(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 June 2019 (06.06.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/104716 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/CN2017/114247

(22) International Filing Date:
01 December 2017 (01.12.2017)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: METHODS FOR USING CD137 LIGAND AS BIOMARKER FOR TREATMENT WITH ANTI-CD137 ANTIBODY

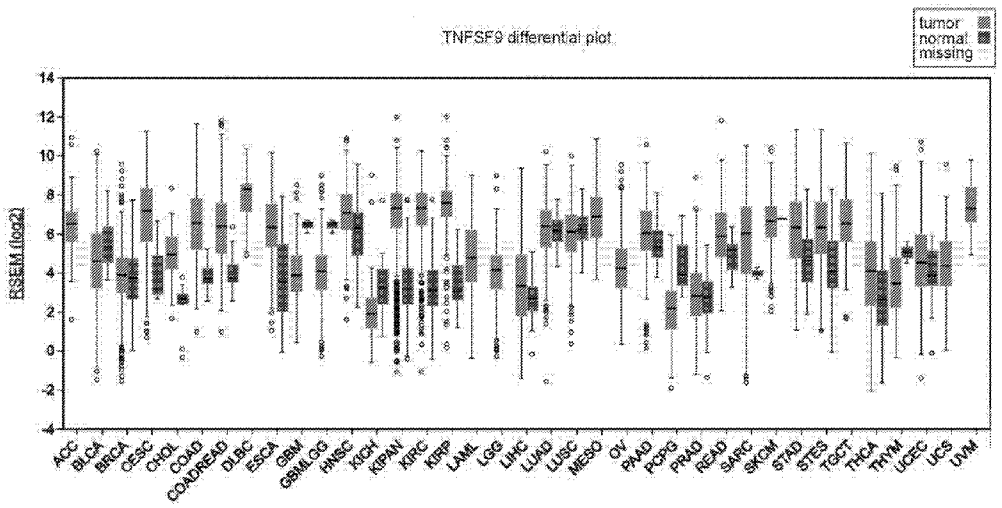

FIG. 1

(57) Abstract: Provided herein are methods relating to the use of CD137 Ligand (CD137L) expression; methods relating to the use of CD137L expression as a biomarker; and methods for predicting, assessing, and/or aiding in assessment of responsiveness of a subject in need thereof to treatment with particular anti-cancer therapies (e.g., an anti-CD137 antibody, a checkpoint blockade immunotherapy).

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EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

METHODS FOR USING CD137 LIGAND AS BIOMARKER FOR TREATMENT WITH ANTI-CD137 ANTIBODY

REFERENCE TO SEQUENCE LISTING

[0001] 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: 695402000440seqlist.txt, date recorded: November 20, 2017, size: 363 KB).

FIELD OF THE INVENTION

[0002] The present disclosure relates to CD137 Ligand (CD137L) expression, and to the use of CD137L expression as a biomarker.

BACKGROUND

[0003] Activation of T cells plays a central role in antitumor immunity. Two key signals are required to activate naïve T cells. Signal one is provided through the T-cell receptor (TCR), while signal two is that of co-stimulation. The CD28:B7 molecules are some of the best-studied costimulatory pathways, thought to be the main mechanism through which primary T cell stimulation occurs. However, a number of other molecules have been identified which serve to amplify and diversify the T cell response following initial T cell activation. These include CD137:CD137 ligand (CD137L) molecules, also known as 4-1BB:4-1BB ligand (4-1BBL). CD137:CD137L are members of the Tumor Necrosis Factor (TNF) Receptor (TNFR):TNF ligand family, which are expressed on T cells and antigen-presenting cells (APCs), respectively. Therapies targeting the CD137:CD137L signaling pathway have been shown to have antitumor effects in a number of model systems, and agonistic anti-CD137 antibodies have also entered clinical development (Yonezawa *et al.* Clin. Cancer Res. 2015 Jul 15;21(14):3113-20; Tolcher *et al.* Clin Cancer Res. 2017 Sep 15;23(18):5349-5357).

[0004] Previous studies indicated that CD137L gene delivery into multiple mouse tumor models can enable the host mice to develop long-term immunity against wild-type tumors (Melero *et al.* Eur. J. Immunol. 1998 Mar;28(3):1116-21), prevent tumor formation or induce tumor regression from the transfectants (Guinn *et al.* J Immunol. 1999 Apr 15;162(8):5003-10; Xiang Cancer Biother. Radiopharm. 1999 Oct;14(5):353-61), or improve host survival

(Martinet *et al.* J Natl Cancer Inst. 2000 Jun 7;92(11):931-6). These results suggest that CD137L expression may modify the tumor cells for whole cell vaccination by improving their ability to act as APCs for their tumor antigens, with the costimulatory CD137L molecules providing an abundance of signal two. However, suitable development of strategies using CD137L expression as a biomarker for informing decisions regarding immunotherapy treatment in cancer patients remains lacking.

[0005] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0006] To meet the above and other needs, disclosed herein are methods for using CD137L expression as a biomarker for: 1) determining whether a subject is likely to respond to certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy); 2) predicting responsiveness and/or monitoring, assessing or aiding in assessment of treatment/responsiveness of a subject to certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy); 3) selecting a subject to receive/not receive certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy); 4) treating or delaying progression of cancer in a subject by administering an effective amount of an anti-cancer therapy (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy); 5) negatively stratifying cancer patients for immunotherapy comprising agonistic anti-CD137 antibodies; and/or 6) positively stratifying cancer patients for immunotherapy comprising checkpoint blockade-directed antibodies (*e.g.*, anti-PD-1/anti-PD-L1 antibodies).The present disclosure is based, in part, on the surprising finding that CD137L can be used as a biomarker to stratify patients, where low levels of CD137L is predictive for responsiveness to anti-CD137 antibody therapy, while high levels of CD137L is predictive for non-responsiveness to anti-CD137 antibody therapy and responsiveness to checkpoint blockade immunotherapies (*See e.g.*, FIGS. 4 and 5, as well as Example 3 below).

[0007] Accordingly, in one aspect, provided herein are methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of an anti-CD137 antibody to the subject if the level of expression of CD137 ligand (CD137L) in a sample obtained from the subject is lower than a reference level. In some embodiments, the method further comprises the steps of a) obtaining the sample from the subject, and b) measuring the level of expression of CD137L in the sample prior to administration of the anti-CD137 antibody to the subject.

[0008] In another aspect, provided herein are methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of an anti-CD137 antibody to the subject, wherein it has been determined that the subject is likely to respond to the anti-CD137 antibody when the level of expression of CD137L in a sample obtained from the subject is lower than a reference level.

[0009] In another aspect, provided herein are methods of determining if a subject is likely to respond to an anti-CD137 antibody comprising a) obtaining a sample from the subject, b) measuring the level of expression of CD137L in the sample, and c) determining that the subject is likely to respond to the anti-CD137 antibody when the level of expression of CD137L in the sample is lower than a reference level.

[0010] In another aspect, provided herein are methods of selecting a subject having cancer for treatment with an anti-CD137 antibody comprising a) measuring the level of expression of CD137L in a sample obtained from the subject, and b) selecting the subject for treatment with the anti-CD137 antibody if the level of expression of CD137L in the sample is lower than a reference level.

[0011] In some embodiments that may be combined with any of the preceding embodiments, the level of expression of CD137L in the sample is below the limit of detection.

[0012] In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody 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: 531. In some embodiments that

may be combined with any of the preceding embodiments, the anti-CD137 antibody binds to one or more amino acid residues within amino acid residues 34-93 of SEQ ID NO: 531. In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody 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: 531. In some embodiments, the anti-CD137 antibody 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: 531. In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody 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: 531. In some embodiments, the anti-CD137 antibody does not bind to amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO: 531.

[0013] In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody 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. In some embodiments, the anti-CD137 antibody binds to cynomolgus monkey CD137. In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody is a human antibody. In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody is an anti-CD137 agonist antibody. In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody blocks binding of CD137L to CD137.

[0014] In some embodiments that may be combined with any of the preceding embodiments, the anti-CD137 antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-60, an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-120, and an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 121-180, and wherein the light chain variable region comprises an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 181-240, an HVR-L2 comprising an amino acid sequence selected from the group

consisting of SEQ ID NOS: 241-300, and an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 301-360. In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 361-420, and wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 421-480. In some embodiments, the anti-CD137 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: 481-504, and wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 505-528.

[0015] In some embodiments, the anti-CD137 antibody 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: 15, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 75, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 135, and wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 195, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 255, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 315. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 375, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 435. In some embodiments, the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 483, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 507.

[0016] In some embodiments, the anti-CD137 antibody 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: 25, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 85, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 145, and wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 205, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 265, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 325. In some embodiments, the heavy chain variable

region comprises the amino acid sequence of SEQ ID NO: 385, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 445. In some embodiments, the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 484, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 508.

[0017] In some embodiments, the anti-CD137 antibody 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: 30, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 90, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 150, and wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 210, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 270, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 330. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 390, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 450. In some embodiments, the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 503, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 527.

[0018] In some embodiments that may be combined with any of the preceding embodiments, the methods further comprise administering to the subject a therapeutically effective amount of at least one additional therapeutic agent. In some embodiments, the at least one additional therapeutic agent is selected from 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 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.

[0019] In another aspect, provided herein are methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject if the level of expression of CD137L in a sample obtained from the subject is higher than a reference level. In some embodiments, the method further comprises a) obtaining the sample from the subject, and b) measuring the level of expression of CD137L in the sample prior to administration of the checkpoint blockade immunotherapy to the subject.

[0020] In another aspect, provided herein are methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject, wherein it has been determined that the subject is likely to respond to the checkpoint blockade immunotherapy when the level of expression of CD137L in a sample obtained from the subject is higher than a reference level.

[0021] In another aspect, provided herein are methods of determining if a subject is likely to respond to a checkpoint blockade immunotherapy comprising a) obtaining a sample from the subject, b) measuring the level of expression of CD137L in the sample, and c) determining that the subject is likely to respond to the checkpoint blockade immunotherapy when the level of expression of CD137L in the sample is higher than a reference level.

[0022] In another aspect, provided herein are methods of selecting a subject having cancer for treatment with a checkpoint blockade immunotherapy comprising a) measuring the level of CD137L expression in a sample obtained from the subject, and b) selecting the subject for treatment with the checkpoint blockade immunotherapy if the level of expression of CD137L in the sample is higher than a reference level.

[0023] In some embodiments that may be combined with any of the preceding embodiments, the checkpoint blockade immunotherapy comprises administering an anti-PD-1

antibody or an anti-PD-L1 antibody. In some embodiments that may be combined with any of the preceding embodiments, the subject is further administered an effective amount of an anti-CD137 antibody.

[0024] In some embodiments that may be combined with any of the preceding embodiments, the subject is a human. In some embodiments that may be combined with any of the preceding embodiments, the sample is a serum sample. In some embodiments that may be combined with any of the preceding embodiments, the sample is a tumor sample. In some embodiments, the tumor sample is a tumor biopsy. In some embodiments that may be combined with any of the preceding embodiments, the sample comprises one or more cancer cells.

[0025] In some embodiments that may be combined with any of the preceding embodiments, the level of expression of CD137L is the level of protein expression of CD137L. In some embodiments, the level of protein expression is measured by a method selected from immunoassay, PET imaging, Western blotting, ELISA, immunohistochemistry, and flow cytometry. In some embodiments that may be combined with any of the preceding embodiments, the level of expression of CD137L is the level of RNA transcript expression of CD137L. In some embodiments, the level of transcript expression is measured by a method selected from RT-PCR, *in situ* hybridization, and next generation sequencing.

[0026] 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

[0027] **FIG. 1** shows CD137 ligand transcriptional expression status in tumor vs. normal tissues from clinical samples. The data were obtained from the Cancer Genome Atlas (TCGA). ACC, Adrenocortical carcinoma; BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical and endocervical cancers; CHOL, Cholangiocarcinoma; COAD,

Colon adenocarcinoma; COADREAD, Colorectal adenocarcinoma; DLBC, Lymphoid Neoplasm Diffused Large-B cell lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; GBMLGG, Glioblastoma multiforme/low grade glioma; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIPAN, Kidney Chromophobe/kidney renal clear cell carcinoma/kidney renal papillary cell carcinoma; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Low grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; STES, Stomach and esophageal carcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine carcinosarcoma; and UVM, Uveal Melanoma.

[0028] **FIGS. 2A-D** show CD137 ligand-mediated cell signaling in co-cultures of human B-cell lymphoma (Daudi or Raji) cells and 293T cells with a stably-integrated NFκB-dependent luciferase reporter that were transiently transfected with a plasmid expressing human CD137. **FIG. 2A** shows luciferase activity in co-cultures of Daudi and 293T reporter cells incubated at various cell ratios (0:1, 1:1, 3:1, and 10:1 Daudi:293T cells), and treated with the indicated concentrations of a human IgG4 isotype control antibody. **FIG. 2B** shows luciferase activity in co-cultures of Daudi and 293T reporter cells incubated at various cell ratios (0:1, 1:1, 3:1, and 10:1 Daudi:293T cells), and treated with the indicated concentrations of a blocking anti-CD137 antibody. **FIG. 2C** shows luciferase activity in co-cultures of Raji and 293T reporter cells incubated at various cell ratios (0:1, 1:1, 3:1, and 10:1 Raji:293T cells), and treated with the indicated concentrations of a human IgG4 isotype control antibody. **FIG. 2D** shows luciferase activity in co-cultures of Raji and 293T reporter cells incubated at various cell ratios (0:1, 1:1, 3:1, and 10:1 Raji:293T cells), and treated with the indicated concentrations of a blocking anti-CD137 antibody. NFκB-dependent luciferase activity was measured, after overnight incubation in the co-cultured cells.

[0029] **FIG. 3** shows expression profiles of CD137L on various mouse tumor cell lines, as measured by flow cytometry. The grey line indicates control staining; the black line indicates anti-mouse CD137L staining.

[0030] **FIGS. 4A-4B** show the *in vivo* anti-tumor activity of an anti-CD137 agonist antibody (αCD137-AB1), or isotype control antibody, in various syngenic mouse tumor models. **FIG. 4A** shows the syngenic mouse tumor models that were categorized as non-responders to the anti-CD137 agonist antibody treatment (models showing a tumor growth inhibition (TGI) < 20% after administration of αCD137-AB1 vs. isotype control antibody). **FIG. 4B** shows the syngenic mouse tumor models that were categorized as responders to the anti-CD137 agonist antibody treatment (models showing a TGI >35% after administration of αCD137-AB1 vs. isotype control antibody).

[0031] **FIGS. 5A-5B** show the *in vivo* anti-tumor activity of checkpoint blockade immunotherapies, or vehicle controls (PBS), in the syngenic Pan02 mouse pancreatic tumor model. **FIG. 5A** shows the *in vivo* activity of a surrogate anti-mouse PD-L1 antibody (clone 10F.9G2) in the syngenic Pan02 mouse pancreatic tumor model. **FIG. 5B** shows the *in vivo* activity of a surrogate anti-mouse PD-1 antibody (clone RMP1-14) in the syngenic Pan02 mouse pancreatic tumor model.

DETAILED DESCRIPTION

I. General techniques

[0032] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana

Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

II. Definitions

[0033] Before describing the present disclosure in detail, it is to be understood that this present disclosure is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0034] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0035] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0036] It is understood that aspects and embodiments of the present disclosure described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0037] The term "and/or" as used herein a phrase such as "A and/or B" is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used herein a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0038] 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.

[0039] 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_L) 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.

[0040] 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. 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.

[0041] 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)).

[0042] 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.

[0043] 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 an antibody (*e.g.*, a CD137 antibody), such as conservation amino acid substitutions, additions, and insertions.

[0044] 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).

[0045] The term "binding molecule" encompasses (1) antibody, (2) antigen-binding fragment of an antibody, and (3) derivative of an antibody, each as defined herein.

[0046] 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, with an affinity (K_D) of 100 nM or less.

[0047] 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.

[0048] The term "CD137 antibody" refers to an antibody, as defined herein, capable of binding to human CD137 receptor.

[0049] 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.

[0050] 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).

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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."

[0055] 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.

[0056] 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.

[0057] The term "illustrative antibody" refers to any one of the antibodies described in the disclosure. 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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."

[0067] 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.

[0068] 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)).

[0069] The terms "polypeptide," "protein," and "peptide" are used interchangeably herein and may refer to polymers of two or more amino acids.

[0070] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, *etc.*) and with charged

linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), those containing pendant moieties, such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, ply-L-lysine, *etc.*), those with intercalators (*e.g.*, acridine, psoralen, *etc.*), those containing chelators (*e.g.*, metals, radioactive metals, boron, oxidative metals, *etc.*), those containing alkylators, those with modified linkages (*e.g.*, alpha anomeric nucleic acids, *etc.*), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0071] 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.

[0072] As used herein, the term "biomarker" or "marker" refers generally to a molecule (*e.g.*, pre-mRNA, mRNA, protein, *etc.*), the expression of which in or on a subject's tissue or cell, or secreted by the subject's tissue or cell, can be detected by known methods (or methods disclosed herein) and is predictive or can be used to predict (or aid prediction) for a subject's sensitivity to, and in some embodiments, to predict (or aid prediction) a subject's responsiveness to, treatment regimens (*e.g.*, treatments comprising anti-CD137 antibodies, treatments comprising checkpoint blockade immunotherapy, *etc.*).

[0073] As used herein, the term "sample", refers to a composition that is obtained or derived from a subject of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics.

[0074] As used herein, the term "tissue or cell sample" refers to a collection of similar cells obtained from a tissue of a subject or patient. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells. Optionally, the tissue or cell sample is obtained from a disease tissue or organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

[0075] As used herein, a "subject", "patient", or "individual" may refer to a human or a non-human animal. A "non-human animal" may refer to any animal not classified as a human, such as domestic, farm, or zoo animals, sports, pet animals (such as dogs, horses, cats, cows, *etc.*), as well as animals used in research. Research animals may refer without limitation to nematodes, arthropods, vertebrates, mammals, frogs, rodents (*e.g.*, mice or rats), fish (*e.g.*, zebrafish or pufferfish), birds (*e.g.*, chickens), dogs, cats, and non-human primates (*e.g.*, rhesus monkeys, cynomolgus monkeys, chimpanzees, *etc.*). In some embodiments, the subject, patient, or individual is a human.

[0076] 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.

[0077] As used herein, a "reference value" or "reference level" may be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a particular level or baseline level.

[0078] An "effective amount" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve a one or more desired or indicated effect, including a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. For purposes of the present disclosure, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

III. Methods

Overview

[0079] Certain aspects of the present disclosure relate to methods of treating or delaying progression of cancer in a subject by administering an effective amount of an anti-cancer therapy (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy) based on the level of expression of CD137L in one or more samples obtained from the subject.

[0080] Other aspects of the present disclosure relate to methods of determining whether a subject is likely to respond to certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy,

checkpoint blockade immunotherapy) by measuring CD137L expression in one or more samples obtained from the subject.

[0081] Other aspects of the present disclosure relate to methods of treating or delaying progression of cancer in a subject by administering an effective amount of an anti-cancer therapy (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy) after it has been determined that the subject is likely to respond to the therapy.

[0082] Other aspects of the present disclosure relate to selecting a subject to receive or not receive certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy) based on the level of expression of CD137L in one or more samples obtained from the subject.

[0083] Other aspects of the present disclosure relate to methods for predicting responsiveness and/or monitoring treatment and/or responsiveness of a subject to certain anti-cancer therapies (*e.g.*, anti-CD137 antibody therapy, checkpoint blockade immunotherapy) by measuring CD137L expression in one or more samples obtained from the subject.

[0084] Yet other aspects of the present disclosure relate to methods of positively and/or negatively stratifying patients into particular treatment regimen groups based upon the level of CD137L expression in one or more samples obtained from the patients.

Measuring CD137L expression

[0085] In some embodiments, the present disclosure relates to measuring the level of CD137L expression in a sample. In some embodiments, measuring the level of expression of CD137L in a sample comprises measuring the level of expression of a nucleic acid molecule encoding CD137L (*e.g.*, measuring the level of RNA (such as pre-mRNA or mRNA) transcript expression from a gene encoding CD137L) and/or measuring the level of protein expression of CD137L. The nucleic acid sequence of an exemplary nucleic acid encoding human CD137L, as well as the amino acid sequence of an exemplary human CD137L polypeptide, are shown below:

CD137L nucleic acid sequence:

Atggaatacgcctctgacgcttcactggaccccgaagccccgtggcctcccgcgccccgcgctcgcgcctgccgcgtactgccttggg
ccctggtcgcggggctgctgctgctgctgctgctcgctgccgcctgcgccgtcttcctcgcctgcccctgggccgtgtccggggctcgc
gcctcgcccggctccgcggccagcccgagactccgcgagggtcccgagctttcgcccgacgatcccgccggcctcttggacctgcgg
cagggcatgtttgcgcagctggtggcccaaaatgttctgctgatcgatgggcccctgagctggtacagtgacccaggcctggcaggcgt
gtccctgacgggggcctgagctacaaagaggacacgaaggagctggtggtggccaaggctggagtctactatgtcttctttcaactag
agctgcggcgcgtggtggccggcgagggctcaggctccgtttcacttgcgctgcacctgcagccactgcgctctgctgctggggccgc
cgccctggctttgaccgtggacctgccacccgcctcctccgaggctcggaactcggccttcggtttccagggccgcttgctgcacctgag
tgccggccagcgcctgggcgtccatcttcacactgaggccagggcacgccatgcctggcagcttacccagggcgccacagtcttggg
actcttccgggtgacccccgaaatcccagccggactcccttcaccgaggtcggaa (SEQ ID NO: 529)

CD137L polypeptide sequence:

MEYASDASLD PEAPWPPAPR ARACRVLPWA LVAGLLLLLL LAAACAVFLA CPWAVSGARA SPGSAASPRL REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO: 530)

[0086] In some embodiments, the level of expression of CD137L in a sample is measured by determining the level of RNA transcript expression of CD137L. Suitable methods of measuring RNA transcript levels in a sample are known in the art, including, for example, by Northern blot analysis, nuclease protection assays, *in situ* hybridization, PCR analysis (*e.g.*, qPCR, RT-PCR, RT-qPCR, *etc.*), and next generation sequencing (*e.g.*, RNAseq). In some embodiments, the level of transcript expression of CD137L is measured by RT-PCR, *in situ* hybridization, and/or RNAseq.

[0087] In some embodiments, the level of expression of CD137L in a sample is measured by determining the level of protein expression of CD137L. Suitable methods of measuring protein expression in a sample are known in the art, including, for example, immunoassays, immunohistochemistry (IHC), PET imaging, Western blotting, enzyme-linked immunosorbent assays (ELISAs), flow cytometry, and mass spectrometry. In some

embodiments, the level of CD137L protein expression is measured by immunoassay, Western blotting, ELISA, IHC, and/or flow cytometry.

Subjects

[0088] In some embodiments, the present disclosure relates to subjects suffering from or believed to be suffering from cancer. In some embodiments, the subject has been diagnosed with cancer. In some embodiments, the subject has not been diagnosed with cancer. In some embodiments, the subject is at risk of developing cancer.

[0089] The subject may be suffering from, or believed to be suffering from, any cancer known in the art, including, for example, 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), mesothelioma, 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; adrenal cancers (*e.g.*, adrenocortical carcinoma); cancers in digestive system such as esophageal carcinoma, leiomyoma, epidermoid carcinoma, adenocarcinoma, leiomyosarcoma, stomach adenocarcinomas, intestinal lipomas, intestinal neurofibromas, intestinal fibromas, polyps in large intestine, colon 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 papillary cell carcinoma, renal cell carcinoma, renal clear 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, Diffused Large B cell lymphoma, T 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, germ cell tumors, and choriocarcinoma); breast cancer; female reproductive system cancers such as uterine cancer (endometrial carcinoma, uterine carcinosarcoma), cervical cancer (cervical carcinoma), cancer of the ovaries (ovarian carcinoma, ovarian serous cystadenocarcinoma), vulvar carcinoma, vaginal carcinoma, fallopian tube cancer, and hydatidiform mole; thyroid cancer (including papillary, follicular, anaplastic, or medullary cancer) and thymoma; pheochromocytomas (adrenal gland) and paraganglioma; noncancerous growths of the parathyroid glands; and pancreatic cancers.

[0090] In some embodiments, the subject has not previously received one or more anti-cancer therapies. In some embodiments, the subject has previously received and/or is currently receiving one or more anti-cancer therapies.

[0091] In some embodiments, the methods of the present disclosure are useful for: identifying subjects with cancers that are likely or unlikely to respond to treatment with an anti-CD137 antibody therapy (*e.g.*, as described herein) and/or a checkpoint blockade immunotherapy (*e.g.*, as described herein); aiding in subject selection for administering an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy; predicting the likelihood of success when treating a subject with an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy; assessing or monitor disease progression in a subject treated with an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy; assessing or monitor treatment efficacy using an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy; and/or determining prognosis of a subject.

Samples obtained from a subject

[0092] In some embodiments, the present disclosure relates to measuring the level of CD137L expression in a sample obtained from a subject. In some embodiments, the level of expression of CD137L is measured in one or more (*e.g.*, one or more, two or more, three or more, four or more, *etc.*) samples obtained from a subject. Any suitable sample in the form of tissues and/or fluids that are known or believed to contain diseased cells and/or the target of interest (*e.g.*, full length CD137L, CD137L fragments including soluble CD137L fragments) may be used in the methods described herein, including, for example, sputum, pleural fluid, lymph fluid, bone marrow, blood, plasma, serum, urine, tissue samples (samples known or expected to contain cancer cells), tumor samples, tumor biopsies, *etc.* In some embodiments, the sample is a serum sample. In some embodiments, the sample is a tumor sample. In some embodiments, the sample is a tumor biopsy. In some embodiments, the sample comprises one or more cancer cells.

[0093] Methods of obtaining suitable tissue and/or fluid samples (*e.g.*, methods that are appropriate for obtaining a representative sample from a particular type, location, disease tissue, *etc.*) are well known to one of ordinary skill in the art, including, for example, by resection, bone marrow biopsy or bone marrow aspiration, endoscopic biopsy or endoscopic aspiration (*e.g.*, cystoscopy, bronchoscopy, colonoscopy, *etc.*), needle biopsy or needle aspiration (*e.g.*, fine needle aspiration, core needle biopsy, vacuum-assisted biopsy, image-guided biopsy, *etc.*) skin biopsy (*e.g.*, shave biopsy, punch biopsy, incisional biopsy, excisional biopsy, *etc.*), various other surgical tissue (*e.g.*, tumor tissue) biopsy and/or excision strategies, and fluid collections (*e.g.*, collecting urine, blood, serum, plasma, sputum, *etc.*).

[0094] In some embodiments, the one or more samples obtained from the subject are enriched for diseased (*e.g.*, cancerous) cells. Methods of enriching a tissue or fluid preparation for diseased (*e.g.*, cancerous) cells are known in the art, including, for example, by separating diseased (*e.g.*, cancerous) cells from normal cells by flow cytometry. In some embodiments, the level of expression of CD137L is measured in the enriched samples. In some embodiments, the level of expression of CD137L is measured in samples that have not been enriched or otherwise altered after isolation.

[0095] In some embodiments, the one or more samples are fixed (*i.e.* preserved) by conventional methodology (*See e.g.*, "Manual of Histological Staining Method of the Armed Forces Institute of Pathology," 3rd edition (1960) Lee G. Luna, HT (ASCP) Editor, The Blakston Division McGraw-Hill Book Company, New York; *The Armed Forces Institute of Pathology Advanced Laboratory Methods in Histology and Pathology* (1994) Ulreka V. Mikel, Editor, Armed Forces Institute of Pathology, American Registry of Pathology, Washington, D.C.). The choice of a fixative may be determined by one of ordinary skill in the art for the purpose for which the sample is to be analyzed. The length of fixation will depend upon the size and type of the tissue sample and the fixative used (*e.g.*, neutral buffered formalin, paraformaldehyde, *etc.*), as will be appreciated by one of ordinary skill in the art. In some embodiments, the level of expression of CD137L is measured in a sample that is fixed. In some embodiments, the level of expression of CD137L is measured in samples that have not been fixed or otherwise altered after isolation.

[0096] In some embodiments, one or more samples are obtained from the subject prior to administration with an anti-cancer therapy (*e.g.*, an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy). In some embodiments, one or more samples are obtained from the subject after administration of a first and/or subsequent does of an anti-cancer therapy (*e.g.*, an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy). In some embodiments, one or more samples are obtained from the subject after completion of an anti-cancer therapy regimen (*e.g.*, an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy). In some embodiments, one or more samples are obtained from the subject, prior to, during, and after completion of an anti-cancer therapy regimen (*e.g.*, an anti-CD137 antibody therapy and/or a checkpoint blockade immunotherapy).

Comparison to a reference level

[0097] In some embodiments, the present disclosure relates to comparing the level of expression of CD137L in a sample obtained from a subject to a reference level of expression of CD137L. In some embodiments, the reference level is the level of expression of CD137L in a reference sample (*e.g.*, a reference cell (such as a cell line), a corresponding sample taken from one or more patients determined to be responsive to anti-CD137 antibody therapy, a

corresponding sample taken from one or more patients determined to be non-responsive to anti-CD137 antibody therapy, a corresponding adjacent normal tissue, *etc.*). In some embodiments, the reference level is measured in the reference sample using the same method as was used to measure the level of expression of CD137L in the subject's sample. In some embodiments, the reference level is measured in the reference sample using a different method than was used to measure the level of expression of CD137L in the subject's sample.

[0098] In some embodiments, the reference level is the level of expression of CD137L (*e.g.*, average level of expression) on one or more reference cells. In some embodiments, the one or more reference cells are cells taken from a diseased tissue isolated from a cancer patient (*e.g.*, one or more cancer cells from a patient suffering from adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, cervical or endocervical cancers, cholangiocarcinoma, colon adenocarcinoma, colorectal adenocarcinoma, lymphoid neoplasm diffused large-B cell lymphoma, esophageal carcinoma, glioblastoma multiforme and/or low grade glioma, head and Neck squamous cell carcinoma, kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, acute myeloid leukemia, low grade glioma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma or paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma, skin cutaneous melanoma, stomach adenocarcinoma, stomach or esophageal carcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, uveal melanoma, *etc.*). In some embodiments, the one or more reference cells are one or more cells from a cancer cell line (*e.g.*, a liver cancer cell line, a colon cancer cell line, a melanoma cell line, a lung cancer cell line, a pancreatic cancer cell line, a prostate cancer cell line, a B cell lymphoma cell line, a T cell lymphoma cell line, *etc.*). In some embodiments, the one or more reference cells are one or more cells of an adjacent normal tissue in the subject (*e.g.*, comparing CD137L expression (such as by immunohistochemical staining) in a tumor sample from the patient to CD137L expression in a normal tissue adjacent to the tumor, *etc.*).

[0099] In some embodiments, the reference level is the level of expression of CD137L in one or more samples isolated from one or more patients determined to be responsive to anti-

CD137 antibody therapy (*e.g.*, one or more samples isolated from one or more patients determined by a clinician to be responsive to anti-CD137 antibody therapy (such as patients receiving treatment with an anti-CD137 antibody in a clinical trial)). In some embodiments, the reference level is the level of expression of CD137L in one or more samples isolated from one or more patients determined to be non-responsive to anti-CD137 antibody therapy (*e.g.*, one or more samples isolated from one or more patients determined by a clinician to be non-responsive to anti-CD137 antibody therapy (such as patients receiving treatment with an anti-CD137 antibody in a clinical trial)).

[0100] In some embodiments, the reference level is a pre-determined level of CD137L expression (*e.g.*, the average level of expression of CD137L in a database of diseased samples (such as tissue biopsies or serum samples) isolated from multiple reference patients; the average level of expression of CD137L in a database of samples (such as tissue biopsies or serum samples) isolated from multiple healthy reference patients; *etc.*).

[0101] In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be lower than the reference level when the level of expression of CD137L in the sample is at least about 25% lower than the reference level. For example, the level of expression of CD137L measured in the sample obtained from the subject is considered to be lower than the reference level when the level of expression of CD137L in the sample is at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% lower than the reference level. In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be lower than the reference level when the level of expression of CD137L in the sample is at least about 1-fold lower than the reference level. For example, the level of expression of CD137L measured in the sample obtained from the subject is considered to be lower than the reference level when the level of expression of CD137L in the sample is at least about 1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least

about 7.5 fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 100-fold, or at least about 1000-fold lower than the reference level. In some embodiments, the level of expression of CD137L in the sample obtained from the subject is below the limit of detection. In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be lower than the reference level when the level of expression of CD137L in the sample is below the limit of detection while the reference level is above the limit of detection, is detectable, and/or is not zero. In some embodiments, a level is considered to be below the limit of detection when the level does not give an appreciable signal, a detectable signal, and/or is not significantly different than an appropriate negative control when performing an assay for measuring the level of CD137L expression (*e.g.*, below the limit of detection of an assay measuring RNA transcript expression of CD137L (such as RT-PCR, in situ hybridization, and/or next generation sequencing), below the limit of detection of an assay measuring CD137L protein expression (such as an immunoassay, PET imaging, Western blotting, ELISA, immunohistochemistry, and/or flow cytometry), *etc.*).

[0102] In some embodiments, a subject is administered an effective amount of an anti-CD137 antibody when the level of expression of CD137L in a sample obtained from the subject is lower than the reference level. In some embodiments, a subject is determined to be likely to respond to an anti-CD137 antibody when the level of expression of CD137L in a sample obtained from the subject is lower than the reference level. In some embodiments, a subject is administered an effective amount of an anti-CD137 antibody after the subject has been determined to be likely to respond to the anti-CD137 antibody. In some embodiments, a subject having cancer is selected for treatment with an anti-CD137 antibody when the level of expression of CD137L in a sample obtained from the subject is lower than the reference level. In some embodiments, a subject is positively stratified for enrollment into an anti-CD137 antibody therapy when the level of expression of CD137L in a sample obtained from the subject is lower than the reference level.

[0103] In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be higher than the reference level when the level of expression of CD137L in the sample is at least about 5% higher than the reference level. For example, the level of expression of CD137L measured in the sample obtained from the subject is

considered to be higher than the reference level when the level of expression of CD137L in the sample is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% higher than the reference level. In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be higher than the reference level when the level of expression of CD137L in the sample is at least about 1-fold higher than the reference level. For example, the level of expression of CD137L measured in the sample obtained from the subject is considered to be higher than the reference level when the level of expression of CD137L in the sample is at least about 1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5 fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 100-fold, or at least about 1000-fold higher than the reference level. In some embodiments, the level of expression of CD137L in the reference sample is below the limit of detection. In some embodiments, the level of expression of CD137L measured in the sample obtained from the subject is considered to be higher than the reference level when the level of expression of CD137L in the sample is above the limit of detection, is detectable, and/or is not zero while the level of expression of CD137L in the reference sample is below the limit of detection. In some embodiments, a level is considered to be below the limit of detection when the level does not give an appreciable signal, a detectable signal, and/or is not significantly different than an appropriate negative control when performing an assay for measuring the level of CD137L expression (e.g., below the limit of detection of an assay measuring RNA transcript expression of CD137L (such as RT-PCR, in situ hybridization, and/or next generation sequencing), below the limit of detection of an assay measuring CD137L protein expression (such as an immunoassay, PET imaging, Western blotting, ELISA, immunohistochemistry, and/or flow cytometry), etc.).

[0104] In some embodiments, a subject is administered an effective amount of a checkpoint blockade immunotherapy when the level of expression of CD137L in a sample

obtained from the subject is higher than the reference level. In some embodiments, a subject is determined to be likely to respond to a checkpoint blockade immunotherapy when the level of expression of CD137L in a sample obtained from the subject is higher than the reference level. In some embodiments, a subject is administered an effective amount of a checkpoint blockade immunotherapy after the subject has been determined to be likely to respond to the checkpoint blockade immunotherapy. In some embodiments, a subject having cancer is selected for treatment with a checkpoint blockade immunotherapy when the level of expression of CD137L in a sample obtained from the subject is higher than the reference level. In some embodiments, a subject is positively stratified for enrollment into a checkpoint blockade immunotherapy when the level of expression of CD137L in a sample obtained from the subject is higher than the reference level. In some embodiments, a subject is negatively stratified for enrollment into an anti-CD137 antibody therapy when the level of expression of CD137L in a sample obtained from the subject is higher than the reference level.

CD137L expression and anti-CD137 antibody therapies

[0105] In some embodiments, the present disclosure relates to methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of an anti-CD137 antibody to the subject if the level of expression of CD137L in a sample obtained from the subject is lower than a reference level. In some embodiments, the method comprises obtaining a sample from the subject, and measuring the level of expression of CD137L in the sample prior to administration of the anti-CD137 antibody. In some embodiments, the level of expression of CD137L in the sample obtained from the subject is below the limit of detection. In some embodiments, the subject is administered the anti-CD137 antibody when CD137L expression is below the limit of detection. In some embodiments, the anti-CD137 antibody is any one or more of the anti-CD137 antibodies described herein.

[0106] In some embodiments, the present disclosure relates to methods of determining whether a subject is likely to respond to an anti-CD137 antibody. In some embodiments, the method comprises obtaining a sample from the subject, measuring the level of expression of CD137L in the sample, and determining that the subject is likely to respond to the anti-CD137 antibody when the level of expression of CD137L in the sample is lower than a reference level.

In some embodiments, the level of expression of CD137L in the sample obtained from the subject is below the limit of detection. In some embodiments, the subject is determined to be likely to respond to the anti-CD137 antibody when the CD137L expression is below the limit of detection.

[0107] In some embodiments, the present disclosure relates to methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of an anti-CD137 antibody to the subject after it is determined that the subject is likely to respond to the anti-CD137 antibody. In some embodiments, the anti-CD137 antibody is any one or more of the anti-CD137 antibodies described herein. In some embodiments, responsiveness of the subject comprises treatment efficacy. In some embodiments, responsiveness of the subject comprises reduced tumor volume. In some embodiments, responsiveness of the subject comprises serological responsiveness.

[0108] In some embodiments, the present disclosure relates to methods of selecting a subject having cancer for treatment with an anti-CD137 antibody. In some embodiments, the method comprises measuring the level of expression of CD137L in a sample obtained from the subject, and selecting the subject for treatment with the anti-CD137 antibody if the level of expression of CD137L in the sample is lower than a reference level. In some embodiments, the level of expression of CD137L in the sample obtained from the subject is below the limit of detection. In some embodiments, the subject is selected for treatment with the anti-CD137 antibody when the CD137L expression is below the limit of detection. In some embodiments, the anti-CD137 antibody is any one or more of the anti-CD137 antibodies described herein.

Anti-CD137 antibodies

[0109] In some embodiments, the present disclosure relates to the use of an anti-CD137 antibody. In some embodiments, the anti-CD137 antibody binds to human CD137. In some embodiments, 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.: 531. 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.

[0110] 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:532), 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:533), 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:534), 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:535), 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:536), wherein X1 is D or E, X2 is N or S, and X3 is N or S; and Formula (VI): VSX1ISGX2GX3X4TYYADSVKGRF (SEQ ID NO:537), 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:538), 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:539), 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:540), 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:541), 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:542), 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.

[0111] In some embodiments, the antibody can comprise an HVR_H1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 1-60-312, an HVR_H2 having

the amino acid sequence selected from the group consisting of SEQ ID NOs: 61-120, an HVR_H3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 121-180, an HVR_L1 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 181-240, an HVR_L2 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 241-300, and/or an HVR_L3 having the amino acid sequence selected from the group consisting of SEQ ID NOs: 301-360.

[0112] 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: 361-480. 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: 481-504.

[0113] 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.

[0114] Further, the antibodies provided by the present disclosure can be monoclonal or polyclonal, but preferably monoclonal.

[0115] Examples of specific isolated antibodies (or antigen binding fragments thereof) provided by the present disclosure include those listed in Tables 1-3. The 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. In some embodiments, the anti-CD137 antibody is any anti-CD137 antibody described in PCT International Application No. PCT/CN2017/098332 (incorporated herein by reference in its entirety).

[0116] In some embodiments, the anti-CD137 antibody binds to human CD137 and has 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, or all eight) of the following functional properties: (a) binds to human CD137 with a KD of 500 nM or less; (b) has agonist activity on human CD137; (c) does not bind to human OX40, CD40, GITR and/or CD27 receptor at concentration up to 1000 nM; (d) is cross-reactive with monkey (*e.g.,* GenBank Gene ID 102127961), mouse (*e.g.,* GenBank Gene ID 21942), rat (*e.g.,* GenBank Gene ID 500590), or dog (*e.g.,* GenBank Gene ID 608274) CD137; (e) does not induce ADCC effects; (f) is capable of inhibiting tumor cell growth; (g) has therapeutic effect on a cancer; and (h)blocks binding between CD137 and CD137L. In some embodiments, the antibody binds to cynomolgus monkey CD137. In some embodiments, the anti-CD137 antibody is an agonist antibody. In some embodiments, the anti-CD137 antibody is a human antibody. In some embodiments, the human antibody possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences.

[0117] Human CD137 is a 255 amino acid protein (GenBank Accession No. NM_001561; NP_001552; SEQ ID NO.: 531). In some embodiments, 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).

[0118] The amino acid sequences of exemplary CD137 polypeptides are shown below:

Human CD137 polypeptide sequence:

MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG CSCRFPEEEE GGCEL (SEQ ID NO: 531);

Mouse CD137 polypeptide sequence:

MGNNCYNVVV IVLLLVGCEK VGAVQNSCDN CQPGTFCRKY NPVCKSCPPS TFSSIGGQPN CNICRVCAGY FRFKKFCSST HNAECECIEG FHCLGPQCTR CEKDCRPGQE LTKQGCKTCS LGTFNDQNGT GVCRPWTNCS LDGRSVLKTG TTEKDVVCGP PVVSFSPSTT ISVTPEGGPG GHSLQVLTLF LALTSALLLA LIFITLLFSV LKWIRKKFPH IFKQPFKKTT GAAQEEDACS CRCPQEEEGG GGGYEL (SEQ ID NO: 543);

Cynomolgus monkey CD137 polypeptide sequence:

MGNSCYNIVA TLLLVLNFER TRSLQDLCSN CPAGTFCDNN RSQICSPCPP NSFSSAGGQR TCDICRQCKG VFKTRKECSS TSNAECDCIS GYHCLGAECS MCEQDCKQGQ ELTKKGCKDC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SATPPAPARE PGHSPQIIFF LALTSTVVLF LLFFLVLRFS VVKRSRKKLL YIFKQPFMRP VQTTQEEDGC SCRFPEEEEG GCEL (SEQ ID NO: 544).

Table 1: HVR sequences of anti-CD137 antibodies

AB 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	1	61	121	181	241	301
4072	VH2/VL2	2	62	122	182	242	302
7074	VH3/VL3	3	63	123	183	243	303
4076	VH4/VL4	4	64	124	184	244	304
4079	VH5/VL5	5	65	125	185	245	305
4134	VH6/VL6	6	66	126	186	246	306
4137	VH7/VL7	7	67	127	187	247	307
4139	VH8/VL8	8	68	128	188	248	308
4140	VH9/VL9	9	69	129	189	249	309
4217	VH10/VL10	10	70	130	190	250	310
5299	VH11/VL11	11	71	131	191	251	311
5300	VH12/VL12	12	72	132	192	252	312
5302	VH13/VL13	13	73	133	193	253	313
5303	VH14/VL14	14	74	134	194	254	314
5310	VH15/VL15	15	75	135	195	255	315
5314	VH16/VL16	16	76	136	196	256	316
5316	VH17/VL17	17	77	137	197	257	317
5318	VH18/VL18	18	78	138	198	258	318
5323	VH19/VL19	19	79	139	199	259	319
5341	VH20/VL20	20	80	140	200	260	320
5342	VH21/VL21	21	81	141	201	261	321
5346	VH22/VL22	22	82	142	202	262	322
5348	VH23/VL23	23	83	143	203	263	323

5349	VH24/VL24	24	84	144	204	264	324
5351	VH25/VL25	25	85	145	205	265	325
5353	VH26/VL26	26	86	146	206	266	326
5359	VH27/VL27	27	87	147	207	267	327
5360	VH28/VL28	28	88	148	208	268	328
5363	VH29/VL29	29	89	149	209	269	329
5365	VH30/VL30	30	90	150	210	270	330
5367	VH31/VL31	31	91	151	211	271	331
5370	VH32/VL32	32	92	152	212	272	332
5371	VH33/VL33	33	93	153	213	273	333
5404	VH34/VL34	34	94	154	214	274	334
5407	VH35/VL35	35	95	155	215	275	335
5408	VH36/VL36	36	96	156	216	276	336
5409	VH37/VL37	37	97	157	217	277	337
5413	VH38/VL38	38	98	158	218	278	338
5417	VH39/VL39	39	99	159	219	279	339
7077	VH40/VL40	40	100	160	220	280	340
7078	VH41/VL41	41	101	161	221	281	341
7079	VH42/VL42	42	102	162	222	282	342
7080	VH43/VL43	43	103	163	223	283	343
7081	VH44/VL44	44	104	164	224	284	344
7087	VH45/VL45	45	105	165	225	285	345
7088	VH46/VL46	46	106	166	226	286	346
7090	VH47/VL47	47	107	167	227	287	347
7092	VH48/VL48	48	108	168	228	288	348
7097	VH49/VL49	49	109	169	229	289	349
7100	VH50/VL50	50	110	170	230	290	350
7105	VH51/VL51	51	111	171	231	291	351
7109	VH52/VL52	52	112	172	232	292	352
7120	VH53/VL53	53	113	173	233	293	353
7128	VH54/VL54	54	114	174	234	294	354
7131	VH55/VL55	55	115	175	235	295	355
7133	VH56/VL56	56	116	176	236	296	356
7135	VH57/VL57	57	117	177	237	297	357
7159	VH58/VL58	58	118	178	238	298	358
7163	VH59/VL59	59	119	179	239	299	359
7166	VH60/VL60	60	120	180	240	300	360

[0119] In some embodiments, the anti-CD137 antibody comprises an HVR-H1 comprising the amino acid sequence of FSLSTSGVGVGWI (SEQ ID NO: 15), an HVR-H2 comprising the amino acid sequence of LALIDWDDDKYYSPSLKSRL (SEQ ID NO: 75), and an HVR-H3 comprising the amino acid sequence of ARGGSDTVLGDWFAY (SEQ ID NO: 135), an HVR-L1 comprising the amino acid sequence of RASQSVSPYLA (SEQ ID NO: 195), an HVR-L2 comprising the amino acid sequence of DASSLESGV (SEQ ID NO: 255), and an HVR-L3 comprising the amino acid sequence of YCQQGYSLWT (SEQ ID NO: 315). In some embodiments, the anti-CD137 antibody comprises an HVR-H1 comprising the amino acid

sequence of YSITSGHYWAWI (SEQ ID NO: 25), an HVR-H2 comprising the amino acid sequence of VSSISGYGSTTYYADSVKGRF (SEQ ID NO: 85), and an HVR-H3 comprising the amino acid sequence of ARGGSDAVLGDWFAY (SEQ ID NO: 145), an HVR-L1 comprising the amino acid sequence of RASQGIGSFLA (SEQ ID NO: 205), an HVR-L2 comprising the amino acid sequence of DASNLETGV (SEQ ID NO: 265), and an HVR-L3 comprising the amino acid sequence of YCQQGYYLWT (SEQ ID NO: 325). In some embodiments, the anti-CD137 antibody comprises an HVR-H1 comprising the amino acid sequence of FSLSTGGVGVGWI (SEQ ID NO: 30), an HVR-H2 comprising the amino acid sequence of LALIDWADDKYYSPSLKSRL (SEQ ID NO: 90), and an HVR-H3 comprising the amino acid sequence of ARGGSDTVIGDWFAY (SEQ ID NO: 150), an HVR-L1 comprising the amino acid sequence of RASQSIGSYLA (SEQ ID NO: 210), an HVR-L2 comprising the amino acid sequence of DASNLETGV (SEQ ID NO: 270), and an HVR-L3 comprising the amino acid sequence of YCQQGYYLWT (SEQ ID NO: 330).

Table 2: VH and VL sequences of anti-CD137 antibodies

Hit ID	VH and VL ID	SEQ ID NO.
3760	VH1	361
	VL1	421
4072	VH2	362
	VL2	422
4074	VH3	363
	VL3	423
4076	VH4	364
	VL4	424
4079	VH5	365
	VL5	425
4134	VH6	366
	VL6	426
4137	VH7	367
	VL7	427
4139	VH8	368
	VL8	428
4140	VH9	369
	VL9	429
4217	VH10	370
	VL10	430
5299	VH11	371
	VL11	431
5300	VH12	372
	VL12	432
5302	VH13	373
	VL13	433

5303	VH14	374
	VL14	434
5310	VH15	375
	VL15	435
5314	VH16	376
	VL16	436
5316	VH17	377
	VL17	437
5318	VH18	378
	VL28	438
5323	VH19	379
	VL19	439
5341	VH20	380
	VL20	440
5342	VH21	381
	VL21	441
5346	VH22	382
	VL22	442
5348	VH23	383
	VL23	443
5349	VH24	384
	VL24	444
5351	VH25	385
	VL25	445
5353	VH26	386
	VL26	446
5359	VH27	387
	VL27	447
5360	VH28	388
	VL28	448
5363	VH29	389
	VL29	449
5365	VH30	390
	VL30	450
5367	VH31	391
	VL31	451
5370	VH32	392
	VL32	452
5371	VH33	393
	VL33	453
5404	VH34	394
	VL34	454
5407	VH35	395
	VL35	455
5408	VH36	396
	VL36	456
5409	VH37	397
	VL37	457
5413	VH38	398
	VL38	458
5417	VH39	399
	VL39	459
7077	VH40	400

	VL40	460
7078	VH41	401
	VL41	461
7079	VH42	402
	VL42	462
7080	VH43	403
	VL43	463
7081	VH44	404
	VL44	464
7087	VH45	405
	VL45	465
7088	VH46	406
	VL46	466
7090	VH47	407
	VL47	467
7092	VH48	408
	VL48	468
7097	VH49	409
	VL49	469
7100	VH50	410
	VL50	470
7105	VH51	411
	VL51	471
7109	VH52	412
	VL52	472
7120	VH53	413
	VL53	473
7128	VH54	414
	VL54	474
7131	VH55	415
	VL55	475
7133	VH56	416
	VL56	476
7135	VH57	417
	VL57	477
7159	VH58	418
	VL58	478
7163	VH59	419
	VL59	479
7166	VH60	420
	VL60	480

[0120] In some embodiments, the anti-CD137 antibody comprises a heavy chain variable region comprising the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGLEWLALIDWDDD KYYSPSLKSRLTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDTVLGDWFAYWGQG TLVTVSS (SEQ ID NO: 375), and a light chain variable region comprising the amino acid sequence of:

DIQLTQSPSSLSASVGDRVTITCRASQSVSPYLAWYQQKPGKAPKLLIYDASSLESGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQGYSLWTFGQGTKVEIKR (SEQ ID NO: 435). In some embodiments, the anti-CD137 antibody comprises a heavy chain variable region comprising the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHYWAWIRQAPGKGLEWVSSISGYGSTT YYADSVKGRFTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDAVLGDWFAYWGQG TLVTVSS (SEQ ID NO: 385), and a light chain variable region comprising the amino acid sequence of: DIQLTQSPSSLSASVGDRVTITCRASQGIGSFLAWYQQKPGKAPKLLIYDASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQGYYLWTFGQGTKVEIKR (SEQ ID NO: 445). In some embodiments, the anti-CD137 antibody comprises a heavy chain variable region comprising the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWIRQAPGKGLEWLALIDWADD KYYSPSLKSRLTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDTVIGDWFAYWGQG TLVTVSS (SEQ ID NO: 390), and a light chain variable region comprising the amino acid sequence of: DIQLTQSPSSLSASVGDRVTITCRASQSIGSYLAWYQQKPGKAPKLLIYDASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQGYYLWTFGQGTKVEIKR (SEQ ID NO: 450).

Table 3: Heavy chain and light chain sequences of anti-CD137 antibodies

Hit ID	IgG ID	Heavy chain (HC) and Light Chain (LC) ID	SEQ ID NO.
4072	AG10054	HC1	481
		LC 1	505
5303	AG10057	HC 2	482
		LC 2	506
5310	AG10058	HC 3	483
		LC 3	507
5351	AG10059	HC 4	484
		LC 4	508
5359	AG10060	HC 5	485
		LC 5	509
5370	AG10061	HC 6	486
		LC 6	510
5404	AG10062	HC 7	487
		LC 7	511
5413	AG10063	HC 8	488
		LC 8	512
4074	AG10079	HC 9	489

		LC 9	513
4217	AG10080	HC 10	490
		LC 10	514
5299	AG10081	HC 11	491
		LC 11	515
5300	AG10082	HC 12	492
		LC 12	516
5323	AG10083	HC 13	493
		LC 13	517
5360	AG10084	HC 14	494
		LC 14	518
5367	AG10085	HC 15	495
		LC 15	519
5409	AG10086	HC 16	496
		LC 16	520
5302	AG10124	HC 17	497
		LC 17	521
5314	AG10125	HC 18	498
		LC 28	522
5316	AG10126	HC 19	499
		LC 19	523
5318	AG10127	HC20	500
		LC 20	524
5342	AG10128	HC 21	501
		LC 21	525
5353	AG10129	HC 22	502
		LC 22	526
5365	AG10131 (αCD137-AB1)	HC 23	503
		LC 23	527
5408	AG10132	HC 24	504
		LC24	528

[0121] In some embodiments, the anti-CD137 antibody comprises a heavy chain comprising the amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGLEWLALIDWDDD KYYSPSLKSRLTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDTVLGDWFAYWGQG TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 483), and a light chain comprising the amino acid sequence of:

DIQLTQSPSSLSASVGDRVTITCRASQSVSPYLAWYQQKPGKAPKLLIYDASSLESGVPSR

FSGSGSGTDFTLTISSLQPEDFATYYCQQGYSLWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 507). In some embodiments, the anti-CD137 antibody comprises a heavy chain comprising the amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHYWAWIRQAPGKGLEWVSSISGYGSTT
YYADSVKGRFTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDAVLGDWFAYWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE
FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 484), and a light chain comprising the amino acid sequence of:
DIQLTQSPSSLSASVGDRVTITCRASQGIGSFLAWYQQKPGKAPKLLIYDASNLETGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQGYYLWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 508). In some embodiments, the anti-CD137 antibody comprises a heavy chain comprising the amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWIRQAPGKGLEWLALIDWADD
KYYSPSLKSRLTISRDNSKNTLYLQLNSLRAEDTAVYYCARGGSDTVIGDWFAYWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE
FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 503), and a light chain comprising the amino acid sequence of:
DIQLTQSPSSLSASVGDRVTITCRASQSIGSYLAWYQQKPGKAPKLLIYDASNLETGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQGYYLWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 527).

Antigen binding fragments

[0122] In some aspects, the present disclosure provides antigen-binding fragments of any of the CD137 antibodies provided by the present disclosure.

[0123] 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.

[0124] In some particular embodiments, the disclosure provides an antigen-binding fragment of an antibody selected from those listed in Tables 1-3.

[0125] 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.

[0126] In some particular embodiments, the antigen-binding fragment is a Fab fragment selected from those listed in Table 1.

Antibody derivatives

[0127] The anti-CD137 antibodies as described herein may include any antibody derived from the anti-CD137 antibodies of the present disclosure.

[0128] In some further aspects, the present disclosure provides derivatives of any of the CD137 antibodies provided by the present disclosure.

[0129] 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.

[0130] 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: 361-480. 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: 1-60. 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: 61-120. 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: 121-180. In some embodiments, the antibody derivative comprises an HVR_L1 amino acid sequence for any hit shown in Tables 1-2, which can be found in SEQ ID NOs: 181-240. In some embodiments, the antibody derivative comprises an HVR_L2 amino acid sequence for any hit shown in Tables 1-2, which can be found in SEQ ID NOs: 241-300.

[0131] In some embodiments, the antibody derivative comprises an HVR_L3 amino acid sequence for any hit shown in Tables 1-2, which can be found in SEQ ID NOs: 301-360. 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: 361-480.

[0132] 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: 481-528.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] 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.

[0139] 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.

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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.

Antibody production

[0144] Antibodies of the present disclosure may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acids encoding any antibody described herein are provided. Such nucleic acids may encode an amino acid sequence comprising the V_L and/or an amino acid sequence comprising the V_H of the antibodies (*e.g.*, the light and/or heavy chains of the antibodies). In some embodiments, one or more vectors (*e.g.*, expression vectors) comprising such nucleic acids are provided herein. In some embodiments, a host cell comprising such nucleic acids is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L of the antibody and an amino acid sequence comprising the V_H of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or a lymphoid cell (*e.g.*, Y0, NS0, Sp20 cells). In some embodiments, antibodies of the present disclosure are produced in CHO cells. In some embodiments, antibodies of the present disclosure are modified, and do not include a C-terminal lysine residue (*e.g.*, the C-terminal lysine residue of an antibody heavy chain described herein is removed (such as before or during antibody production)). For example, an antibody of the present disclosure may include an antibody heavy chain having the amino acid sequence of SEQ ID NO: 503, or may include an antibody heavy chain having an amino acid sequence identical to SEQ ID NO: 503 except for the amino acid sequence lacks the C-terminal lysine residue at

position 450 of SEQ ID NO: 503. In some embodiments, a method of making an antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0145] For recombinant production of antibodies of the present disclosure, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0146] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and may be further purified.

[0147] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See* Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0148] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0149] Plant cell cultures can also be utilized as hosts. *See, e.g.,* US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0150] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.,* in Graham *et al., J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.,* in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather *et al., Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.,* Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

[0151] 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 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. For example, 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: 531. 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.

CD137L expression and checkpoint blockade immunotherapies

[0152] In some embodiments, the present disclosure relates to methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject if the level of expression of CD137L in a sample obtained from the subject is higher than a reference level. In some embodiments, the method comprises obtaining a sample from the subject, and measuring the level of expression of CD137L in the sample prior to administration of the checkpoint blockade

immunotherapy. In some embodiments, the checkpoint blockade immunotherapy is any one or more of the checkpoint blockade immunotherapies described herein.

[0153] In some embodiments, the present disclosure relates to methods of determining whether a subject is likely to respond to a checkpoint blockade immunotherapy. In some embodiments, the method comprises obtaining a sample from the subject, measuring the level of expression of CD137L in the sample, and determining that the subject is likely to respond to the checkpoint blockade immunotherapy when the level of expression of CD137L in the sample is higher than a reference level.

[0154] In some embodiments, the present disclosure relates to methods of treating or delaying progression of cancer in a subject in need thereof comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject after it is determined that the subject is likely to respond to the checkpoint blockade immunotherapy. In some embodiments, the checkpoint blockade immunotherapy is any one or more of the checkpoint blockade immunotherapies described herein. In some embodiments, responsiveness of the subject comprises treatment efficacy. In some embodiments, responsiveness of the subject comprises reduced tumor volume. In some embodiments, responsiveness of the subject comprises serological responsiveness.

[0155] In some embodiments, the present disclosure relates to methods of selecting a subject having cancer for treatment with a checkpoint blockade immunotherapy. In some embodiments, the method comprises measuring the level of expression of CD137L in a sample obtained from the subject, and selecting the subject for treatment with the checkpoint blockade immunotherapy if the level of expression of CD137L in the sample is higher than a reference level. In some embodiments, the checkpoint blockade immunotherapy is any one or more of the checkpoint blockade immunotherapies described herein.

Checkpoint blockade immunotherapy

[0156] In some embodiments, the present disclosure relates to the use of a checkpoint blockade immunotherapy. In some embodiments, use of a checkpoint blockade immunotherapy comprises administering to a subject an antibody targeting one or more immune checkpoint

pathways (*e.g.*, the PD-1:PD-L1 pathway). Any checkpoint blockade immunotherapy known in the art may be used in the methods of the present disclosure, including, for example, a therapy comprising one or more antibodies targeting CTLA4, PD-1, PD-L1, TIM3, LAG3, CD27, CD28, CD40, OX40, GITR, BTLA, VISTA, B7-H3, B7-H4, IDO, and/or KIR. In some embodiments, the checkpoint blockade immunotherapy comprises administering an anti-PD-1 antibody. In some embodiments, the checkpoint blockade immunotherapy comprises administering an anti-PD-L1 antibody. In some embodiments, the checkpoint blockade immunotherapy is used in combination with an anti-CD137 antibody (as described herein).

Additional therapeutic agents

[0157] The anti-cancer therapies described herein (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) may be administered alone as monotherapy, or may comprise one or more additional therapeutic agents or therapies. In some embodiments, the one or more (*e.g.*, one or more, two or more, three or more, four or more, five or more, *etc.*) additional therapeutic agents are one or more of a viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, and/or chemotherapies. In some embodiments, the present disclosure provides a combination therapy, which comprises an anti-cancer therapy described herein (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) in combination with one or more additional therapies or therapeutic agents for separate, sequential or simultaneous administration. The term "additional therapy" or "additional therapeutic agent" may refer to a therapy or therapeutic agent which does not employ the same immunotherapy as is provided in the anti-cancer therapy. In some embodiments, the present disclosure provides a combination therapy for treating cancer in a mammal, which comprises administering to the mammal an effective amount of an anti-cancer therapy of the present disclosure (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) in combination with one or more additional therapeutic agents.

[0158] 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.

[0159] 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).

[0160] 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).

[0161] 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.

[0162] 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.

[0163] 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.

Administering immunotherapies

[0164] In some embodiments, the present disclosure relates to the administration of an effective amount of an anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy). In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is used to treat or delay progression of cancer in a subject. In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) delays the onset of cancer, including biochemical, histological and/or behavioral symptoms of cancer, its complications and intermediate pathological phenotypes presenting during development of cancer. In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) delays development of cancer and/or slows the progression of cancer and/or prolongs survival of the subject.

[0165] In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of inhibiting tumor cell growth and/or proliferation. In some embodiments, the tumor cell growth and/or proliferation is inhibited by at least about 5% when contacted with the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a

checkpoint blockade immunotherapy) relative to corresponding tumor cells not contacted with the anti-cancer therapy. For example, the tumor cell growth and/or proliferation is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% when contacted with the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) relative to corresponding tumor cells not contacted with the anti-cancer therapy. In some embodiments, the tumor cell growth and/or proliferation is inhibited by at least about 1-fold when contacted with the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) relative to corresponding tumor cells not contacted with the anti-cancer therapy. For example, the tumor cell growth and/or proliferation is inhibited by at least about 1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5 fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 100-fold, or at least about 1000-fold when contacted with the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) relative to corresponding tumor cells not contacted with the anti-cancer therapy.

[0166] In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of reducing tumor volume in a subject when the subject is administered the anti-cancer therapy. In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of reducing tumor volume in a subject by at least about 5% relative to the initial tumor volume in the subject (*e.g.*, prior to administration of the anti-cancer therapy). For example, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of reducing tumor volume in a subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% relative to the initial tumor volume in the subject (*e.g.*,

prior to administration of the anti-cancer therapy). In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of reducing tumor volume in a subject by at least about 1-fold relative to the initial tumor volume in the subject (*e.g.*, prior to administration of the anti-cancer therapy). For example, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is capable of reducing tumor volume in a subject by at least about 1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5 fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 100-fold, or at least about 1000-fold relative to the initial tumor volume in the subject (*e.g.*, prior to administration of the anti-cancer therapy). Methods of monitoring tumor cell growth and/or proliferation, tumor volume, and/or tumor inhibition are known in the art, including, for example, via the methods described in Example 3 below.

[0167] In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) has therapeutic effect on a cancer. In some embodiments, the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) reduces 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 anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy).

[0168] Binding molecules and pharmaceutical compositions of 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. In some embodiments, the present disclosure provides methods of treating a disorder in a mammal (*e.g.*, after measuring CD137L expression in a sample taken from the mammal), which comprises administering to the mammal in need of treatment an effective amount of an anti-cancer therapy described herein.

[0169] The anti-cancer therapies of the present disclosure (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) may be administered via any suitable enteral or parenteral

route of administration. The term "enteral route" of administration may refer to the administration via any part of the gastrointestinal tract. Enteral routes of administration include, for example, oral, mucosal, buccal, rectal, intragastric, *etc.* The term "Parenteral route" of administration may refer to a route of administration other than enteral route. Parenteral routes of administration include, for example, intravenous, intramuscular, intradermal, intraperitoneal, intratumor, intravesical, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, transtracheal, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal, subcutaneous, topical administration, *etc.* The anti-cancer therapies of the disclosure (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) may 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

[0170] An effective amount of an anti-cancer therapy of the present disclosure (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) may range from about 0.001 to about 500 mg/kg, including, for example, about 0.01 to about 100 mg/kg, of the body weight of the subject. For example, the amount may 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 subject. In some embodiments, the effective amount of the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is in the range of about 0.01-30 mg/kg of body weight of the subject. In some other embodiments, the effective amount of the anti-cancer therapy (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) is in the range of about 0.05-15 mg/kg of body weight of the subject. 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 anti-cancer therapy 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.

[0171] An anti-cancer therapy of the present disclosure (*e.g.*, an anti-CD137 antibody, a checkpoint blockade immunotherapy) may be administered on multiple occasions. Intervals between single doses can be, for example, daily, 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. The precise timing of dosages to be administered can be readily determined by a person skilled in the art.

[0172] 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.

EXAMPLES

Example 1: CD137L expression in multiple types of human cancers

[0173] To begin to identify whether an association exists between CD137L expression and cancer, the expression status of CD137L (also known as TNFSF9) in human cancers was checked against The Cancer Genome Atlas (TCGA) database. As shown in **FIG. 1**, CD137 ligand was frequently overexpressed at the transcript level in multiple human tumor panels when compared to the corresponding matched normal tissues.

Example 2: human cancer cells express functional CD137L

Methods

[0174] To examine whether some human cancer cell express functional CD137L, 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.

Results

[0175] CD137L can signal through CD137 to activate downstream NFκB signaling. To determine whether human cancer cells express functional CD137L, human Daudi and Raji B cell lymphoma cell lines were tested to identify whether these cells could specifically activate CD137-mediated signaling, using a cellular NFκB luciferase reporter assay. As shown in **FIGS. 2A and 2C**, when Daudi (**FIG. 2A**) or Raji (**FIG. 2C**) cells were mixed with the CD137-expressing 293T reporter cells, NFκB signaling was activated in a cell ratio-dependent manner. To exclude the possibility that factors other than the CD137 ligand expressed on the Daudi or Raji cells were stimulating NFκB signaling in the 293T reporter cells, an anti-CD137 antibody capable of blocking CD137 ligand binding was added in this assay. As shown in **FIGS. 2B and 2D**, the antibody significantly inhibited the NFκB signaling stimulated by both cell types, suggesting that both Daudi and Raji B lymphoma cells express functional CD137L that can trigger NFκB-dependent signaling in neighboring cells through CD137.

Example 3: CD137 ligand expression in certain mouse tumor cells

Methods

[0176] To examine the expression status of CD137L in mouse tumor cells, a series of mouse tumor cell lines (including the liver cancer H22, colon cancer CT26 and MC38, melanoma B16F10, lung cancer LL/2, pancreatic cancer Pan02, prostate cancer RM-1, B lymphoma A20, T lymphoma L5178-S and L5178-R cell lines) were expanded in respective growth medium according to manufacturer's instructions, and were subjected to flow cytometry analysis to detect CD137L expression. Briefly $5X10^6$ cells per cell line (in exponential growth phase) were resuspended in 1 mL FACS buffer (2% BSA in PBS), and 200 μL/well were aliquoted into a 96-well plate. Cells were collected by centrifugation, and the supernatants were discarded. 100 μL/well of a 1:100 dilution of PE-conjugated isotype control antibody or anti-mouse CD137L

antibody (BioLegend) was added to the wells, and the samples were incubated for 30 minutes on ice in the dark. After washing the samples with FACS buffer, cells were resuspended in 200 μL of FACS buffer and were subjected to flow cytometry analysis.

[0177] To examine the *in vivo* anti-tumor responses of anti-CD137 immunotherapy, 10 different mouse syngeneic tumor models that were tested for CD137L surface expression described above were profiled with αCD137-AB1 antibody treatment, in comparison with an isotype control antibody treatment. Briefly, these different mouse tumor cells were maintained *in vitro* as a monolayer culture in DMEM or RPMI-1640 medium supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2 in air. The tumor cells were routinely subcultured. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Female mice at 7-8 weeks in age were inoculated subcutaneously at the right flank region with corresponding tumor cells in 0.1 ml of PBS for tumor development, 2×10^5 - 3×10^6 cells per inoculation site based on the tumor growth kinetics determined in pilot experiments. Depending on the origin of each tumor cell line, the corresponding mouse strain was used for tumor model development: BALB/c mice were used for the H22, CT26, EMT6, A20 tumor models; C57BL/6 mice were used for the B16F10, MC38, RM-1 and Pan02 tumor models; and DBA/2 mice were used for the L5178-R and L5178-S T lymphoma models. The treatments were started when the mean tumor size reached ~100 mm^3 with isotype control or αCD137-AB1 antibody, 8-10 mice per treatment group. The antibodies were dosed at levels between 10-50mg/kg through intraperitoneal injection, twice a week for 3 weeks, or until the average group tumor volume reach 2000 mm^3. The Pan02 tumor model was examined with the surrogate anti-mouse PD-1 or anti-mouse PD-L1 antibody. Tumor growth inhibition (TGI): TGI(%) was an indication of antitumor effectiveness, and was expressed as: TGI (%)=100 × (1-T/C). T and C were the mean tumor volume (or weight) of the treated and control groups, respectively, on a given day.

Results

[0178] To detect CD137 ligand expression in mouse tumor cells, some commonly used mouse tumor cell lines were profiled by flow cytometry using an anti-mouse CD137 ligand antibody (**FIG. 3**). Of the ten tumor cell lines tested, six cell lines (H22, CT26, B16F10, LL/2,

A20, and L5178-S cells) had little to no detectable CD137L on their surface, while four cells lines had appreciable CD137L levels (MC38, L5178-R, RM-1, and Pan02 cells).

[0179] Next, the *in vivo* anti-tumor activity of targeting CD137 with an agonist antibody that recognizes mouse CD137 (antibody αCD137-AB1) was examined for the 10 syngenic mouse tumor models tested for CD137L surface expression described above (*i.e.*, the H22, CT26, B16F10, LL/2, MC38, A20, L5178-S, L5178-R, RM-1, and Pan02 cell lines). αCD137-AB1 is a fully human IgG4 anti-CD137 monoclonal antibody, which is capable of activating CD137-mediated signaling from human, monkey, and rodent CD137 (*See* PCT International Application No. PCT/CN2017/098332, incorporated herein by reference in its entirety). The anti-tumor efficacy of αCD137-AB1 for the 10 syngeneic mouse tumor models was profiled (**FIG. 4A-B**). As shown in **FIGS. 4A and 4B**, the tumor responses to αCD137-AB1 were categorized into groups of non-responders (tumor growth inhibition (TGI) < 20%) and responders (TGI > 35%), respectively. Interestingly, an inverse correlation between CD137L expression and responsiveness to the agonist anti-CD137 antibody was observed. As shown in **Table 4** below, the $CD137L^+$ cancer cells were resistant to anti-CD137 monotherapy, while >70% (5/7 mouse models tested) of the $CD137L^-$ cancers were susceptible to the anti-CD137 monotherapy.

Table 4: CD137L expression status and responsiveness to anti-CD137 antibody therapy in syngenic mouse tumor models

Tumor Model	CD137L expression status	Responsiveness to anti-CD137 antibody
Pan02	(+)	Non-responder
RM-1	(+)	Non-responder
L5178-R	(+)	Non-responder
B16F10	(-)	Non-responder
MC38	(-)	Non-responder
H22	(-)	Responder
CT26	(-)	Responder
LL/2	(-)	Responder
A20	(-)	Responder
L5178-S	(-)	Responder

[0180] The Pan02 cell line was positive for CD137L expression (**FIG. 3**) and resistant to anti-CD137 monotherapy in the syngenic mice model (**FIG. 4A**). However, this same syngenic

mouse tumor model was responsive to anti-PD-L1 (Bio X Cell) monotherapy (**FIG. 5A**) or anti-PD-1 (Bio X Cell) monotherapy (**FIG. 5B**).

[0181] Taken together, the data in the experiments provided above may suggest that $CD137L^+$ cancer cells may be resistant to treatment with anti-CD137 antibody therapy, but may be susceptible to checkpoint blockade immunotherapy (*e.g.*, anti-PD-L1 or anti-PD-1 therapy). As such, CD137L expression in tumor cells may serve as a biomarker to negatively stratify patients for enrollment into anti-CD137 antibody therapies and/or positively stratify patients for enrollment into checkpoint blockade immunotherapies (such as anti-PD-1 and/or anti-PD-L1 immunotherapies).

CLAIMS

What is claimed is:

1. A method of treating or delaying progression of cancer in a subject in need thereof, the method comprising administering an effective amount of an anti-CD137 antibody to the subject if the level of expression of CD137 ligand (CD137L) in a sample obtained from the subject is lower than a reference level.

2. The method of claim 1, further comprising the steps of:

a) obtaining the sample from the subject; and

b) measuring the level of expression of CD137L in the sample prior to administration of the anti-CD137 antibody to the subject.

3. A method of treating or delaying progression of cancer in a subject in need thereof, the method comprising administering an effective amount of an anti-CD137 antibody to the subject, wherein it has been determined that the subject is likely to respond to the anti-CD137 antibody when the level of expression of CD137L in a sample obtained from the subject is lower than a reference level.

4. A method of determining if a subject is likely to respond to an anti-CD137 antibody, the method comprising:

a) obtaining a sample from the subject;

b) measuring the level of expression of CD137L in the sample; and

c) determining that the subject is likely to respond to the anti-CD137 antibody when the level of expression of CD137L in the sample is lower than a reference level.

5. A method of selecting a subject having cancer for treatment with an anti-CD137 antibody, the method comprising:

a) measuring the level of expression of CD137L in a sample obtained from the subject; and

b) selecting the subject for treatment with the anti-CD137 antibody if the level of expression of CD137L in the sample is lower than a reference level.

6. The method of any one of claims 1-5, wherein the level of expression of CD137L in the sample is below the limit of detection.

7. The method or use of any one of claims 1-6, wherein the anti-CD137 antibody 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: 531.

8. The method or use of any one of claims 1-7, wherein the anti-CD137 antibody binds to one or more amino acid residues within amino acid residues 34-93 of SEQ ID NO: 531.

9. The method or use of any one of claims 1-8, wherein the anti-CD137 antibody 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: 531.

10. The method or use of claim 9, wherein the anti-CD137 antibody 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: 531.

11. The method or use of any one of claims 1-10, wherein the anti-CD137 antibody 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: 531.

12. The method or use of claim 11, wherein the anti-CD137 antibody does not bind to amino acid residues 109-112, 125, 126, 135-138, 150 and 151 of SEQ ID NO: 531.

13. The method or use of any one of claims 1-12, wherein the anti-CD137 antibody 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.

14. The method or use of claim 13, wherein the anti-CD137 antibody binds to cynomolgus monkey CD137.

15. The method or use of any one of claims 1-14, wherein the anti-CD137 antibody is a human antibody.

16. The method or use of any one of claims 1-15, wherein the anti-CD137 antibody is an anti-CD137 agonist antibody.

17. The method or use or use of any one of claims 1-16, wherein the anti-CD137 antibody blocks binding of CD137L to CD137.

18. The method or use of any one of claims 1-17, wherein the anti-CD137 antibody comprises a heavy chain variable region and a light chain variable region,

wherein the heavy chain variable region comprises an HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-60, an HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-120, and an HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 121-180; and

wherein the light chain variable region comprises an HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 181-240, an HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 241-300, and an HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 301-360.

19. The method or use of claim 18, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 361-420, and wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 421-480.

20. The method or use of claim 19, wherein the anti-CD137 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: 481-504, and wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 505-528.

21. The method or use of any one of claims 1-17, wherein the anti-CD137 antibody 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: 15, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 75, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 135; and

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 195, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 255, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 315.

22. The method or use of claim 21, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 375, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 435.

23. The method or use of claim 22, wherein the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 483, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 507.

24. The method or use of any one of claims 1-17, wherein the anti-CD137 antibody 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: 25, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 85, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 145; and

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 205, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 265, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 325.

25. The method or use of claim 24, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 385, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 445.

26. The method or use of claim 25, wherein the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 484, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 508.

27. The method or use of any one of claims 1-17, wherein the anti-CD137 antibody 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: 30, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 90, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 150; and

wherein the light chain variable region comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 210, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 270, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 330.

28. The method or use of claim 27, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 390, and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 450.

29. The method or use of claim 28, wherein the anti-CD137 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 503, and wherein the light chain comprises the amino acid sequence of SEQ ID NO: 527.

30. The method of any one of claims 1-29, further comprising administering to the subject a therapeutically effective amount of at least one additional therapeutic agent.

31. The method of claim 30, 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.

32. The method of claim 30 or claim 31, 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.

33. A method of treating or delaying progression of cancer in a subject in need thereof, the method comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject if the level of expression of CD137L in a sample obtained from the subject is higher than a reference level.

34. The method of claim 33, further comprising the steps of:

a) obtaining the sample from the subject; and

b) measuring the level of expression of CD137L in the sample prior to administration of the checkpoint blockade immunotherapy to the subject.

35. A method of treating or delaying progression of cancer in a subject in need thereof, the method comprising administering an effective amount of a checkpoint blockade immunotherapy to the subject, wherein it has been determined that the subject is likely to respond to the checkpoint blockade immunotherapy when the level of expression of CD137L in a sample obtained from the subject is higher than a reference level.

36. A method of determining if a subject is likely to respond to a checkpoint blockade immunotherapy, the method comprising:

a) obtaining a sample from the subject;

b) measuring the level of expression of CD137L in the sample; and

c) determining that the subject is likely to respond to the checkpoint blockade immunotherapy when the level of expression of CD137L in the sample is higher than a reference level.

37. A method of selecting a subject having cancer for treatment with a checkpoint blockade immunotherapy, the method comprising:

a) measuring the level of expression of CD137L in a sample obtained from the subject; and

b) selecting the subject for treatment with the checkpoint blockade immunotherapy if the level of expression of CD137L in the sample is higher than a reference level.

38. The method or use of any one of claims 33-37, wherein the checkpoint blockade immunotherapy comprises administering an anti-PD-1 antibody or an anti-PD-L1 antibody.

39. The method or use of any one of claims 33-38, further comprising administering an effective amount of an anti-CD137 antibody to the subject.

40. The method or use of any one of claims 1-39, wherein the subject is a human.

41. The method of any one of claims 1-40, wherein the sample is a serum sample.

42. The method of any one of claims 1-40, wherein the sample is a tumor sample.

43. The method or use of claim 42, wherein the tumor sample is a tumor biopsy.

44. The method or use of any one of claims 1-43, wherein the sample comprises one or more cancer cells.

45. The method or use of any one of claims 1-44, wherein the level of expression of CD137L is the level of protein expression of CD137L.

46. The method of claim 45, wherein the level of protein expression is measured by a method selected from the group consisting of immunoassay, PET imaging, Western blotting, ELISA, immunohistochemistry, and flow cytometry.

47. The method of any one of claims 1-44, wherein the level of expression of CD137L is the level of RNA transcript expression of CD137L.

48. The method of claim 47, where the level of transcript expression is measured by a method selected from the group consisting of RT-PCR, *in situ* hybridization, and next generation sequencing.

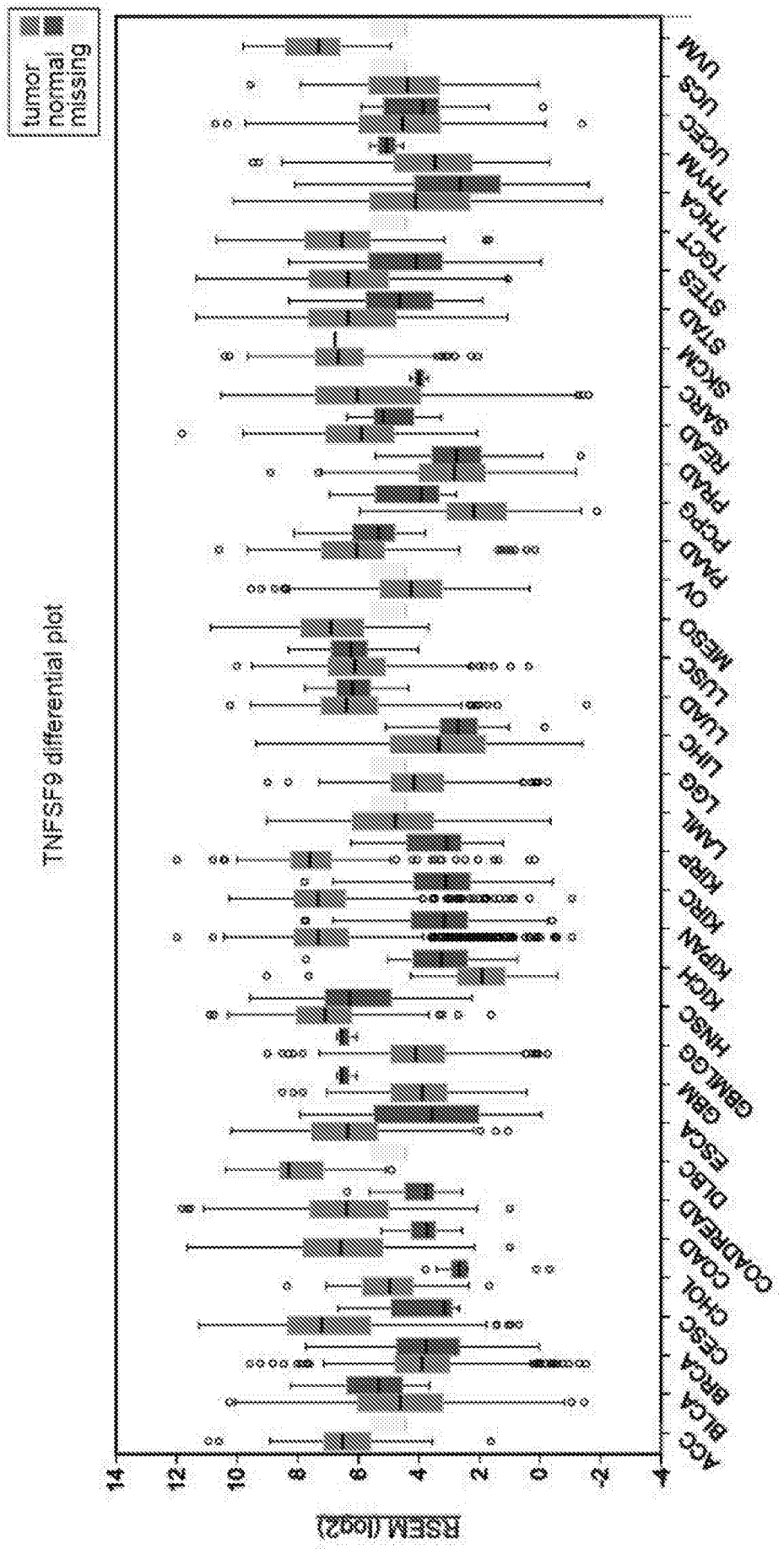
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FIG. 1

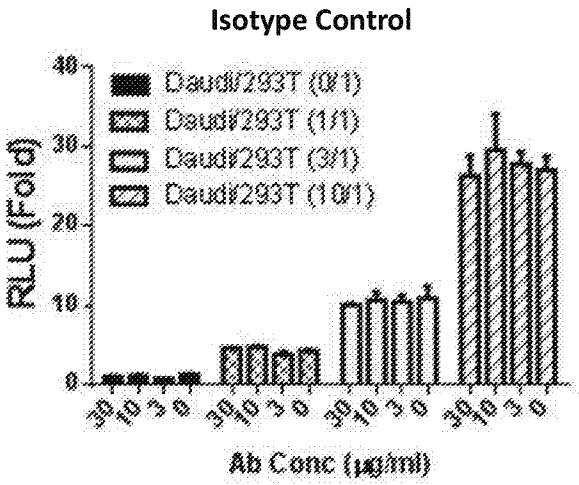

FIG. 2A

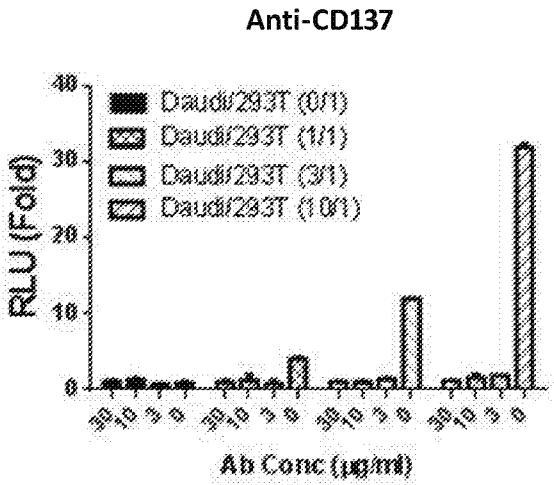

FIG. 2B

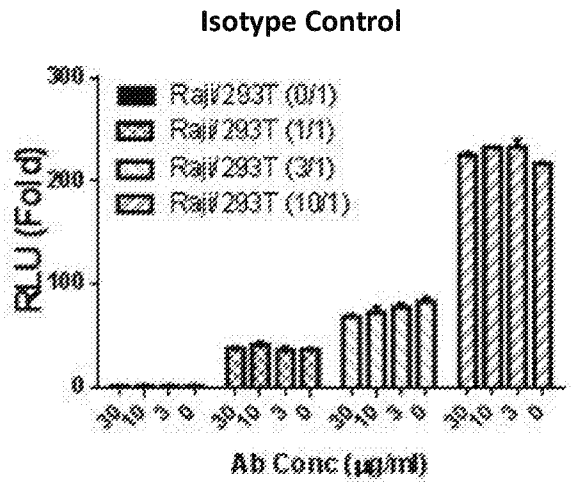

FIG. 2C

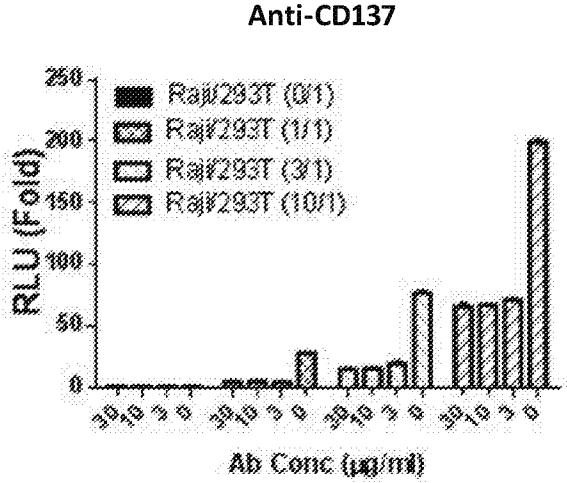

FIG. 2D

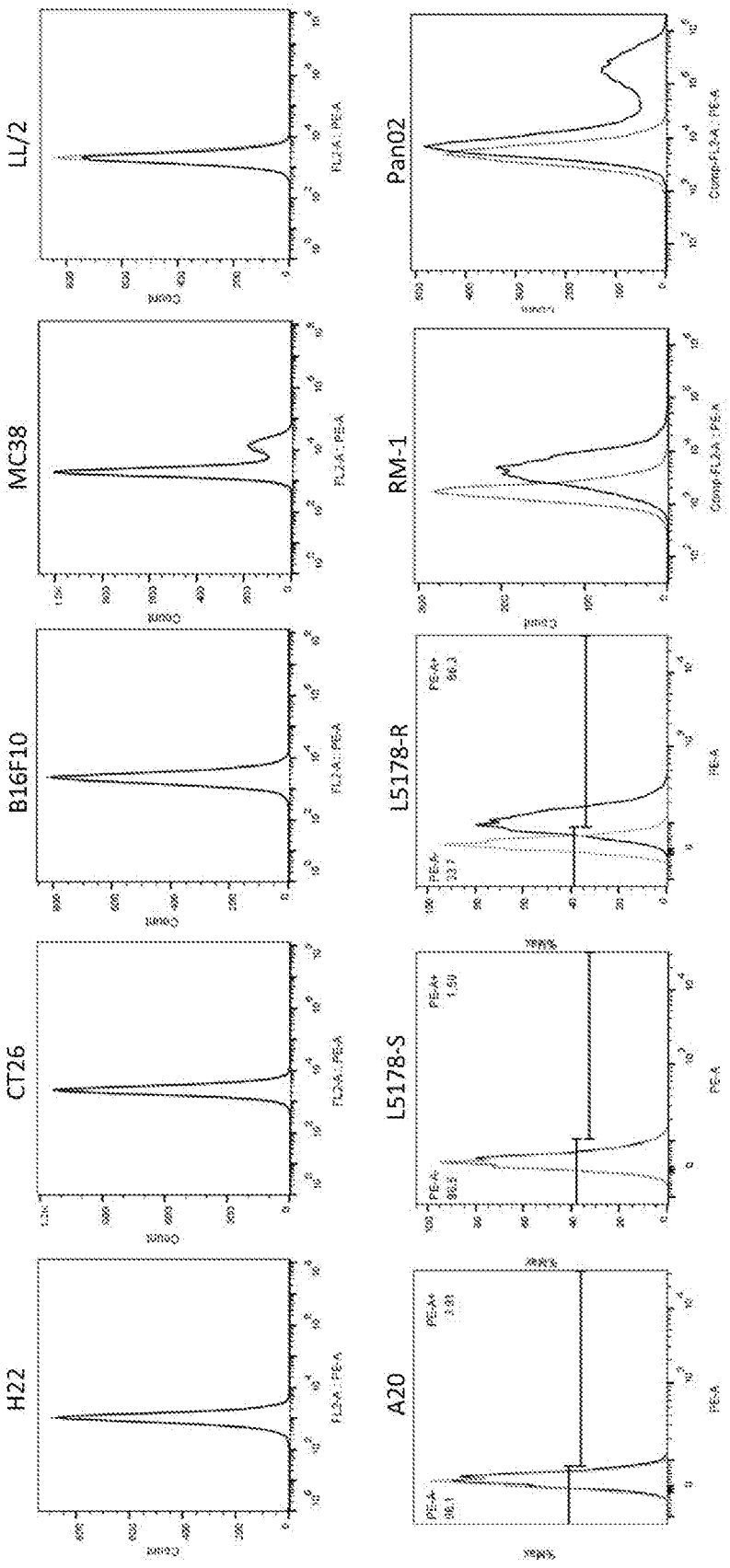

FIG. 3

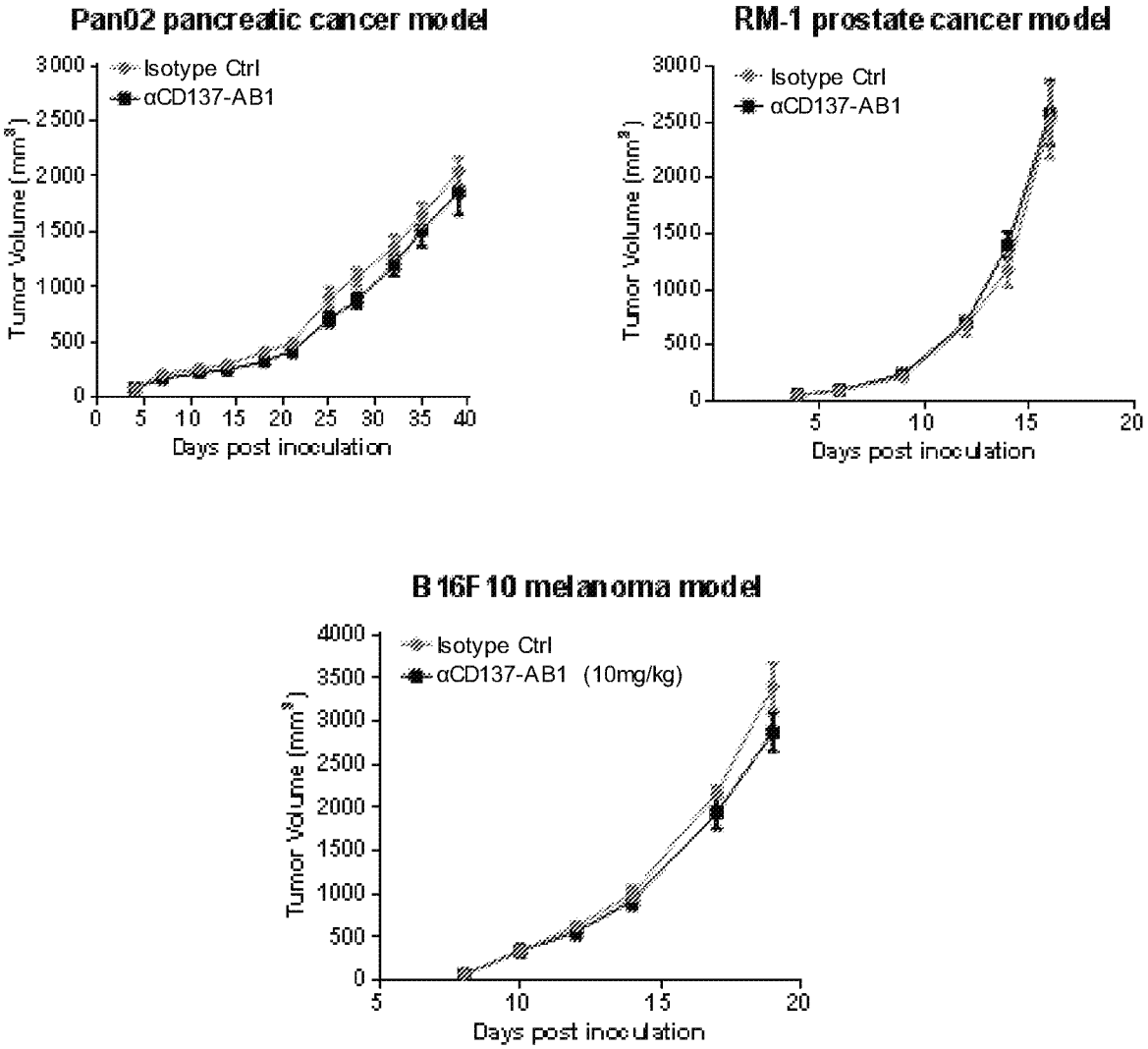
Pan02 pancreatic cancer model
Isotype Ctrl
αCD137-AB1
Tumor Volume (mm³)
Days post inoculation
RM-1 prostate cancer model
Isotype Ctrl
αCD137-AB1
Tumor Volume (mm³)
Days post inoculation
B16F10 melanoma model
Isotype Ctrl
αCD137-AB1 (10mg/kg)
Tumor Volume (mm³)
Days post inoculation

FIG. 4A

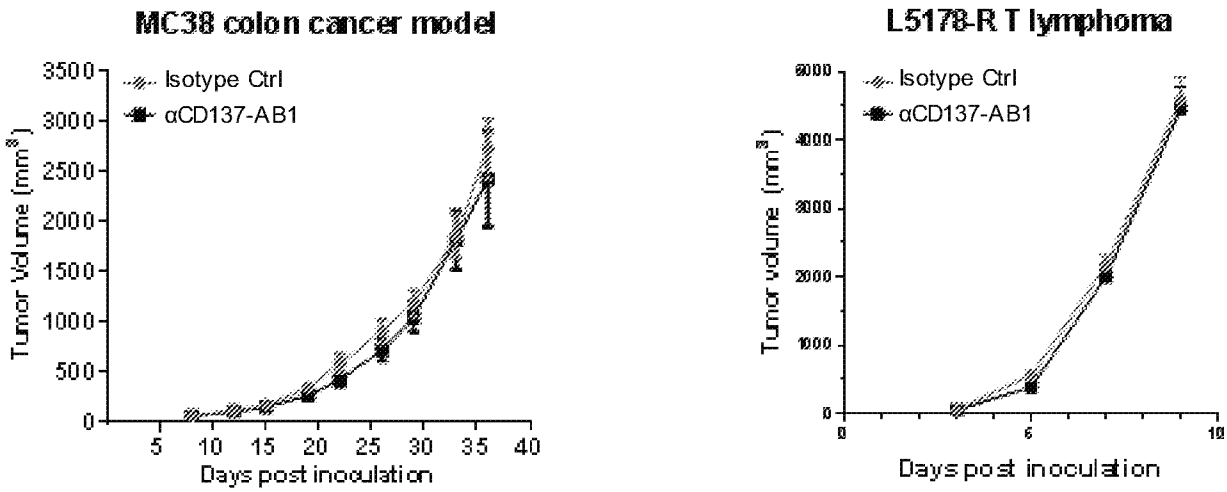
MC38 colon cancer model
Isotype Ctrl
αCD137-AB1
Tumor Volume (mm³)
3500
3000
2500
2000
1500
1000
500
0
5
10
15
20
25
30
35
40
Days post inoculation
L5178-R T lymphoma
Isotype Ctrl
αCD137-AB1
Tumor volume (mm³)
6000
4000
2000
1000
0
0
6
10
Days post inoculation

FIG. 4A cont.

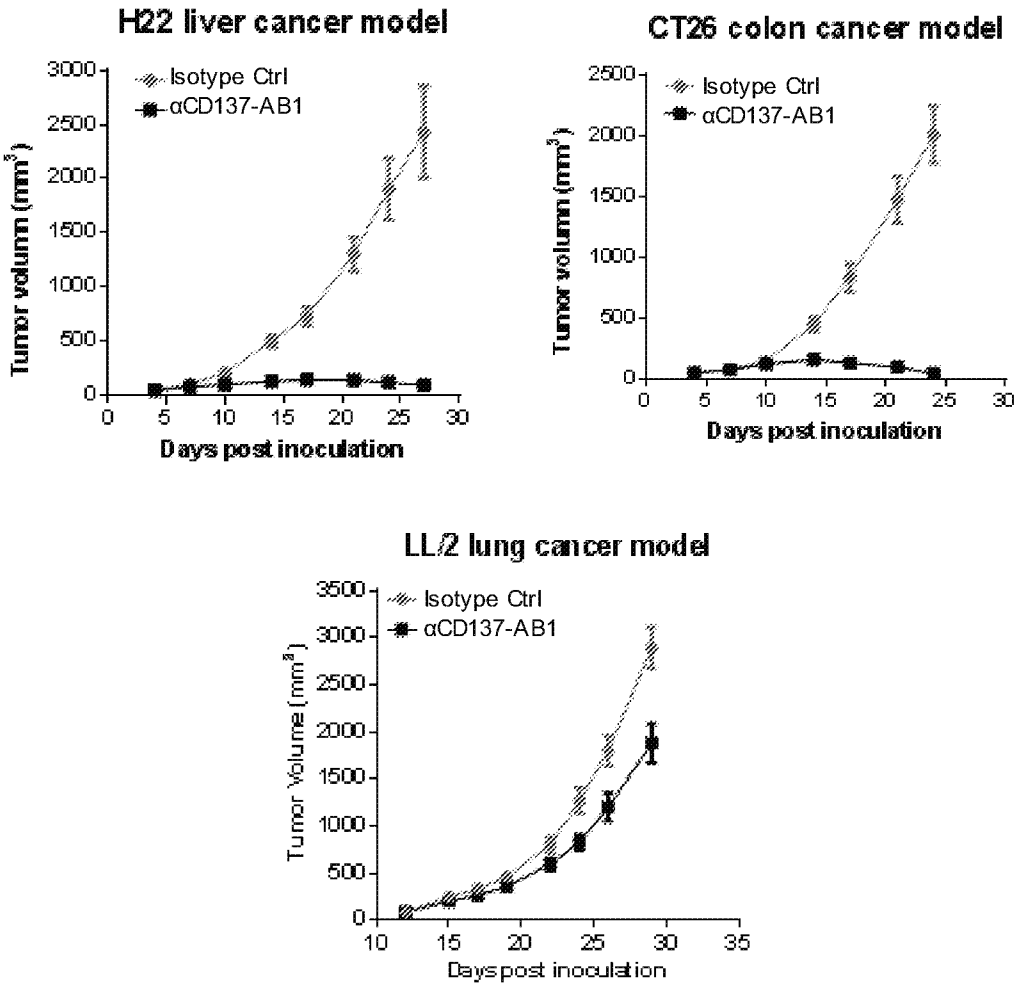
H22 liver cancer model
Isotype Ctrl
αCD137-AB1
Tumor volumn (mm3)
Days post inoculation
CT26 colon cancer model
Isotype Ctrl
αCD137-AB1
Tumor volumn (mm3)
Days post inoculation
LL/2 lung cancer model
Isotype Ctrl
αCD137-AB1
Tumor Volume (mm3)
Days post inoculation

FIG. 4B

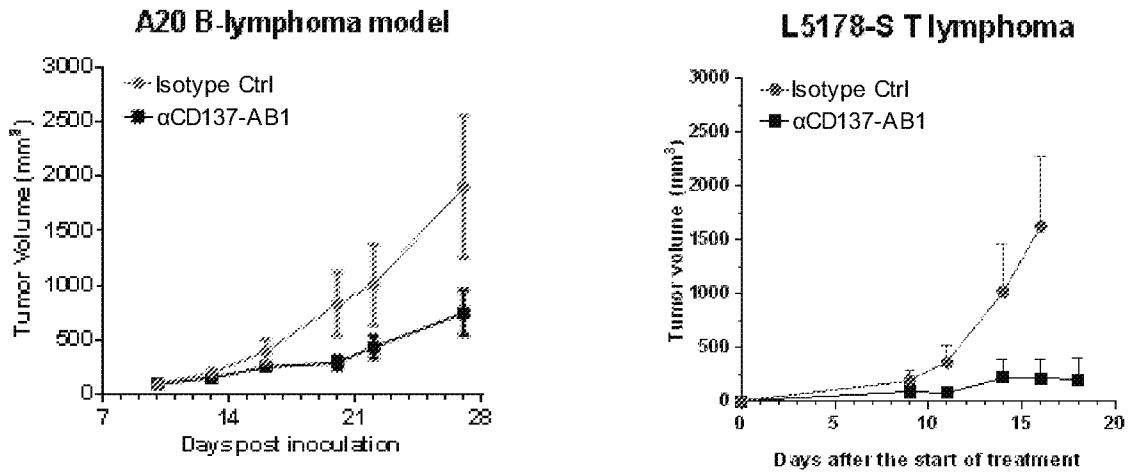
A20 B-lymphoma model
Isotype Ctrl
αCD137-AB1
Tumor Volume (mm³)
3000
2500
2000
1500
1000
500
0
7
14
21
28
Days post inoculation
L5178-S T lymphoma
Isotype Ctrl
αCD137-AB1
Tumor volume (mm³)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
Days after the start of treatment

FIG. 4B cont.

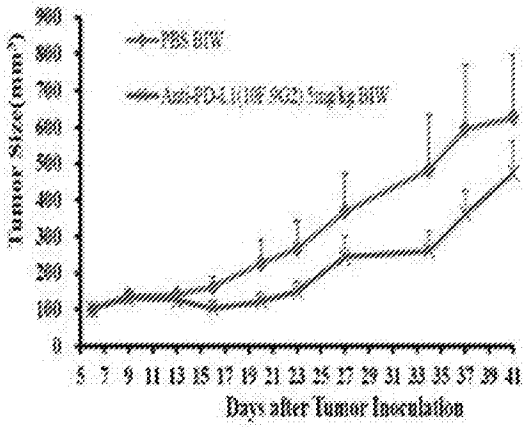
PBS, BIW
Tumor Size(mm³)
Days after Tumor Inoculation

FIG. 5A

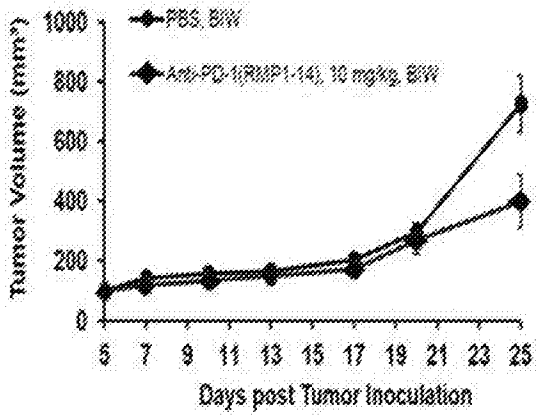
PBS, BIW
Anti-PD-1(RMP1-14), 10 mg/kg, BIW
Tumor Volume (mm³)
1000
800
600
400
200
0
5 7 9 11 13 15 17 19 21 23 25
Days post Tumor Inoculation

FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/114247

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/00(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNMED;CPRSABS;CNABS;DWPI;SIPOABS;CNTXT;WOTXT;EPTXT;JPTXT;CNKI;Pubmed;Google scholar:CD137, CD137L, ligand, 4-1BB, immune, receptor, antibody, anti-CD137, CDR, HVR, epitope, cancer, carcinoma, detect+, express+, metasta+, checkpoint, immunotherapy, PD-1, PD-L1, combinat+, sequence search based on SEQ ID NOs: 1-544

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016134358 A1 (SORRENTO THERAPEUTICS INC) 25 August 2016 (2016-08-25) see claims 1-10	1-6, 13-17
X	WEI H. et al. "Combinatorial PD-1 Blockade and CD137 Activation Has Therapeutic Efficacy in Murine Cancer Models and Synergizes with Cisplatin" *PLOS ONE*, Vol. 8, No. 12, 19 December 2013 (2013-12-19), Article e84927 see the abstract	30-48
A	WO 2016134358 A1 (SORRENTO THERAPEUTICS INC) 25 August 2016 (2016-08-25) see the whole document	7-12, 18-29

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 2018	**07 September 2018**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**LI,Kangqi**
Facsimile No. **(86-10)62019451**	Telephone No. **62411034**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/114247

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **1-3,6-35,38-48**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Although claims 1,3,33,35 and dependent claims 2,6-32,34,38-48 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged uses for the manufacture of pharmaceutical compositions for treating or preventing diseases of the antibody or compositions thereof.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2017/114247

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2016134358 A1	25 August 2016	AU 2016219772 A1	21 September 2017
		US 2016244528 A1	25 August 2016
		US 2017226215 A1	10 August 2017
		JP 2018508509 A	29 March 2018
		EP 3258959 A1	27 December 2017
		CA 2977257 A1	25 August 2016
		CN 107921104 A	17 April 2018
		AR 105313 A1	27 September 2017
		TW 201632559 A	16 September 2016
		KR 20180016972 A	20 February 2018
		IL 254088 D0	31 October 2017