were expressed in *E.coli* and purified. Their

affinities against human CD137 were measured by ForteBio Octet RED96 Systems. Briefly, the AHC sensors (Anti-Human IgG Fc Capture Dip and Read Biosensors) were used to capture CD137-hisFc fusion protein (Sino Biological # Cat 10041-H03H), and dipped into wells containing purified Fabs that were diluted to 5-10 μg/ml with kinetic buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20, pH 7.4). The acquired ForteBio data were processed with Data Acquisition software 7.1, and kinetic data were fitted to a 1:1 Langmuir binding model. The affinity and kinetic parameters (with background subtracted) are listed in Table 1a. The affinity of their corresponding IgGs to human CD137 was measured by Biacore and shown in Table 1b.

Table 1a. Affinity of selected Fabs against human CD137 and corresponding amino acid sequences (in SEQ ID NO.)

Hit ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (upper row VH; lower row VL)
3760	1.26E-08	3.52E+05	4.44E-03	13
				14
4072	6.95E-09	1.54E+05	1.07E-03	15
				16
4074	1.95E-08	4.80E+04	9.37E-04	17
				18
4076	7.44E-09	6.38E+04	4.75E-04	19
				20
4079	3.15E-08	6.93E+04	2.19E-03	21
				22
4134	1.30E-08	5.93E+04	7.69E-04	23
				24
4137	<1.0E-12	8.56E+04	<1.0E-07	25
				26
4139	1.65E-09	4.96E+04	8.17E-05	27
				28
4140	<1.0E-12	2.57E+04	<1.0E-07	29
				30
4217	9.67E-08	5.64E+05	5.45E-02	31
				32
5299	1.37E-08	5.55E+05	7.60E-03	33
				34
5300	1.53E-08	5.96E+05	9.10E-03	35
				36
5302	1.21E-09	3.54E+05	4.26E-04	37
				38
5303	5.12E-09	9.95E+05	5.09E-03	39
				40
5310	5.72E-09	8.13E+05	4.65E-03	41
				42
5314	8.39E-09	2.10E+05	1.77E-03	43
				44
5316	1.14E-08	140600	0.001605	45

Hit ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (upper row VH; lower row VL)
				46
5318	1.90E-08	1.41E+05	2.69E-03	47
				48
5323	1.04E-08	7.82E+05	8.12E-03	49
				50
5341	2.93E-08	6.42E+04	1.88E-03	51
				52
5342	3.89E-08	1.57E+05	6.12E-03	53
				54
5346	1.61E-08	6.05E+05	9.77E-03	55
				56
5348	1.02E-08	1.31E+06	1.33E-02	57
				58
5349	6.20E-09	1.62E+05	1.01E-03	59
				60
5351	7.29E-09	4.66E+05	3.40E-03	61
				62
5353	1.61E-08	3.70E+05	5.97E-03	63
				64
5359	7.10E-10	4.64E+05	3.30E-04	65
				66
5360	2.41E-08	1.20E+05	2.89E-03	67
				68
5363	9.87E-09	8.37E+04	8.26E-04	69
				70
5365	2.56E-09	7.01E+05	1.79E-03	71
				72
5367	1.49E-08	4.07E+05	6.08E-03	73
				74
5370	1.91E-09	5.24E+05	1.00E-03	75
				76
5371	3.97E-09	1.21E+06	4.79E-03	77
				78
5404	3.30E-09	3.95E+05	1.30E-03	79
				80
5407	1.76E-09	2.48E+05	4.37E-04	81
				82
5408	2.36E-08	3.18E+05	7.50E-03	83
				84
5409	1.70E-08	2.51E+05	4.27E-03	85
				86
5413	9.93E-10	5.55E+05	5.51E-04	87
				88
5417	4.04E-08	5.72E+04	2.31E-03	89
				90
7077	1.88E-08	4.98E+05	9.34E-03	91
				92
7078	2.52E-08	3.45E+05	8.70E-03	93
				94

Hit ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (upper row VH; lower row VL)
7079	2.99E-08	1.00E+05	3.00E-03	95
				96
7080	2.44E-08	3.06E+05	7.46E-03	97
				98
7081	4.31E-08	2.87E+05	1.23E-02	99
				100
7087	6.96E-08	1.23E+05	8.55E-03	101
				102
7088	4.36E-08	2.55E+05	1.11E-02	103
				104
7090	5.55E-08	3.12E+05	1.73E-02	105
				106
7092	4.57E-08	4.31E+05	1.97E-02	107
				108
7097	2.43E-08	5.42E+05	1.32E-02	109
				110
7100	3.50E-08	4.62E+05	1.62E-02	111
				112
7105	3.33E-08	3.30E+05	1.10E-02	113
				114
7109	3.20E-08	1.73E+05	5.55E-03	115
				116
7120	3.45E-08	2.64E+05	9.11E-03	117
				118
7128	3.97E-08	3.09E+05	1.23E-02	119
				120
7131	3.04E-08	2.66E+05	8.10E-03	121
				122
7133	4.03E-08	1.01E+05	4.05E-03	123
				124
7135	3.17E-08	1.02E+05	3.22E-03	125
				126
7159	3.79E-08	1.06E+05	4.03E-03	127
				128
7163	1.26E-08	2.99E+05	3.78E-03	129
				130
7166	1.24E-08	3.45E+05	4.29E-03	131
				132

The corresponding DNA sequences encoding the amino acid sequences of SEQ ID NOs:13-132 can be found in SEQ ID NOs: 133-252, respectively. The HVR_H1 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 253-312, respectively. The HVR_H2 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 313-372, respectively. The HVR_H3 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 373-432, respectively. The HVR_L1 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 433-492, respectively. The HVR_L2 amino acid

sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 493-552, respectively. The HVR_L3 amino acid sequences for all Fab hits shown in Table 1a can be found in SEQ ID NOs: 553-612, respectively (*See also*, Table 1c).

Table 1b Affinity of Fabs and the corresponding IgGs against human CD137

Hits ID	Fab			IgG ID	IgG			IgG SEQ ID NO. (upper row heavy chain; lower row light chain)
	KD (M)	ka(1/Ms)	kd(1/s)		KD (M)	Ka(1/Ms)	kd (1/s)	
4072	7.0E-09	1.5E+05	1.1E-03	AG10054	1.3E-08	1.4E+05	1.9E-03	613 614
5303	5.1E-09	1.0E+06	5.1E-03	AG10057	7.9E-09	7.7E+05	6.1E-03	615 616
5310	5.7E-09	8.1E+05	4.7E-03	AG10058	5.9E-09	4.1E+05	2.4E-03	617 618
5351	7.3E-09	4.7E+05	3.4E-03	AG10059	3.8E-08	1.6E+05	6.3E-03	619 620
5359	7.1E-10	4.6E+05	3.3E-04	AG10060	1.1E-09	2.2E+05	2.5E-04	621 622
5370	1.9E-09	5.2E+05	1.0E-03	AG10061	3.6E-09	2.2E+05	7.8E-04	623 624
5404	3.3E-09	4.0E+05	1.3E-03	AG10062	5.9E-09	1.6E+05	9.4E-04	625 626
5413	9.9E-10	5.6E+05	5.5E-04	AG10063	9.9E-10	3.9E+05	3.9E-04	627 628
4074	2.0E-08	4.8E+04	9.4E-04	AG10079	1.4E-09	1.9E+05	2.7E-04	629 630
4217	9.7E-08	5.6E+05	5.5E-02	AG10080	1.0E-08	1.2E+06	1.2E-02	631 632
5299	1.4E-08	5.6E+05	7.6E-03	AG10081	6.9E-09	2.4E+05	1.7E-03	633 634
5300	1.5E-08	6.0E+05	9.1E-03	AG10082	1.3E-08	5.6E+05	7.2E-03	635 636
5323	1.0E-08	7.8E+05	8.1E-03	AG10083	1.2E-08	5.7E+05	6.9E-03	637 638
5360	2.4E-08	1.2E+05	2.9E-03	AG10084	4.3E-08	6.6E+04	2.8E-03	639 640
5367	1.5E-08	4.1E+05	6.1E-03	AG10085	5.4E-08	1.5E+05	7.9E-03	641 642
5409	1.7E-08	2.5E+05	4.3E-03	AG10086	4.6E-08	1.0E+05	4.5E-03	643 644
5302	1.2E-09	3.5E+05	4.3E-04	AG10124	6.0E-09	5.0E+05	3.0E-03	645 646
5314	8.4E-09	2.1E+05	1.8E-03	AG10125	1.5E-08	1.1E+05	1.7E-03	647 648
5316	1.1E-08	1.4E+05	1.6E-03	AG10126	1.4E-08	5.4E+09	7.3E+01	649 650
5318	1.9E-08	1.4E+05	2.7E-03	AG10127	9.6E-09	3.0E+05	2.9E-03	651

								652
5342	3.9E-08	1.6E+05	6.1E-03	AG10128	3.0E-09	1.2E+05	3.7E-04	653
								654
5353	1.6E-08	3.7E+05	6.0E-03	AG10129	1.9E-08	3.1E+05	6.0E-03	655
								656
5365	2.6E-09	7.0E+05	1.8E-03	AG10131	3.7E-09	5.1E+05	1.9E-03	657
								658
5408	2.4E-08	3.2E+05	7.5E-03	AG10132	6.9E-08	2.0E+05	1.4E-02	659
								660

The corresponding DNA sequences encoding the amino acid sequences of SEQ ID NOs: 613-660 can be found in SEQ ID NOs: 661-708, respectively. The HVR_H1 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 709-732, respectively. The HVR_H2 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 733-756, respectively. The HVR_H3 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 757-780, respectively. The HVR_L1 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 781-804, respectively. The HVR_L2 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 805-828, respectively. The HVR_L3 amino acid sequences for all IgG sequences shown in Table 1b can be found in SEQ ID NOs: 829-852, respectively.

Table 1c: CDR sequences of Fabs

Hit ID	VH/VL	HVR-H1 SEQ ID NO.	HVR-H2 SEQ ID NO.	HVR-H3 SEQ ID NO.	HVR-L1 SEQ ID NO.	HVR-L2 SEQ ID NO.	HVR-L31 SEQ ID NO.
3760	VH1/VL1	253	313	373	433	493	553
4072	VH2/VL2	254	314	374	434	494	554
7074	VH3/VL3	255	315	375	435	495	555
4076	VH4/VL4	256	316	376	436	496	556
4079	VH5/VL5	257	317	377	437	497	557
4134	VH6/VL6	258	318	378	438	498	558
4137	VH7/VL7	259	319	379	439	499	559
4139	VH8/VL8	260	320	380	440	500	560
4140	VH9/VL9	261	321	381	441	501	561
4217	VH10/VL10	262	322	382	442	502	562
5299	VH11/VL11	263	323	383	443	503	563
5300	VH12/VL12	264	324	384	444	504	564
5302	VH13/VL13	265	325	385	445	505	565
5303	VH14/VL14	266	326	386	446	506	566
5310	VH15/VL15	267	327	387	447	507	567
5314	VH16/VL16	268	328	388	448	508	568
5316	VH17/VL17	269	329	389	449	509	569
5318	VH18/VL18	270	330	390	450	510	570
5323	VH19/VL19	271	331	391	451	511	571
5341	VH20/VL20	272	332	392	452	512	572
5342	VH21/VL21	273	333	393	453	513	573
5346	VH22/VL22	274	334	394	454	514	574
5348	VH23/VL23	275	335	395	455	515	575

5349	VH24/VL24	276	336	396	456	516	576
5351	VH25/VL25	277	337	397	457	517	577
5353	VH26/VL26	278	338	398	458	518	578
5359	VH27/VL27	279	339	399	459	519	579
5360	VH28/VL28	280	340	400	460	520	580
5363	VH29/VL29	281	341	401	461	521	581
5365	VH30/VL30	282	342	402	462	522	582
5367	VH31/VL31	283	343	403	463	523	583
5370	VH32/VL32	284	344	404	464	524	584
5371	VH33/VL33	285	345	405	465	525	585
5404	VH34/VL34	286	346	406	466	526	586
5407	VH35/VL35	287	347	407	467	527	587
5408	VH36/VL36	288	348	408	468	528	588
5409	VH37/VL37	289	349	409	469	529	589
5413	VH38/VL38	290	350	410	470	530	590
5417	VH39/VL39	291	351	411	471	531	591
7077	VH40/VL40	292	352	412	472	532	592
7078	VH41/VL41	293	353	413	473	533	593
7079	VH42/VL42	294	354	414	474	534	594
7080	VH43/VL43	295	355	415	475	535	595
7081	VH44/VL44	296	356	416	476	536	596
7087	VH45/VL45	297	357	417	477	537	597
7088	VH46/VL46	298	358	418	478	538	598
7090	VH47/VL47	299	359	419	479	539	599
7092	VH48/VL48	300	360	420	480	540	600
7097	VH49/VL49	301	361	421	481	541	601
7100	VH50/VL50	302	362	422	482	542	602
7105	VH51/VL51	303	363	423	483	543	603
7109	VH52/VL52	304	364	424	484	544	604
7120	VH53/VL53	305	365	425	485	545	605
7128	VH54/VL54	306	366	426	486	546	606
7131	VH55/VL55	307	367	427	487	547	607
7133	VH56/VL56	308	368	428	488	548	608
7135	VH57/VL57	309	369	429	489	549	609
7159	VH58/VL58	310	370	430	490	550	610
7163	VH59/VL59	311	371	431	491	551	611
7166	VH60/VL60	312	372	432	492	552	612

Example 2

Selection of Fab hits that are cross-reactive with mouse CD137

The species cross-reactivity of Fab hits was determined using ELISA. Briefly, 200 µL 5 µg/mL anti-human IgG (Fab specific) (Sigma #I5260) was coated on Maxisorp microplate (Thermo Scientific 446469) at 4℃ overnight. After blocking, 100 µL Fab 5310 (5 µg/mL), 5351 (2.8 µg/mL) and 5365 (5 µg/mL) were added and incubated for 1 hr. After washing for three times, serial dilutions of human or mouse CD137 antigens fused with human FC fragments were added and incubated for 1 hr. After washing, HRP labelled goat anti-human FC were diluted 1:2000 with PBS, and added to each well for 1 hr incubation. Plates were washed three times and incubated with TMB substrate for 20 min at room temperature. Absorbance at 450 nm was measured after the reaction was stopped. The result is presented in Figure 1b, lower panel showing that Fab 5310 and 5365 bind to both human and mouse CD137, whereas Fab 5351 binds to human CD137, but not to

mouse CD137.

Example 3

IgG conversion and expression: AG10058, AG10059 and AG10131

The heavy chains and light chains of the Fabs 5310, 5351, and 5365 were cloned into the mammalian expression vector pCDNA3.3 (Thermo Fisher Scientific) separately in IgG4 isotype with S241P mutation. The heavy and light chains of two reference antibodies were also cloned into pCDNA3.3 in IgG4 and IgG2 isotype respectively.

The heavy chain variable region used in reference antibody AC1097 comprised the sequence EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYWISWVRQMPGKGLEWMGKIYPGDSYTNY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGYGIFDYWGQGTLVTVSS (SEQ ID NO: 862), and the light chain variable region in reference antibody AC1097 comprised the sequence SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVLVIYQDKNRPSGIPERFS GSNSGNTATLTISGTQAMDEADYYCATYTGFGSLAVFGGGTKLTVL (SEQ ID NO: 863). The heavy chain variable region used in reference antibody AC1121 comprised the sequence QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLEWIGEINHGGYVTYN PSLESRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYGPGNYDWYFDLWGRGTLVTVS S (SEQ ID NO: 864), and the light chain variable region in reference antibody AC1121 comprised the sequence EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARF SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPALTFGGGTKVEIK (SEQ ID NO: 865). IgGs used in herein are shown in Table 2.

Table 2: List of IgGs

IgG	Fab	Isotype	Description
AC1097	Reference 1	IgG2	Reference Ab
AC1121	Reference 2	IgG4 (S241P)	Reference Ab
AG10058	5310	IgG4 (S241P)	Adagene mAb
AG10059	5351	IgG4 (S241P)	Adagene mAb
AG10131	5365	IgG4 (S241P)	Adagene mAb
AG10154		IgG4 (S241P)	Isotype control

Pairs of plasmids were transiently transfected into HEK293F cells following manufacturer's

instructions. The supernatants were harvested, cleared by centrifugation and filtration, and IgGs were purified with standard protein A affinity chromatography (MabSelect SuRe, GE Healthcare). The proteins were eluted and neutralized, and buffer exchanged into PB buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0). Protein concentrations were determined by UV-spectrophotometry, and IgG purity was analyzed under denaturing, reducing and non-reducing conditions by SDS-PAGE or SEC-HPLC.

Example 4

Binding affinity to human, monkey and mouse CD137

The binding affinity of IgGs to human, monkey and mouse CD137 were measured by BIAcore, ELISA and flow cytometry. The results were summarized in Table 3.

Table 3. Binding affinity of antibodies to human, monkey and mouse CD137

KD (nM)	Biacore			ELISA			HEK293F Cell surface		
	Human	Cyno	Mouse	Human	Cyno	Mouse	Human	Cyno	Mouse
AG10131	3.7	12.5	64.5	0.2	0.3	23.9	1.3	1.2	49.4
AG10058	5.9	9.3	15.2	0.2	0.3	0.3	1.8	2	10.1
AG10059	24.2	23.1	NC	0.8	0.4	NC	5	2.6	NC
AC1097	20.9	37.6	NC	0.2	0.4	NC	1.9	2.9	NC
AC1121	9.6	NC	NC	0.2		NC	3.3	NC	NC

NC: not cross-reactive

4a. Measurement of binding affinity and kinetics by SPR

Binding affinity and kinetics of antibodies against human, monkey and mouse CD137 protein were examined by surface plasmon resonance (SPR) analysis using a Biacore™ T200 instrument (Biacore AB, Uppsala, Sweden) according to the manufacturer's guidelines. Anti-Human IgG (Fc) antibody from Human Antibody Capture Kit (GE BR-1008-39) was immobilized on CM5 chips by coupling of its amine groups onto carboxylated surfaces of sensor chips according to the instructions of Amine Coupling kit (GE Biacore #BR-1000-50). The immobilized Anti-Human IgG (Fc) antibody was used to capture AG10058, AG10059, AG10131, AC1121 and AC1097. Finally, six concentrations (3.13, 6.25, 12.5, 25, 50, 100) (nM) (diluted in running buffer) of human CD137-His6 (Sino Biological #10041-H08H) were injected at a flow rate of 30 μl/min for 300 seconds, and the dissociation time was 300 seconds. The running buffer used was 1×HBS-EP (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20, pH 7.4 at 25 ℃). Corresponding controls were conducted in each case using a blank flow cell with no protein immobilized for "background" subtraction. The association and dissociation curves were fitted to a 1: 1 Langmuir binding model using Biacore T200 Evaluation Software (Biacore AB, Uppsala, Sweden) according to the manufacturer's guidelines. As shown in Table 3, all antibodies bind to

human CD137. AG10058 and AG10059 show higher affinity than both reference antibodies. Except AC1121 reference mAb, all antibodies bind to monkey CD137. Only AG10058 and AG10131 bind to mouse and rat CD137. AG10058 has higher affinity (15.2 nM) than AG10131 (64.5 nM).

4b. Measurement of binding affinity to soluble CD137 using ELISA assay

A serial dilution of human, monkey or mouse CD137 fused with human FC fragment were prepared and used to coat the ELISA plate at 37℃ for 1 hr. After blocking, 100 μL IgGs (5 μg/mL) were added and incubated at 37℃ for 1 hr. Plates were washed for three times and then incubated with HRP-conjugated protein L (1:2000 dilution) at 37℃ for 1 hr. Plates were washed again for three times and incubated with TMB substrate for 20 min at room temperature. Absorbance at 450 nm was measured after the reaction was stopped. The data was analyzed by Graphpad Prism 6 with nonlinear fitting. As shown in Figure 2, all antibodies bind to human CD137 (FC fusion protein) with similar sub nM affinity. Except AC1121 reference mAb, all antibodies bind to monkey CD137 with similar sub nM affinity. Consistent with results from Biacore, only AG10058 and AG10131 bind to mouse CD137. AG10058 has higher affinity (0.3 nM) than AG10131 (23.9 nM).

4c. Measurement of binding affinity to CD137 overexpressed on cell surface by flow cytometry

The affinity of antibodies were also assessed against human, monkey and mouse CD137 that are transiently expressed on the surface of HEK293F cells. Briefly, HEK293F cells were transfected with a plasmid expressing full-length human, monkey or mouse CD137 from a bicistronic IRES vector, EGFP was used to identify the transfected cells. After 48 hrs, the transfected cells were harvested and then washed once with cold FACS buffer (PBS supplemented with 1% BSA). Cells were then incubated with various IgGs (each at 100 nM) for 1 hr on ice, washed twice with pre-chilled FACS buffer, and incubated with Alexa Fluor® 647 conjugated mouse anti-human FC antibodies for 30 min on ice. The cells were washed once prior to analysis by flow cytometry (Beckman® CytoFlex). As shown in Figure 3a, all antibodies bind to human CD137 expressed on cell surface with low nM affinity. AG10058, AG10059 and AG10131 are slightly better than both reference antibodies. Except AC1121 reference mAb, all antibodies bind to monkey CD137 with low nM affinity, AG10058, AG10059 and AG10131 are slightly better than AC1097 reference antibody. Consistent with results from Biacore and ELISA, only AG10058 and AG10131 bind to mouse and rat CD137. AG10058 has higher affinity than AG10131 against mouse CD137. Additionally, AG10058 and AG10131 (each at 100 nM) also bind to rat and canine CD137 overexpressed on HEK293F cell surface (Figure 3b).

4d. *Binding of IgGs to activated human, monkey, mouse and rat T cells.*

The species-cross reactivity of the exemplary antibodies was further confirmed using PMA and Ionomycin stimulated PBMC or T cells of human, monkey, mouse and rat. Human and cynomolgus monkey PBMC were isolated by Ficoll-density gradient centrifugation. Briefly, fresh whole blood from healthy donors or cynomolgus monkeys were diluted with equal volume of PBS and carefully loaded on to the top of Histopaque 1077 (14ml in 50ml centrifuge tube). Centrifuge at 1,200×g for 30 minutes at room temperature with brake off. After centrifugation, carefully aspirate the upper layer with a pipette to within 0.5 cm of the opaque interface containing mononuclear cells. Discard upper layer. Carefully transfer the opaque interface (about 3-5 ml) with a pipette into a clean 50 ml conical centrifuge tube. Wash the cells with 20 ml of PBS, collect cells by centrifuge at 400 × g for 5 minutes, and resuspend cells into 20 ml of PBS. Count the cells with hemocytometer, and collect cells again by centrifuge at 400 × g for 5 minutes. Mouse or rat splenocytes were isolated by passing spleens through a 45μm cell strainer attached to a 50-mL conical tube to get single cell suspension, and wash cells through the strainer with PBS. Centrifuge at 1600 rpm for 5 min and discard the supernatant. Resuspend cell pellet in 2ml red blood cell lysing solution for 2min. Add in excess of 10-fold volume PBS and collect cells at 1600rpm centrifugation for 5min. Discard supernatant and resuspend splenocytes in RPMI1640/10%FBS. Pan-T cells were enriched from PBMC (human/monkey), or splenocytes (mouse/rat) by negative selection with magnetic beads in commercial kits (Stemcell Technologies) specific for human, monkey, mouse and rat, respectively. Activation of human/monkey PBMC, or mouse/rat splenocytes was performed by incubating cells with 50ng/ml PMA + 1μM Ionomycin at 37°C, 5% CO2 for overnight.

Activated cells (~$2x10^5$ cells/tube) were washed in pre-chilled staining buffer (PBS supplemented with 2% FBS) and incubated with 100 nM tested antibodies for 1 hr on ice. Cells were then washed twice using 1 mL staining buffer and resuspended in 100 μL staining buffer containing Alexa Fluor® 647 conjugated mouse anti-human FC antibody and the species-specific T cell marker antibodies. The T cell marker antibodies were used as follows: CD3, CD4 or CD8. The cells were washed twice with staining buffer after incubation in dark for 30 minutes. Finally, cells were resuspended in 300 μL staining buffer and analyzed by Beckman CytoFlex. The data analysis was performed using Flowjo 10 software. As shown in Figure 4a, all tested antibodies bind to both activated human and monkey T cells, but not to the naïve human T cells. The binding ability of AG10131 to activated mouse and rat T cells were further evaluated (Figure 4b). AG10131 binds to both activated mouse and rat T cells.

In summary, AG10058 and AG10131 antibodies show higher affinity to human and

monkey CD137. They exhibit broad species-cross reactivity, including human, cynomolgus monkey, mouse, rat and dog for AG10131, but human, cynomolgus monkey, mouse, and dog for AG10058, allowing quick assessment of in vivo efficacy in mouse syngeneic models.

Example 5

Binding selectivity of antibodies for CD137

The selectivity of antibodies for CD137 was evaluated using flow cytometry analysis of their binding ability to members of TNFR superfamily. The TNFRSF receptors including CD137, OX40, CD40, GITR and CD27 were transiently overexpressed on the surface of HEK293F cells. Transfected cells were washed in pre-chilled staining buffer (PBS supplemented with 2% FBS), then incubated with 100 nM test antibodies for 1 hr on ice. Cells were washed twice with staining buffer, and Alexa Fluor® 647 conjugated mouse anti-human FC antibodies were added and incubated for 30 min on ice. Samples were washed once with staining buffer prior to analysis by flow cytometry. As shown in Figure 5, AG10058, AG10059 and AG10131 bind specifically to CD137, not to any other tested family members or parent cells transfected with empty vectors.

Example 6

Ligand competition using ELISA and flow cytometry

Antibodies were tested for their ability to block the binding of CD137 to its cognate ligand CD137L by both ELISA and flow cytometry assay. As shown in Figures 6a and 6b, all tested antibodies block the binding of CD137 and CD137L.

6a. Ligand competition binding by ELISA

Recombinant human CD137 (fused with human Fc and His tag) was diluted to 1 μg/mL in PBS and coated on Maxisorp plate at 4°C overnight. Plates were blocked with PBS supplemented with 3% non-fat milk at 37°C for 1 hr. After washing, a total volume of 100 uL mixture of 50 uL biotinylated CD137L (4 μg/mL) and various concentrations of test antibodies (eight 1:2 serial dilutions ranging from 500 μg/mL to 2 μg/mL) were added to each well and incubated at 37°C for 1 hr. Plates were washed three times and 100 μL HRP conjugated neutravidin (1:1000) were added to each well and incubated at 37°C for 1 hr. Plates were washed as previously described and 50 μL TMB substrate solution was added and incubated at room temperature for 20 minutes before the reaction was stopped by 50 μL H_2SO_4. As shown in Figure 6a, all test antibodies AG10058, AG10059 and AG10131 block the binding of CD137 to CD137L. AG10131 shows the strongest or complete blocking ability, at about uM range, followed by AG10058 for significant blocking at >uM; and AG10059 for effective blocking at uM range. These data suggest that, under the

conditions tested and with the reagents used, the broad species cross-reactive antibodies AG10131 and AG10058 are highly effective inhibitors of the interaction between CD137 and its ligand CD137L, whereas AG10059 only shows moderately effective blocking of the interaction between CD137 and its ligand CD137L. It should be noted that the reference antibody AC1097, which cross-reacts with both human and monkey CD137, while AC1121, which only reacts with human CD137, shows almost no blocking at all.

6b. Ligand competition binding by flow cytometry

The plasmid encoding full-length human CD137 was transiently expressed in HEK293F cells. Cells were washed with staining buffer (PBS supplemented with 1% BSA) and resuspended in staining buffer containing 100 nM test antibodies. After incubation on ice for 30 min, 33 nM biotinylated CD137L were added to each well and incubated for another 1 hr on ice. Cells were washed with staining buffer twice, and 50 μL staining buffer containing Alexa fluor 647 conjugated streptavidin were added and incubated on ice for 30 min. Cells were washed once and analyzed by CytoFlex flow cytometry. As shown in Figure 6b, all three tested antibodies can block binding between CD137 and CD137L in a concentration dependent manner. AG10131 shows the strongest blocking capability, followed by AG10058 with significant blocking; and AG10059 with less effective blocking. These data suggest that the broad species cross-reactive antibodies AG10131 and AG10058 are highly effective in blocking the interaction between CD137 and its ligand CD137L, while AG10059 shows partial blocking of the interaction between CD137 and its ligand CD137L. In contrast, the AC1097 reference antibody, which cross-reacts with both human and monkey CD137, shows only partial blocking, while the AC1121 reference antibody, which only reacts with human CD137, shows no blocking.

Example 7

Epitope mapping

To determine the binding regions of the tested antibodies at amino acid residue level, a series of mutations (Table 5) were made at the extracellular domain of human CD137. These CD137 mutation plasmids were used to transfect HEK293F cells. The binding of antibodies to the human CD137 mutants were assessed by flow cytometry analysis as previously described in Example 5 and shown in Figure 7A. The results are summarized in Table 5, together with the cross-reactivity of these antibodies with human, monkey, mouse, and rat CD137 in interesting differentiation, indicating the fine epitopes from hits derived from Adagene libraries. AG10131 binds to all 4 species, whereas AG10058 binds all 3 CD137 but not rat CD137. AG10058, AG10059 and AG10131 lost the binding ability to GFT34AAA, FSS53AAA, and FH92AA

mutations, indicating that their binding epitopes are within these regions, e.g., amino acid residues 34-93 or 34-108 of SEQ ID NO.: 1 (*See also*, figure 7B). AG10058 and AG10131 may bind the same or highly similar epitope, while AG10059 may bind different epitopes from AG10058 and AG10131.

The mutant constructs were meant to differentiate the epitopes by AG10058, AG10059 and AG10131 from the reference antibodies by AC1121 and AC1097. It is clear that all three antibodies AG10058, AG10059 and AG10131 target very different epitopes from AC1121 and AC1097. AG10058, AG10059 and AG10131 differ from AC1121 in regions defined by mutants Hu_FH92AA and Hu_FSS53AAA and possibly Hu_GTF34AAA, whereas AG10058, AG10059 and AG10131 differ from AC1097 in regions defined by most of the mutants used, except for Hu_FH92AA and their species cross-reactivity with Monkey but different in other species cross-reactivity such as mouse, rat and dog CD137. In some embodiments, AG10058, AG10059 and AG10131 or other antibodies disclosed herein do not bind to an epitope located within amino acid residues 115-156 of SEQ ID NO.: 1. Also shown in FIG. 7A and Table 5 is that binding of the human CD137 ligand to the wild-type vs. mutant human CD137 matches well with binding pattern of the tested antibodies, consisting with the observation that these antibodies block CD137 ligand binding to its receptor.

Table 5 Epitope Mapping

Mutations	AG10058	AG10059	AG10131	AC1121	AC1097	**HuCD137L**
Hu_WT	+	+	+	+	+	+
Cyno_WT	+	+	+	-	+	
Mouse_WT	+	-	+	-	-	
Rat_WT	-	-	+	-	-	
Hu_GTF34AAA	-	-	-	-/+	+	-
Hu_FSS53AAA	-	-	-	+	+	-
Hu_FH92AA	-	-	-	+	-	-
Hu_GQ109AA	+	+	+	+	-	+
Hu_EL111AA	+	+	+	+	-	+
Hu_F125A	+	+	+	+	-	+
Hu_FN125AA	+	+	+	+	-	+
Hu_PW135AA	+	+	+	+	-	+
Hu_TN137AA	+	+	+	+	-	+
Hu_GT150AA	+	+	+	+	-	+

Example 8

Agonist activity of antibodies in NFκB luciferase reporter assay

Agonist activity of antibodies was evaluated using NFκB reporter assay. 293T cells were

transfected with plasmid expressing human, monkey or mouse CD137 along with NFκB luciferase reporter plasmids. After 4h, 50 μL cells were plated into each assay well of a 96-well plate at density of 0.4 x 10^6/mL. A total volume of 50 μL antibody mixture containing test antibodies and 3:1 ratio of cross linking antibody (Fab' goat anti-human IgG FC) was added and incubated for 18h. After medium is removed, 50 μL Passive Lysis Buffer (Promega E1980) were added and incubated at 37℃ for 30 min. 20 μL lysate were transferred to a white plate and the luciferase substrates were added. The luminescence signal of firefly and Renina was measured and their ratio was used for data analysis by GraphPad Prism 6.0 software. As shown in Figure 8, compare to isotype control antibody, all test antibodies activate NFκB reporter gene expression when human and monkey CD137 is expressed. When mouse CD137 is expressed, AG10058 and AG10131, but not AG10059, activate NFκB reporter gene expression. This is consistent with the prior observation that AG10058 and AG10131 bind to mouse CD137 whereas AG10059 does not.

Example 9

Agonist activity of antibodies in T cell activation assay

Agonist activity of antibodies was further confirmed in the T cell activation assay. 96-well cell culture plates were coated with 50 μL of the anti-CD3 antibody (2 μg/ml) alone or along with 50 μL of the test antibodies (60 μg/mL, 20 μg/mL, 6 μg/mL, 2 μg/mL, and 0 μg/mL) in 1×PBS at 4°C overnight. CD8+ T cells were isolated using protocols according to the manufacture's instruction. Cells were prepared at density of 1×10^7 cells/mL in RPMI1640 media supplemented with 10% FBS. 200 μL cells were plated to each assay well and incubated for 4 days in a 37°C, 5% CO2 incubator. Cells were checked daily under microscope for proliferation. After 96 hr incubation, 100 μL of supernatant were transferred to a new 96-well plate for IFN-γ detection. T cell proliferation was assayed using Cell Titer Glow kit (Promega). As shown in Figure 9, compared to isotype control antibody, all tested antibodies induced both CD8+ T cell proliferation and IFN-γ secretion in a dose-dependent manner.

Example 10

Anti-tumor activity in mouse syngeneic models

The species cross-reactivity with mouse CD137 allows quick *in vivo* functional assessment. AG10058 and AG10131 have been tested in multiple mouse syngeneic models. BALB/c mice (n=8 per group) were transplanted subcutaneously with 2×10^6 H22 liver cancer cells (Xiao et.al, Soluble PD-1 facilitates 4-1BBL-triggered antitumor immunity against murine H22 hepatocarcinoma in vivo. Clin Cancer Res. 2007;13(6):1823-30.), 5×10^5 CT26 colon cancer cells, or 5×10^5 EMT6

breast cancer cells. When tumors were established (>50mm^3), treatment began with isotype control antibody, AG10058, or AG10131 by intraperitoneal injection, twice a week for up to 3 weeks. Tumor growth was monitored twice a week and reported as the mean tumor volume±s.e.m. over time. As shown in Figures 10-13, compared to the isotype control antibody, both AG10058 and AG10131 exhibited potent in vivo anti-tumor activity in these different syngeneic mouse tumor models.

10a. CD137 agonist antibodies exhibit anti-tumor efficacy in H22 mouse liver cancer model

First, AG10058 or AG10131 was administrated twice a week for 3 weeks at dosage of 50 mg/kg. Both molecules showed almost 100% TGI (tumor growth inhibition) (Figure 10, panel a). Immunohistochemistry staining of CD4 and CD8 markers showed that AG10131 significantly increased the infiltration of both CD4+ and CD8+ T cells in H22 tumor (Xiao et.al, Soluble PD-1 facilitates 4-1BBL-triggered antitumor immunity against murine H22 hepatocarcinoma in vivo. Clin Cancer Res. 2007;13(6):1823-30.) microenvironment (Figure 10, panel b). Further dose titrations down to 3 mg/kg still showed ~100% TGI, suggesting both molecules have potent anti-tumor activity (Figure 10, panels c and d). Further dose titration of AG10131 down to 1 and 0.1 mg/kg showed greater than 50% TGI at 0.1 mg/kg and 1 mg/kg (Figure 10, panel e).

10b. CD137 agonist antibodies exhibit anti-tumor efficacy in CT26 mouse colon cancer model

As shown in Figure 10, both AG10058 and AG10131 showed almost 100% TGI (tumor growth inhibition) at dose of 50 mg/kg (Figure 11, panel a) in CT26 mouse colon cancer model (Martinez-Forero et.al, T cell costimulation with anti-CD137 monoclonal antibodies is mediated by K63-polyubiquitin-dependent signals from endosomes. J Immunol. 2013;190(12):6694-706). Further dose titration of AG10131 (Figure 11, panel b) showed almost 100% TGI at doses of 5 mg/kg and 1 mg/kg. At 0.1 mg/kg dosage, approximately 40% TGI was achieved, indicating a dose-dependent anti-tumor activity.

10c. EMT6 breast cancer model

Anti-tumor activity is further evaluated in EMT6 mouse breast cancer syngeneic model (Shi and Siemann, Augmented antitumor effects of radiation therapy by 4-1BB antibody (BMS-469492) treatment. Anticancer Res. 2006;26:3445-53) (Figure 12). Both AG10058 and AG10131 exhibited almost ~100% tumor growth inhibition.

10d. Mice with complete response to CD137 agonist antibody treatment maintain tumor free after re-challenge with new tumor cells

After treatment with AG10058 or AG10131 for 3 weeks in the CT26 tumor model, the mice with complete tumor regression were maintained without treatment for over an additional month. Mice that maintained complete response were then re-challenged on Day 62 subcutaneously with 5×10^5 CT26 tumor cells in the opposite flank, and monitored for tumor growth. Re-challenge control group was set up at the same time with naïve mice inoculated with the same number of CT26 tumor cells. As shown in Figures 13, treatment with AG10131 (at 1 and 5 mg/kg, see Figure 13, top and bottom panel, respectively) exhibited potent antitumor activity in CT26 tumor model, 5/8 in AG10131 (1 mg/kg group), 6/8 in AG10131 (5 mg/kg group) showing complete response over 60 days before re-challenged with CT26 tumor cells again. Furthermore, these mice remained tumor free after re-challenge with the same tumor cells, suggesting that specific anti-tumor memory was developed in these mice.

To prove this hypothesis, splenocytes were collected from these tumor-rejecting re-challenged mice and control mice and co-cultivated with the mitomycin C-arrested CT26 tumor cells in vitro for 7 days to amplify the tumor-specific memory T cells. These splenocytes were then recovered and mixed with fluorescence labeled live CT26 tumor cells at different E/T ratio for 4h and tumor cell killing was detected by the live/dead staining and FACS analysis. As shown in Figure 14, significantly increased tumor cell killing was observed with splenocytes from tumor-rejecting re-challenged mice with prior treatment of both AG10058 and AG10131.

Example 11

AG10131-IgG4 does not induce ADCC effect

Human $CD8^+$ T cells were isolated from peripheral blood from a healthy donor with the EasySep human $CD8^+$ T cell enrichment kit (StemCell Technologies), and then stimulated with PMA (50ng/ml) + Ionomycin (1uM) for 18 hours *in vitro*. These activated $CD8^+$ T cells were then labeled with Calcein-AM and served as the target cells. NK cells from different healthy donors were isolated with the human NK isolation kit (StemCell Technologies), and served as effector cells. For the antibody-dependent cytotoxicity (ADCC) assay, effector (NK) and target (activated $CD8^+$ T) cells were mixed at 5:1 ratio in a 96-well plate in the absence and presence of serially diluted antibodies for 4 hours under culture condition. Supernatant from each well was then collected, and fluorescence signal was detected by plate-reader SpectraMax i3x (Ex 488nm, Em 520nm). An isotype hIgG4 mAb was used as a negative control, whereas the humanized OKT3 (an anti-CD3 hIgG1 from Novoprotein) was used as a positive control. The % lysis was then calculated using the following formula: %Lysis = [(Experimental Release) –Ave (Target+ NK)]/[Ave (Target Max) – Ave (Target only)] ×100% (FIG. 15).

Example 12

Developability profile of antibodies

For developability assessment, purified AG10058, AG10059, AG10131 and AC1097 were exchanged into PB buffer (20mM PB, 150 mM NaCl, pH 7.0). All experiments, including filtration, concentration, accelerated stress tests, were performed in PB buffer. For all the SEC-HPLC analyses, the TSKgel columns (Tosoh Bioscience G3000SWxl) were used.

12a. Solubility

All three antibodies can be concentrated to higher than 100 mg/ml in PB buffer without obvious precipitation (Table 6). Antibodies then were adjusted to 20 mg/ml in PB buffer. Samples (10 μg each) were then assayed through SEC-HPLC for detection of high molecular weight (HMW) aggregate. As shown in the chromatograms (Figure 16), no increase of HMW aggregate was observed at high concentration (20 mg/ml) for all test antibodies.

Table 6 Solubility of antibodies

Sample	Concentration /(mg/mL)	Aggregation (HMW %)
AG10058	108	1.0
AG10059	134	1.4
AG10131	110	2.0

12b. Antibody stability under accelerated stress conditions

Antibody stabilities were also examined under accelerated stress conditions, result is summarized in Table 7. All antibodies remain stable after six cycles of freezing (-80 ℃) and thawing (Room temperature) (Figure 17). After seven days at 50 ℃, there was little change of HMW aggregate or LMW fragments (Figure 17). In longer-term time course experiments (40 ℃ for up to 28 days), all antibodies remain stable, and there were no significant increase of HMW aggregate or LMW fragments (Figure 17).

Table 7 Changes of HMW under accelerated conditions

	AG10058	AG10059	AG10131
Freeze-thaw 6X	4.6%	1.2%	0.4%
50℃ 7 d	0.7%	1.2%	0%
40℃ 28 d	0.9%	0%	0%

Furthermore, thermostability as measured by differential scanning calorimetry (DSC) shows that both AG10131 and AG10058 are stable up to at least about 59 °C. The transition midpoint, Tm (the characteristic temperature at which the unfolding transition for almost all protein domains occur) is shown in Figure 18 and Table 8 below.

Table 8 Thermostability by DSC

	Tm onset (°C)	Tm1 (°C)	Tm2 (°C)
AG10058	61.5	67.3	76.9
AG10131	59.3	67.6	81.5

In addition, the highest achievable concentration of AG10131 and AG10058 after centrifugation was over 180 mg/mL and over 220 mg/mL, respectively.

Example 13

Safety profile in relevant species: mouse and cynomolgus monkey

13a. Repeated dosing toxicity studies of AG10131 in normal C57BL/6 mice.

Repeated dosing toxicity of AG10131 was conducted in normal C57BL/6 mice. Vehicle, AG10131 (100 mg/kg) was administered i.p. (10 mL/kg) on Day1, Day4, Day8, and Day11. Five female mice (7-8 weeks old) were included in each group. Mice were monitored daily for abnormal behaviors and symptoms, and measured daily for food intake and body weight. On day14, animals were euthanized for post-mortem examination and other analysis. Blood was collected from each animal, with 2 blood samples per group used for hematology (RBC, platelet, WBC, WBC differential) and the other 3 blood samples in the group for blood biochemistry (AL, AST, ALB, GLB, A/G, TBIL, ALP, GGT, and LDH) analysis. The following organs from each mouse were collected and preserved in FFPE: Heart, lung, thymus, liver, spleen, and kidneys. FFPE blocks for liver tissues were prepared, sectioned and H&E stained for histopathology analysis.

During the in-life period of the whole study, there was no abnormal behavior observed or un-scheduled animal death. Compared to the vehicle treatment, AG10131 did not affect the food intake and body weight. Post-mortem examination also did not show any obvious lesions in mice of the treatment groups with both AG10131. Hematology analysis did not show any significant changes, so as to the blood biochemistry parameters tested in mice treated with AG10131 (Figure 19). No obvious abnormalities were found in the histopathology sections of the liver from all these mice (Figure 20). Overall, AG10131 was well tolerated in this study and no significant toxicity was observed in mice.

13b. Repeated dosing studies of AG10131 in cynomolgus monkeys

Repeated dosing study of AG10131 was conducted in normal cynomolgus monkeys. Human IgG4 Isotype control (10 mg/kg), AG10131 (0.5 and 10 mg/kg) was administered i.v. (1 mL/kg) on Day0, Day7, Day14, and Day22. One male and one female cynomolgus monkeys (3~5 years old) were included in each group. Animals were monitored daily for abnormal behaviors and clinical signs, and measured daily for food intake. Body weight was measured on predose Day (-15),

Day (-5), and postdose Day6, Day13, Day18 and Day26. Hematology and blood chemistry parameters were measured on predose Day (-12), Day (-5), and postdose Day7, Day14, Day19, and Day27 (10 mg/kg group only), urinalysis was conducted on predose Day (-12), Day (-5), and postdose Day6, Day13, Day18. Animals in the 10 mg/kg groups were euthanized for post-mortem examination and other analysis on Day27. Major organs were dissected and weighed. FFPE liver tissue blocks were prepared, sectioned and H&E stained for histopathology analysis.

During the in-life period of the whole study, there was no abnormal behavior observed or un-scheduled animal death in all groups. Compared to the vehicle treatment, AG10131 treatment at 10 mg/kg did not affect the food intake and body weight. No clinical signs were noted including injection site reactions as well. Post-mortem examinations did not show any obvious lesions and weight abnormalities in all organs examined in cynomolgus monkeys treated with AG10131 at 10 mg/kg. Hematology, blood chemistry and urine parameters are also within normal ranges in all treatment groups (Figure 21). Histopathology analysis of the liver did not show any obvious abnormalities including lymphocyte infiltration after repeated dosing of AG10131 at 10mg/kg (Figure 22). Overall, AG10131 was well tolerated at up to 10 mg/kg weekly doses in cynomolgus monkeys and no obvious toxicity was detected.

Example 14

Pharmacokinetics of AG10131 in cynomolgus monkey

14a. Pharmacokinetics of AG10131 in cynomolgus monkey

A pharmacokinetics study of AG10131 was conducted in naive cynomolgus monkeys. Three dose levels of AG10131 (10 mg/kg, 30 mg/kg and 100 mg/kg) were intravenous bolus administrated to three groups of monkeys. Each group contains 3 males and 3 females. Serum samples were collected pre-dose, 0.083, 0.25, 0.5, 1, 2, 6, 12, 24, 36, 48, 72, 96, 120, 144, 168, 240, 336, 408, 504, 672 and 840 hours post dosing. Serum concentrations of AG10131 were determined by ELISA.

AG10131 was rapidly cleared at day 14 (336hrs) in 12 out of 16 animals, i.e., all animals from low and medium dose groups and 2 of 6 animals from high dose group. On day 21, 2 more animals from high dose group showed a rapid clearance. Serum concentrations in these14 animals are low or below the limit of the quantification. This is consistent with the observation of anti-drug antibody generation in these animals. Data from the two animals with potentially unaffected pharmacokinetics from the high dose group were fitted to predict the pharmacokinetics parameters (Figure 23). The half-life of AG10131 ranges from 7.3 to 8.8 days.

14b. Pharmacokinetics of AG10131 in rat

A pharmacokinetics study of AG10131 was conducted in naïve SD rats. Three dose levels of AG10131 (10 mg/kg, 30 mg/kg and 100 mg/kg) were intravenous bolus administrated to three groups of animals. Each group contains 15 males and 15 females. Serum samples were collected from 3 animals each time point: pre-dose, 0.083, 0.25, 0.5, 1, 2, 6, 12, 24, 36, 48, 72, 96, 120, 144, 168, 240, 336, 408, 504, 672 and 840 hours post dosing. Serum concentrations of AG10131 were determined by ELISA and the data were analyzed by Phoenix Professional V6.3.

Results: The PK parameters from low, mid and high doses are similar (Figure 24). The clearance rate of AG10131 is about 0.004 ml/kg/min. The half-life of AG10131 ranges from 11.5 to 14.6 days.

14c. Pharmacokinetics of AG10131 in mouse

A pharmacokinetics study of AG10131 was conducted in BALB/c mice at about 8-week age. 3 female BALB/c mice per dosing group were intravenously injected the test antibodies including AG10131 at 1 mg/kg through the tail vein. Blood samples (~100ul per sample) were collected at 1h, 8, 48, 168, and 336 hours post dosing. Blank control blood was collected from 3 naive female mice without antibody administration. Serum concentrations of each test antibody including AG10131 were determined by ELISA, in which the anti-human IgG (Fc specific) antibody was used for capture and the HRP-labeled anti-human IgG (Fab specific) antibody for detection.

All test antibodies including the isotype control (AG10154), two benchmark antibodies (AC1020 and AC1021), and three Adagene antibodies (AG10131, AG10058, and AG10059) exhibit comparable pharmacokinetics in mice (Figure 25).

Example 15

Further epitope mapping

To determine the binding epitope of the antibodies shown herein by Adagene and other reference antibodies, we have taken a systematic approach to dissecting the epitopes by three levels of resolution: domain, motif, and residues. The extra cellular domain of CD137 containing 4 CRD motifs and CD137 from 4 different species such as human, monkey, mouse, and rat CD137 were used (Table 9). A series of human CD137 CRD motif (cysteine rich domain) and their constructs containing one, two, and three units of human CRD motifs (Table 9) were displayed. A low copy number, CEN/ARS-based vector was used to express the human CD137 CRDs under the control of the inducible GAL1-10 promoter in the yeast S. cerevisiae (Boder and Wittrup (1997) Nat Biotechnol 15(6):553-7). The binding of antibodies to the human CD137 CRDs were assessed by flow cytometry analysis and other technology as previously described in Example 5 and shown in **FIG. 27**.

The results are summarized in Table 9, these antibodies only bind selectively to CD137 target, but none of the non-CD137 targets listed in the table; however, their species-specific cross-reactivity by these antibodies with CD137 from human, monkey, mouse, and rat species are striking, highlighting the fine epitope coverage by diverse hits screened from Adagene Dynamic Precision Libraries. For example, AG10131 binds to CD137 from human, monkey, mouse, rat, and dog (not shown); AG10058 binds human, monkey, mouse CD137, but not rat CD137; whereas AG10059 binds both human and monkey CD137. In contrast, the reference antibody AC1121 from transgenic mouse only binds to human CD137; whereas another reference antibody by morphosys phage library binds to both human and monkey CD137. For comparison, it should be noted that the human ligand CD137L only interacts with human CD137 receptor, not mouse CD137, and mouse CD137L only interact with mouse CD137 not human CD137 (see table 9).

Table 9

	AG10058	AG10059	AG10131	AC1121	AC1097	Human CD137L	Mouse CD137L
Human WT	+	+	+	+	+	+	-
Mouse_WT	+	-	+	-	-	-	+
Cyno_WT	+	+	+	-	+		NA
Rat_WT	-	-	+	-	-		NA

To further dissect the binding motifs of these antibodies to human CD137 target as summarized in Table 9, distinguished binding sites by Adagene, other reference antibodies, in comparison with CD137 ligand binding with the dissected CD137 CRD motifs and their combination are well separated and noted: AG10058, AG10059 and AG10131 antibodies and human CD137 ligand does not bind to the single CRD or two CRD units (CRD2-CRD3) of human CD137. AG10058, AG10059, and AG10131 antibodies, similar to the human CD137 ligand, can bind to three CRD units (CRD1–CRD2-CRD3) of human CD137. Although the reference antibody AC1121 can also bind to three CRD units (CRD1–CRD2-CRD3) of human CD137, however, it is the specific two CRD units (CRD1-CRD2) of human CD137. In comparison, the three CRD units (CRD1-CRD2-CRD3) of human CD137 are required for binding by AG10058, AG10059, AG10131 antibodies, and the human CD137 ligand. Reference antibody AC1097 can bind two CRD units (CRD3-CRD4), including three CRD units (CRD2-CRD3-CRD4) of human CD137. These indicate Adagene AG10058, AG10059, AG10131 antibodies bind the epitopes covered by the three CRD units (CRD1–CRD2-CRD3) of human CD137, similar to human CD137 ligand, but they are very different from the reference antibody AC1121 that binds to two CRD units (CRD1–CRD2) of human CD137 and reference antibody AC1097 which binds to two CRD units (CRD3–

CRD4) of human CD137. In conclusion, the binding epitope of CD137 by Adagene's antibodies is different from the epitope by the two reference antibodies (AC1121 with CRD1-CRD2; and AC1097 with CRD3-CRD4) as shown by the distinction in terms of the specific CRDs used and their number of CRD units required (see Table 9B) by highly similar, if not overlapping, to the CD137 epitope by human CD137L ligand; the epitope between CD137- CD137L is confirmed by the recently reported crystal structure complex, as is shown in **FIG. 26** (Gilbreth, R.N., Oganesyan, V.Y., Amdouni, H., Novarra, S., Grinberg, L., Barnes, A., Baca, M. (2018) J. Biol. Chem. 293: 9880-9891).

Table 9B.

	AG10058	AG10059	AG10131	AC1121	AC1097	Human CD137L	Mouse CD137L
Hu_CRD1	-	-	-	-	-	-	-
Hu_CRD2	-	-	-	-	-	-	-
Hu_CRD3	-	-	-	-	-	-	-
Hu_CRD4	-	-	-	-	-	-	-
Hu_CRD1-CRD2	-	-	-	+	-	-	-
Hu_CRD2-CRD3	-	-	-	-	-	-	-
Hu_CRD1-CRD2-CRD3	+	+	+	+	-	+	-
Hu_CRD3-CRD4	-	-	-	-	+	-	-
Hu_CRD2-CRD3-CRD4	-	-	-	-	+	-	-

To determine the binding epitope of Adagene's and reference antibodies at amino acid residue level, a series of mutations (Table 5) were made at the extracellular domain of human CD137. These CD137 mutation plasmids were used to transfect HEK293F cells. The binding of antibodies to the human CD137 mutants were assessed by flow cytometry analysis as previously described in Example 5 and shown in Figure 7A. The results are summarized in Table 5, together with the cross-reactivity of these antibodies with human, monkey, mouse, and rat CD137 in interesting differentiation, indicating the fine epitopes from hits derived from Adagene libraries. AG10131 binds to human, monkey, mouse, and rat CD137, whereas AG10058 binds human, monkey and mouse CD137, but not rat CD137. The binding epitope of CD137 by AG10058, AG10059 and AG10131 was mapped onto CRD1-CRD2-CRD3 units of CD137, they lost their binding ability to GFT34AAA, FSS53AAA, and FH92AA mutations, indicating that their binding epitopes are within these regions, e.g., amino acid residues 34-93 or 34-108 of SEQ ID NO.: 1 (*See also*, figure 7B). AG10058 and AG10131 may bind the same or highly similar epitope, while AG10059 may bind different epitopes from AG10058 and AG10131. Single mutants such as T35A, F36A, F53A, R66A, F72A, N83A, and F92A show that the loss of binding by Adagene antibodies AG10058, AG10059 and AG10131 with human CD137, together with the binding by CD137L

except for R66A which still maintains the binding of CD137 by its ligand. Single mutants, P32A and P49A, however, that the binding between CDL137L and CD137 is lost but its impact on the interaction between antibody and CD137 are varied. F125A shows AC1097 does not bind to CD137 anymore, but with no effects on the binding by other antibodies including human CD137L. In conclusion, the overall binding pattern by mutants across CD137 does show a clear message that Adagene antibodies and their reference antibodies are distinguished in terms of their binding sites. The mutant constructs were meant to differentiate the epitopes by AG10058, AG10059 and AG10131 from the reference antibodies by AC1121 and AC1097. Three antibodies AG10058, AG10059 and AG10131 target very different epitopes from AC1121 and AC1097. AG10058, AG10059 and AG10131 differ from AC1121 in regions defined by mutants Hu_FH92AA and Hu_FSS53AAA and possibly Hu_GTF34AAA, whereas AG10058, AG10059 and AG10131 differ from AC1097 in regions defined by most of the mutants used, except for Hu_FH92AA and their species cross-reactivity with Monkey but different in other species cross-reactivity such as mouse, rat and dog CD137. In some embodiments, AG10058, AG10059 and AG10131 or other antibodies disclosed herein do not bind to an epitope located within amino acid residues 115-156 of SEQ ID NO.: 1. Also shown in FIG. 7A and Table 5 is that binding of the human CD137 ligand to the wild-type vs. mutant human CD137 matches well with binding pattern of the tested antibodies, consisting with the observation that these antibodies block CD137 ligand binding to its receptor.

	AG10058	AG10059	AG10131	AC1121	AC1097	Human CD137L	Mouse CD137L
Human WT	+	+	+	+	+	+	-
Mouse_WT	+	-	+	-	-	-	+
Cyno_WT	+	+	+	-	+		NA
Rat_WT	-	-	+	-	-		NA
Hu_N30A	+	+	+	+	+	+	-
Hu_P32A	+	+	+	-	+	-	-
Hu_GTF34AAA	-	-	-	-/+	+	-	-
Hu_T35A	-	-	-	-	+	-	-
Hu_F36A	-	-	-	-	+	-	-
Hu_P49A	+	-	+	+	+	-	-
Hu_P50A	+	+	+	+	+	+	-
Hu_F53A	-	-	-	-	+	-	-
Hu_FSS53AAA	-	-	-	+	+	-	-
Hu_Q59A	+	+	+	+	+	+	-
Hu_I64A	+	+	+	+	+	+	-
Hu_R66A	-	-	-	+	+	+	-
Hu_F72A	-	-	-	+	+	-	-

Hu_N83A	-	-	-	+	+	-	-
Hu_F92A	-	-	-	+	+	-	-
Hu_L95A	+	+	+	+	+	+	-
Hu_FH92AA	-	-	-	+	-	-	-
Hu_GQ109AA	+	+	+	+	-	+	-
Hu_EL111AA	+	+	+	+	-	+	-
Hu_F125A	+	+	+	+	-	+	-
Hu_FN125AA	+	+	+	+	-	+	-
Hu_PW135AA	+	+	+	+	-	+	-
Hu_TN137AA	+	+	+	+	-	+	-
Hu_GT150AA	+	+	+	+	-	+	-
	AG10058	AG10059	AG10131	AC1121	AC1097	Human CD137L	Mouse CD137L
Hu_CRD1	-	-	-	-	-	-	-
Hu_CRD2	-	-	-	-	-	-	-
Hu_CRD3	-	-	-	-	-	-	-
Hu_CRD4	-	-	-	-	-	-	-
Hu_CRD1-CRD2	-	-	-	+	-	-	-
Hu_CRD2-CRD3	-	-	-	-	-	-	-
Hu_CRD1-CRD2-CRD3	+	+	+	+	-	+	-
Hu_CRD3-CRD4	-	-	-	-	+	-	-
Hu_CRD2-CRD3-CRD4	-	-	-	-	+	-	-

Example 16

Native CD137L signaling is blocked by AG10131

An *in vitro* binding assay by ELISA demonstrated that AG10131 can block recombinant CD137 and its ligand interaction. To further functionally validate this ligand-blocking activity of AG10131 , a cellular NFκB luciferase reporter assay was conducted. Briefly, 293T cells stably expressing an NFκB luciferase reporter were transfected with a DNA construct expressing human CD137, and the cells were co-cultivated with the human B-cell lymphoma cells Daudi or Raji at different ratios. The cell mixture was incubated with serial dilutions of isotype control or ligand-blocking anti-CD137 antibodies overnight, and luciferase activity was measured using the Promega luciferase assay kit according to manufacturer's instructions. Relative luciferase units (RLUs) were calculated vs. the levels of luciferase expressed in 293T cells in the absence of antibody treatment.

As shown in **FIG. 28**, both Daudi (top row) and Raji (bottom row) cells expressed functional CD137 ligand to activate the NFκB luciferase reporter in 293T cells. Compared to the isotype control antibody (left column), addition of AG10131 to the co-culture system (right

column) significantly inhibited the NFκB signaling stimulated by both cell types, suggesting that AG10131 antibody can functionally block the CD137 signaling stimulated from CD137 ligand expressed on both Daudi and Raji B lymphoma cells.

Example 17

Anti-CD137 antibody crosslinking in an NFκB reporter assay

Using the functional cellular NFκB reporter assay, three anti-CD137 antibodies (AG10131 , AC1121 and AC1097) were tested. As shown in **FIG. 29**, when crosslinked, all three anti-CD137 antibodies were capable of stimulating human CD137 receptor signaling in a dose-dependent manner at comparable levels. The EC50s of the antibody induced NFκB signaling activation response were at similar range for all three anti-CD137 antibodies. However, AC1121 displayed a unique property that is different from AG10131 and AC1097. AC1121 was able to activate human CD137 receptor signaling significantly in the absence of crosslinking, whereas AG10131 and AC1097 were unable to do so. The EC50 of AC1121 with or without crosslinking in the stimulation of the CD137 receptor signaling was found to be at similar levels.

Example 18

AG10131 does not induce CDC

The CDC activity of AG10131 was determined by the direct binding of AG10131 with the purified C1q component of the human complement with ELISA. As shown in **FIG. 30**, AG10131 and its human IgG4 isotype control antibody lack the ability to bind to human complement C1q component in the concentration range tested, whereas a human IgG1 isotype control antibody is able to bind to C1q. This result suggests that AG10131 is also unlikely to induce complement dependent cytotoxicity, consistent with its IgG4 isotype framework.

Example 19

Anti-CD137 antibody AG10131 enhances tumor-infiltrating T-lymphocytes

The *in vivo* anti-tumor efficacy studies in the syngeneic mouse H22 liver cancer, EMT6 breast cancer, and CT26 colon cancer models shown in Example 10 demonstrated that AG10131 treatment strongly inhibits tumor growth. AG10131 is an agonistic antibody that activates T cells, and thus AG10131 treatment is expected to stimulate tumor infiltrating T cells into the tumor micro-environment, thereby mediating an anti-tumor effect *in vivo*. To evaluate the effect of AG10131 treatment on the tumor infiltrating lymphocytes, tumors from the *in vivo* anti-tumor efficacy study of AG10131 in mouse H22, EMT6, and CT26 cancer models were collected at the end of studies.

FIG. 31 shows representative IHC staining images of mouse CD4+ and CD8+ T cells in H22 tumors (top left), EMT6 tumors (top right), and CT26 tumors (bottom center). As shown in **FIG. 32**, few T lymphocytes (either CD4+ or CD8+ T cells) were present in the vehicle control treated H22, EMT6, and CT26 tumors, whereas AG10131 treatment significantly stimulated the infiltration of both CD4+ and CD8+ T cells (indicated by black arrows in **FIG. 31**) into the tumors. These data are consistent with the function of AG10131 as an immune agonist by stimulating T cell proliferation, activation, and infiltration into the tumor micro-environment to mediate an antitumor effect.

Example 20

Enhanced anti-tumor efficacy by combining anti-CD137 antibody AG10131 and anti-PD1 antibody in the CT26 colon cancer model

The effect of combining the anti-CD137 antibody AG10131 with an anti-PD1 antibody was next tested in the CT26 colon cancer model. Each female BALB/c mouse was inoculated subcutaneously at the right lower flank region with CT26 tumor cells (3×10^5) for tumor development. When the mean tumor volume reached 98 mm^3, 10 mice were assigned to each experimental group. These groups received either vehicle (PBS), AG10131 at 5 or 10 mg/kg, anti-PD-1 at 10 mg/kg, or a combination of 5 or 10 mg/kg of AG10131 and 10 mg/kg of anti-PD-1 mAb by i.p. injection twice weekly for 3 weeks. Tumor volumes were measured and each mouse was euthanized when its tumor reached the endpoint volume of 2000 mm^3, or on the final day (Day 42), whichever came first.

As shown in **FIG. 33**, both AG10131 (5 mg/kg or 20 mg/kg) and anti-PD1 (10 mg/kg) delayed tumor progression, though AG10131 delayed tumor progression by a few more days, and in rare cases, resulted in tumor shrinkage. However, nearly all mice treated with either AG10131 or anti-PD1 eventually died of tumor progression. Importantly, when AG10131 (5 mg/kg or 20 mg/kg) was administered in combination with anti-PD1 (10 mg/kg), most of the mice were essentially cured of tumor, with only 2 (out of 10) or 1 (out of 10) escaped tumor suppression in the combinations of AG10131 (5 mg/kg) with anti-PD1 (10 mg/kg) or AG10131 (20 mg/kg) with anti-PD1 (10 mg/kg) respectively. These results demonstrated the strong synergistic effect of AG10131 and anti-PD1, suggesting that the combination of AG10131 with anti-PD1 could be effective in anti-PD1 resistance tumors.

CLAIMS

What is claimed is:

1. An isolated antibody, or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137, wherein the antibody or the antigen-binding fragment thereof binds to one or more amino acid residues within amino acid residues 34-108 of SEQ ID NO:1.

2. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment binds to one or more amino acid residues within amino acid residues 34-93 of SEQ ID NO:1.

3. The antibody or antigen-binding fragment of claim 1 or claim 2, wherein the antibody or antigen-binding fragment binds to one or more amino acid residues selected from the group consisting of amino acid residues 34-36, 53-55, and 92-93 of SEQ ID NO:1.

4. The antibody or antigen-binding fragment of claim 3, wherein the antibody or antigen-binding fragment binds to one or more of amino acid residues 34-36, one or more of amino acid residues 53-55, and one or more or amino acid residues 92-93 of SEQ ID NO:1.

5. The antibody or antigen-binding fragment of any one of claims 1-4, wherein the antibody or antigen-binding fragment does not bind to one or more of amino acid residues selected from the group consisting of amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1.

6. The antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment does not bind to amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO:1.

7. The antibody or antigen-binding fragment of any one of claims 1-6, wherein the antibody or antigen-binding fragment is cross-reactive with a CD137 polypeptide from at least one non-human species selected from the group consisting of cynomolgus monkey, mouse, rat and dog.

8. The antibody or antigen-binding fragment of claim 7, wherein the antibody or antigen-binding fragment binds to cynomolgus monkey CD137.

9. The antibody or antigen-binding fragment of any one of claims 1-8, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:711, an HVR-H2 comprising the amino acid sequence of SEQ ID

NO:735, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:759; and/or

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:783, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:807, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:831.

10. The antibody or antigen-binding fragment of claim 9, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:41, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:42.

11. The antibody or antigen-binding fragment of claim 10, wherein the antibody comprises a heavy chain and a light chain, and wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:617, and/or the light chain comprises the amino acid sequence of SEQ ID NO:618.

12. The antibody or antigen-binding fragment of any one of claims 1-8, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region,

wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:712, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:736, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:760; and/or

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:784, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:808, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:832.

13. The antibody or antigen-binding fragment of claim 12, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:61, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:62.

14. The antibody or antigen-binding fragment of claim 13, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:619, and/or the light chain comprises the amino acid sequence of SEQ ID NO:620.

15. The antibody or antigen-binding fragment of any one of claims 1-8, wherein the antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region,

wherein the heavy chain variable region comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:731, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:755, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:779; and/or

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:803, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:827,

and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:851.

16. The antibody or antigen-binding fragment of claim 15, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:71, and/or the light chain variable region comprises the amino acid sequence of SEQ ID NO:72.

17. The antibody or antigen-binding fragment of claim 16, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:657, and/or the light chain comprises the amino acid sequence of SEQ ID NO:658.

18. An isolated antibody, or antigen-binding fragment thereof, that binds to an extracellular domain of human CD137, comprising a heavy chain variable region and a light chain variable region,

a) wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3,

wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of:

Formula (I): $X_1TFX_2X_3YX_4IHWV$ (SEQ ID NO:2), wherein X1 is F or Y, X2 is S or T, X3 is G, N, or S, and X4 is A, G, or W;

Formula (II): $YSIX_1SGX_2X_3WX_4WI$ (SEQ ID NO:3), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, G, N, S, or T; and

Formula (III): $FSLSTX_1GVX_2VX_3WI$ (SEQ ID NO:4), wherein X1 is G or S, X2 is A or G, and X3 is A, G, S, or T;

wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of:

Formula (IV): $LALIDWX_1X_2DKX_3YSX_4SLKSRL$ (SEQ ID NO:5), wherein X1 is A, D, or Y, X2 is D or G, X3 is R, S, or Y, and X4 is P or T;

Formula (V): $IGX_1IYHSGX_2TYYX_3PSLKSRV$ (SEQ ID NO:6), wherein X1 is D or E, X2 is N or S, and X3 is N or S; and

Formula (VI): $VSX_1ISGX_2GX_3X_4TYYADSVKGRF$ (SEQ ID NO:7), wherein X1 is A, G, S, V, or Y, X2 is A, D, S, or Y, X3 is D, G, or S, and X4 is S or T; and

wherein the HVR-H3 comprises an amino acid sequence according to Formula (VII): $ARX_1GX_2X_3X_4VX_5GDWFX_6Y$ (SEQ ID NO:8), wherein X1 is E or G, X2 is E or S, X3 is D or T, X4 is A, T, or V, X5 is A, I, L, T, or V, and X6 is A, D, or G; and/or

b) wherein the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3,

wherein the HVR-L1 comprises an amino acid sequence according to Formula (VIII): $X_1ASQX_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:9), wherein X1 is Q or R, X2 is D, G, or S, X3 is I or V, X4 is G, R, S, or T, X5 is P, R, S, or T, X6 is A, D, F, S, V, or Y, X7 is L or V, and X8 is A, G, or N;

wherein the HVR-L2 comprises an amino acid sequence according to Formula (IX): $X_1ASX_2X_3X_4X_5GX_6$ (SEQ ID NO:10), wherein X1 is A or D, X2 is N, S, or T, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and

wherein the HVR-L3 comprises an amino acid sequence according to a formula selected from the group consisting of:

Formula (X): $YCQQX_1YX_2X_3X_4T$ (SEQ ID NO:11), wherein X1 is A, G, S, or Y, X2 is Q, S, or Y, X3 is I, L, T, or Y, and X4 is I, S, V, or W; and

Formula (XI): $YCX_1QX_2X_3X_4X_5PX_6T$ (SEQ ID NO:12), wherein X1 is E or Q, X2 is P, S, or Y, X3 is D, L, S, T, or Y, X4 is D, E, H, S, or T, X5 is D, L T, or W, and X6 is L, P, R, or V.

19. The antibody or antigen-binding fragment of claim 18, wherein the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 253-312, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 313-372, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 373-432, the HVR-L1 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 433-492, the HVR-L2 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 493-552, and/or the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 553-612.

20. The antibody or antigen-binding fragment of claim 18 or claim 19, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, and 131, and/or the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, and 132.

21. The antibody or antigen-binding fragment of claim 18, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of:

Formula (XII): X_1TFSX_2YWIHWV (SEQ ID NO:853), wherein X1 is F or Y, and X2 is N, or S;

Formula (XIII): YSIX_1SGX_2X_3WX_4WI (SEQ ID NO:854), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, G, N, or S; and

Formula (XIV): FSLSTX_1GVX_2VX_3WI (SEQ ID NO:855), wherein X1 is G or S, X2 is A or G, and X3 is A, G, or S;

wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of:

Formula (IV): LALIDWX_1X_2DKX_3YSX_4SLKSRL (SEQ ID NO:5), wherein X1 is A, D, or Y, X2 is D or G, X3 is R, S, or Y, and X4 is P or T; and

Formula (XV): VSX_1ISGX_2GX_3X_4TYYADSVKGRF (SEQ ID NO:856), wherein X1 is G, S, V, or Y, X2 is A, D, S, or Y, X3 is D, G, or S, and X4 is S or T; and

wherein the HVR-H3 comprises an amino acid sequence according to Formula (VII): ARX_1G$X_2X_3X_4$VX_5GDWFX_6Y (SEQ ID NO:8), wherein X1 is E or G, X2 is E or S, X3 is D or T, X4 is A, T, or V, X5 is A, I, L, T, or V, and X6 is A, D, or G; and/or

wherein the HVR-L1 comprises an amino acid sequence according to Formula (XVI): X_1ASQ$X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:857), wherein X1 is Q or R, X2 is D, G, or S, X3 is I or V, X4 is G, R, S, or T, X5 is P, R, S, or T, X6 is A, F, S, V, or Y, X7 is L or V, and X8 is A or G;

wherein the HVR-L2 comprises an amino acid sequence according to Formula (XVII): X_1AS$X_2X_3X_4X_5$GX_6 (SEQ ID NO:858), wherein X1 is A or D, X2 is N or S, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and

wherein the HVR-L3 comprises an amino acid sequence according to Formula (XVIII): YCQQX_1YX_2X_3WT (SEQ ID NO:859), wherein X1 is A or G, X2 is S or Y, and X3 is I, L, or T.

22. The antibody or antigen-binding fragment of claim 21, wherein the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:709-732, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:733-756, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:757-780, the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:781-804, the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:805-828, and the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:829-852.

23. The antibody or antigen-binding fragment of claim 21 or claim 22, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID

NOS:15, 17, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 53, 61, 63, 65, 67, 71, 73, 75, 79, 83, 85, and 87, and/or the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:16, 18, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 54, 62, 64, 66, 68, 72, 74, 76, 80, 84, 86, and 88.

24. The antibody or antigen-binding fragment of any one of claims 21-23, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, and 659, and/or the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, and 660.

25. The antibody or antigen-binding fragment of claim 21, wherein the HVR-H1 comprises an amino acid sequence of SEQ ID NO:711 or 731, the HVR-H2 comprises an amino acid sequence of SEQ ID NO:735 or 755, the HVR-H3 comprises an amino acid sequence of SEQ ID NO:759 or 779, the HVR-L1 comprises an amino acid sequence of SEQ ID NO:783 or 803, the HVR-L2 comprises an amino acid sequence or SEQ ID NO:807 or 827, and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:831 or 851.

26. The antibody or antigen-binding fragment of claim 25, wherein the heavy chain variable region comprises an amino acid sequence of SEQ ID NO:41 or 71, and the light chain variable region comprises an amino acid sequence of SEQ ID NO:42 or 72.

27. The antibody or antigen binding fragment of claim 26, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 617 or 657, and the light chain comprises an amino acid sequence of SEQ ID NO: 618 or 658.

28. The antibody or antigen-binding fragment of 21, wherein the HVR-H1 comprises an amino acid sequence of SEQ ID NO:712, the HVR-H2 comprises an amino acid sequence of SEQ ID NO:736, the HVR-H3 comprises an amino acid sequence of SEQ ID NO:760, the HVR-L1 comprises an amino acid sequence of SEQ ID NO:784, the HVR-L2 comprises an amino acid sequence or SEQ ID NO:808, and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:832.

29. The antibody or antigen-binding fragment of claim 28, wherein the heavy chain variable

region comprises an amino acid sequence of SEQ ID NO:61, and the light chain variable region comprises an amino acid sequence of SEQ ID NO:62.

30. The antibody or antigen binding fragment of claim 29, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence of SEQ ID NO: 619, and the light chain comprises an amino acid sequence of SEQ ID NO:620.

31. The antibody or antigen-binding fragment of any one of claims 1-30, wherein the antibody or antigen-binding fragment binds human CD137 with a K_D of 100 nM or less, as measured by surface plasmon resonance.

32. The antibody or antigen-binding fragment of claim 31, wherein the antibody or antigen-binding fragment binds human CD137 with a K_D of 50 nM or less, as measured by surface plasmon resonance.

33. The antibody or antigen-binding fragment of any one of claims 18-32, wherein the antibody or antigen-binding fragment is cross-reactive with a CD137 polypeptide from at least one non-human species selected from the group consisting of cynomolgus monkey, mouse, rat and dog.

34. The antibody or antigen-binding fragment of claim 33, wherein the antibody or antigen-binding fragment binds to cynomolgus monkey CD137.

35. The antibody or antigen-binding fragment of any one of claims 1-34, wherein an activity of human CD137 expressed on a human cell is decreased when contacted with the antibody or antigen-binding fragment.

36. The antibody or antigen-binding fragment of any one of claims 1-34, wherein the antibody or antigen-binding fragment has a half maximal inhibitory concentration (IC_{50}) of about 100 nM or less for blocking binding of human CD137 to human CD137L *in vitro*.

37. The antibody or antigen-binding fragment of any one of claims 1-34, wherein the antibody or antigen-binding fragment completely blocks binding of human CD137 to human CD137L *in vitro* when the antibody or antigen-binding fragment is provided at a concentration of about 1 μM or greater.

38. The antibody or antigen-binding fragment of any one of claims 1-34, wherein the antibody or antigen-binding fragment blocks one or more aspects of CD137 signaling stimulated by CD137L in a cell that expresses CD137.

39. The antibody or antigen-binding fragment of any one of claims 1-34, wherein an activity of human CD137 expressed on a human cell is increased when contacted with the antibody or antigen-binding fragment.

40. The antibody or antigen-binding fragment of claim 39, wherein contacting a human cell expressing CD137 with the antibody or antigen-binding fragment results in increased NF-κB-dependent transcription.

41. The antibody or antigen-binding fragment of any one of claims 1-40, wherein the antibody comprises a human IgG2 Fc region.

42. The antibody or antigen-binding fragment of any one of claims 1-41, wherein the antibody comprises a human IgG4 Fc region.

43. The antibody or antigen-binding fragment of claim 42, wherein the human IgG4 Fc region comprises an S241P mutation, wherein numbering is according to Kabat.

44. The antibody or antigen-binding fragment of any one of claims 18-20, wherein the heavy chain variable region is encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, and 251, and/or the light chain variable region is encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, and 252.

45. The antibody or antigen-binding fragment of claim 24, wherein the heavy chain is encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs:661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, and 707, and/or the light chain is encoded by a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs:662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, and 708.

46. A polynucleotide encoding the antibody or antigen-binding fragment of any one of claims 1-45.

47. A polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs:133-252.

48. A vector comprising the polynucleotide of any one of claims 46-47.

49. The vector of claim 48, wherein the vector is an expression vector.

50. A host cell comprising the vector of claim 48 or claim 49.

51. A method of making an antibody or antigen-binding fragment, comprising culturing the host cell of claim 50 under conditions suitable for producing the antibody or antigen-binding fragment.

52. The method of claim 51, further comprising recovering the antibody or antigen-binding fragment produced by the cell.

53. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1-45 and a pharmaceutically acceptable carrier.

54. A method for treating a cancer in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment of any one of claims 1-45.

55. The method of claim 54, further comprising administering to the subject a therapeutically effective amount of at least one additional therapeutic agent.

56. The method of claim 55, wherein the at least one additional therapeutic agent is selected from the group consisting of viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, and chemotherapies.

57. The method of claim 55 or claim 56, wherein the at least one additional therapeutic agent is selected from the group consisting of pomalyst, revlimid, lenalidomide, pomalidomide, thalidomide, a DNA-alkylating platinum-containing derivative, cisplatin, 5-fluorouracil, cyclophosphamide, an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-DR5 antibody, an anti-CD1d antibody, an anti-TIM3 antibody, an anti-SLAMF7 antibody, an anti-KIR receptor antibody, an anti-OX40 antibody, an anti-HER2 antibody, an anti-ErbB-2 antibody, an anti-EGFR antibody, cetuximab, rituximab, trastuzumab, pembrolizumab, radiotherapy, single dose radiation, fractionated radiation, focal radiation, whole organ radiation, IL-12, IFNα, GM-CSF, a chimeric antigen receptor, adoptively transferred T cells, an anti-cancer vaccine, and an oncolytic virus.