ANTIBODIES TARGETING CD137 AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

The present invention relates to an isolated antibody which specifically binds human CD137, and pharmaceutical compositions and methods of use thereof. The present invention further relates to a nucleic acid encoding said antibody, a vector comprising said nucleic acid, a host cell comprising said nucleic acid or said vector, and a method of producing said antibody.

BACKGROUND OF THE INVENTION

The tumor necrosis factor receptor superfamily (TNFRSF) is a protein superfamily of receptors characterized by their ability to bind tumor necrosis factors (TNFs) via cysteine-rich pseudorepeats in the extracellular domain (Locksley et al., 2001, Cell. 104: 487–501). At present, 27 TNF family members have been identified. TNFRSF members and their ligands are expressed mostly on immune cells, where they are playing a role of immunomodulators in T-cell-mediated immune responses. TNFRSF members play a role in enhancement of dendritic cell survival and priming capacity of T cells, optimal generation of effector T cells, optimal antibody responses, and amplification of inflammatory reactions.

CD137 (4-1BB, TNF-receptor superfamily 9, TNFRSF9) is a surface glycoprotein of the TNFR superfamily. It is an inducible costimulatory T cell receptor. CD137 expression is activation-dependent, and encompasses a broad subset of immune cells including activated NK and NKT cells, regulatory T cells, dendritic cells (DC) including follicular DC, stimulated mast cells, differentiating myeloid cells, monocytes, neutrophils, eosinophils (Wang et al, Immunol Rev. 229(1): 192-215 (2009)), and activated B cells (Zhang et al, J Immunol. 184(2):787-795 (2010)). In addition, CD137 expression has also been demonstrated on tumor vasculature (Broil K et al., Am J Clin Pathol. 115(4):543-549 (2001); Seaman et al, Cancer Cell 11(6):539-554 (2007)) and atherosclerotic endothelium (Olofsson et al, Circulation 117(10): 1292 1301 (2008)).

CD137-Ligand (CD137L, 4-1BBL or tnfsf9), a molecule of the TNF family, is an intercellular natural ligand known for CD137 (Alderson, M. R., et al., Eur. J. Immunol. 24:2219–2227 (1994); Pollok K., et al., Eur. J. Immunol. 24:367-374 (1994); Goodwin, R. G., et al., Eur. J. Immunol. 23: 2631–2641 (1993)). The ligand for CD137 forms a homotrimer, and the signaling via CD137 proceeds from ligated molecules at the cell surface, which become cross-linked by trimerized ligand (Won, E. Y., et al., J. Biol. Chem. 285:

9202–9210 (2010)). The higher order clustering of CD137 was suggested to be necessary for mediating the signaling. CD137 associates with the adaptors TRAF-2 and TRAF-1 in its cytoplasmic tail, resulting in co-immunoprecipitation, which is enhanced upon CD137 activation in T cells (Saoulli, K., et al., J. Exp. Med. 187: 1849–1862 (1998); Sabbagh, L., et al., J. Immunol. 180: 8093–8101 (2008)). Recruitment of TRAF-1 and TRAF-2 by CD137 results in downstream activation of NF-kB and the Mitogen Activated Protein (MAP) Kinase cascade including ERK, JNK, and p38 MAP kinases. NF-kB activation leads to upregulation of Bfl-1 and Bcl-XL, pro-survival members of the Bcl-2 family. The pro-apoptotic protein Bim is downregulated in a TRAF-1 and ERK dependent manner (Sabbagh et al., J Immunol. 180(12):8093-8101 (2008)). It has been suggested that the main action of CD137 is to place two or more TRAF-2 molecules in close molecular proximity to each other (Sanchez-Paulete, A. R., et al., Eur. J. Immunology 46(3): 513-522 (2016)). Based on this it was postulated that the major factor driving CD137 signaling is the relative density of TRAF-2-assembled CD137 moieties in micropatches of plasma membrane (Sanchez-Paulete, A. R., et al., Eur. J. Immunology 46(3): 513-522 (2016)). Overall, CD137 signaling is fostered by multimerization, and it was proposed that cross-linking CD137 molecules is the key factor in CD137 co-stimulatory activity.

CD137 co-stimulates T cells to carry out effector functions such as eradication of established tumors, broadening primary $CD8^+$T cell responses, and enhancing the memory pool of antigen- specific $CD8^+$ T cells, induction of interferon-gamma (IFN-γ) synthesis. The critical role of CD137 stimulation in $CD8^+$ T-cell function and survival could potentially be utilized for the treatment of tumors through manipulation of the CD137/CD137L interaction. In fact, *in vivo* efficacy studies in mice have demonstrated that treatment with anti-CD137 antibodies led to tumor regressions in multiple tumor models. For example, agonistic anti-mouse CD137 antibody was demonstrated to induce an immune response against P815 mastocytoma tumors, and low immunogenic tumor model Ag104 (I. Melero et al., Nat. Med., 3(6):682-5 (1997)). The efficacy of CD137 agonist mAbs in prophylactic and therapeutic settings for both monotherapy and combination therapy and anti-tumor protective T cell memory responses have been reported in several studies (Lynch et al., Immunol Rev. 222:277-286 (2008)). CD137 agonists also inhibit autoimmune reactions in a variety of autoimmunity models (Vinay et al, J Mol Med 84(9):726-736 (2006)).

Two anti-CD137 antibodies currently in the clinic are urelumab (Bristol-Myers Squibb), a fully humanized IgG4 mAb, and utomilumab (PF-05082566, Pfizer), a fully human IgG2 mAb (Chester C., et al., Cancer Immunol Immunother Oct;65(10):1243-8

(2016)). Although utilization of therapeutic antibodies agonizing CD137 is a very promising treatment strategy, it is coupled to difficulties such as low efficacy of anti-CD137 agonist antibodies, high toxicities and adverse events. CD137 agonist antibodies were shown to lead to alterations in immune system and organ function increasing risks of toxicities. High doses of CD137 agonist antibodies in naïve and tumor-bearing mice have been reported to induce T-cell infiltration to the liver and elevations of aspartate aminotransferase and alanine aminotransferase consistent with liver inflammation (Niu L, et al. J Immunol 178(7):4194–4213 (2007); Dubrot J, et al., Int J Cancer 128(1):105–118 (2011)). Initial clinical studies into the human therapeutic use of CD137 agonist antibody have also demonstrated elevations of liver enzymes and increased incidence of hepatitis (Sznol M., et al., J Clin Oncol 26(115S):3007 (2008); Ascierto PA, et al., Semin Oncol 37(5):508–516 (2010); Chester C., et al., Cancer Immunol Immunother Oct;65(10):1243-8 (2016)). Potentially fatal hepatitis was observed in a Bristol-Myers Squibb (BMS) phase II anti-CD137 study for previously treated stage III/IV melanoma, National Clinical Trial (NCT) 00612664. That study and several others (NCT00803374, NCT00309023, NCT00461110, NCT00351325) were terminated due to adverse events (Chester C., et al., Cancer Immunol Immunother Oct;65(10):1243-8 (2016)). Such adverse events are most probably due to systemic overstimulation of T-cells.

Thus, there is a need in the field to generate improved therapeutic anti-human CD137 antibodies having higher efficacy without the inherent side effects of general anti-proliferative drugs, in particular having lower toxicities comparable to the currently available CD137 antibodies.

SUMMARY

It is an object of the present disclosure to provide antibodies that specifically bind to human CD137 protein, and which have beneficial properties for use in therapies, such as improved affinity, efficacy, safety and improved biophysical properties, e.g. improved solubility, developability and stability. In particular, CD137 antibodies are yet to be found that do not directly and independent of other cell-surface molecules result in CD137 signaling upon binding.

In one aspect, the present disclosure refers to an isolated antibody having a binding specificity for human CD137, which comprises: HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR 2 and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, wherein said antibody binds human CD137 extracellular domain at an epitope located in the distal part of the extracellular domain of CD137 within amino acid residues 24-86 of SEQ ID NO: 32, wherein said epitope is characterized by a set of critical residues, determined in accordance with Example 13, comprising the residues Ala33, Asn40, Arg 41, Gln43, Cys45, Cys48, Pro49, Ser52, and Ser80; provided that amino acid residue Asn42 of CD137 is not a critical residue for binding. In one example, the present disclosure describes a novel CD137 antibody.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody as disclosed herein and a pharmaceutically acceptable carrier. In one example, there is disclosed a pharmaceutical composition comprising the isolated antibody of the disclosure, and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to the antibody of the disclosure, or the composition of the disclosure for use as a medicament.

In one example, the antibody of the disclosure, or the composition of the disclosure is described for use in the treatment of a cancer in a subject in need thereof.

In one aspect, the present disclosure refers to a use of the antibody as disclosed herein, or the composition as disclosed herein in the manufacture of a medicament in the treatment of a cancer.

In another example, the present disclosure describes a method of treating a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of the disclosure, or the composition of the disclosure.

In yet another aspect, the present disclosure refers to a nucleic acid encoding the antibody as disclosed herein. In a further example, the present disclosure describes a vector comprising said nucleic acid. In a further example, the present disclosure describes a host cell comprising said nucleic acid or said vector.

In another aspect, the present disclosure refers to a method of producing the antibody as disclosed herein, the method comprising the step of culturing a host cell comprising the nucleic acid or the vector as disclosed herein.

The aspects, advantageous features and preferred embodiments of the present invention summarized in the following items, respectively alone or in combination, further contribute to solving the object of the invention:

1. An isolated antibody having a binding specificity for human CD137, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 10 or fewer amino acid substitutions from a set of CDRs in which
   HCDR1′ is an amino acid sequence selected from any one of SEQ ID Nos: 1, 4, 7, and 10;
   HCDR2′ is an amino acid sequence selected from any one of SEQ ID Nos: 2, 5, 8, and 11;
   HCDR3′ is an amino acid sequence selected from any one of SEQ ID Nos: 3, 6, 9, and 12;
   LCDR1′ is an amino acid sequence selected from any one of SEQ ID Nos: 16, 19, and 22;
   LCDR2′ is an amino acid sequence selected from any one of SEQ ID Nos: 17, 20, and 23; and

LCDR3′ is an amino acid sequence selected from any one of SEQ ID Nos: 18, 21, and 24.

2. The antibody of item 1 comprising: a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 10 or fewer amino acid substitutions from a set of CDRs in which
HCDR1′ is as set forth in SEQ ID No: 1;
HCDR2′ is as set forth in SEQ ID No: 2;
HCDR3′ is as set forth in SEQ ID No: 3;
LCDR1′ is as set forth in SEQ ID No: 16;
LCDR2′ is as set forth in SEQ ID No: 17;
LCDR3′ is as set forth in SEQ ID No: 18.
3. The antibody of item 1 or item 2, which comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:
    (a) said VH comprises, in sequence, the three complementary determining regions HCDR1, HCDR2 and HCDR3, and
    (b) said VL comprises, in sequence, the three complementary determining regions LCDR1, LCDR2 and LCDR3.
4. The antibody of item 3, wherein
(a) said HCDR1 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 1, 4, 7, and 10, preferably SEQ ID NO: 1; (b) said HCDR2 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 2, 5, 8 and 11, preferably SEQ ID NO: 2; (c) said HCDR3 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 3, 6, 9 and 12, preferably SEQ ID NO: 3; (d) said LCDR1 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 16, 19 and 22, preferably SEQ ID NO: 16; (e) said LCDR2 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 17, 20 and 23, preferably SEQ ID NO: 17; and (f) said LCDR3 comprises, preferably consists of, an amino acid sequence selected from any one of SEQ ID NOs: 18, 21 and 24, preferably SEQ ID NO: 18.
5. The antibody of item 3, wherein the antibody comprises: (a) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively; (b) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 4, 5, and 6, respectively, and

LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 19, 20, and 21, respectively; LCDR1(c) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 7, 8, and 9, respectively, and LCDR1LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively; (d) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 10, 11, and 12, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 22, 23, and 24, respectively.

6. The antibody of item 3, comprising: (a) a HCDR1 comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 1; (b) a HCDR2 comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 2; (c) a HCDR3 comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 3; (d) a LCDR1 comprising, preferably consisting of, the amino acid sequence of SEQ ID NOs: 16; (e) a LCDR2 comprising, preferably consisting of, the amino acid sequence of SEQ ID NOs: 17; and (f) a LCDR3 comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 18.
7. The antibody of any one of items 3 to 6, wherein said VH comprises VH3 frameworks FR1, FR2, FR3 and FR4.
8. The antibody of any one of items 3 to 7, wherein said VL comprises Vκ frameworks FR1, FR2 and FR3, particularly Vκ1 or Vκ3 FR1 to FR3, preferably Vκ1 FR1 to FR3, and a framework FR4, which is selected from a Vκ FR4, particularly a Vκ1 FR4 or a Vκ3 FR4, and a Vλ FR4; particularly a Vλ FR4 comprising the amino acid sequence having at least 60, 70, 80, 90 percent identity to an amino acid sequence selected from any of SEQ ID NO: 33 to SEQ ID NO: 39, preferably a Vλ FR4 as set forth in any of SEQ ID NO: 33 to SEQ ID NO: 39, preferably a Vλ FR4 as set forth in SEQ ID NO: 33 or 34, more preferably the Vλ FR4 as set forth in SEQ ID NO: 33.
9. The antibody of any one of items 3 to 8, wherein said VH comprises an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13 or 15, more preferably SEQ ID NO: 13; and/or said VL comprises an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25 or 27, more preferably SEQ ID NO: 25.
10. The antibody of any one of items 3 to 9, wherein said VH comprises an amino acid sequence selected from any one of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13 or 15, more preferably SEQ ID NO: 13; and/or said VL comprises an amino

acid sequence selected from any one of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25 or 27, more preferably SEQ ID NO: 25.

11. The antibody of any one of the preceding items, comprising: (a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 25; (b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 26; or (c) a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 27.
12. The antibody of any one of the preceding items, wherein said antibody
    a) binds to human CD137 with a dissociation constant (KD) of less than 50 nM, particularly less than 10 nM, more particularly less than 5 nM as measured by surface plasmon resonance; and
    b) optionally, binds to Cynomolgus CD137 with a KD of less than 50 nM, particularly less than 10 nM, more particularly less than 5 nM as measured by surface plasmon resonance; and
    c) optionally, cross-competes with urelumab.
13. The antibody of any one of the preceding items, wherein said antibody does not inhibit the interaction between CD137 and its ligand CD137L, in particular as measured by a competition ELISA.
14. The antibody of any one of the preceding items, wherein said antibody does not bind to human CD40 and/or does not bind to human OX40, in particular as measured by SPR.
15. The antibody of any of the preceding items, wherein said antibody:
    a) when in scFv format, has a melting temperature (Tm), determined by differential scanning fluorimetry, of at least 50°C, e.g., at least 55°C, preferably at least 60°C, more preferably at least 64°C, in particular wherein said antibody is formulated in 50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl; and/or
    b) when in scFv format, has a loss in monomer content, after storage for at least two weeks, particularly for at least four weeks, at 4°C, of less than 7%, e.g. less than 6%, less than 5%, less than 4%, less than 3%, preferably less than 2%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein the antibody of the invention is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or
    d) when in scFv format, has a loss in monomer content, after five consecutive freeze-thaw cycles, of less than 5%, preferably less than 3%, more preferably

less than 1%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein the antibody of the invention is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

16. An isolated antibody wherein said antibody binds human CD137 extracellular domain at an epitope located in the distal part of the extracellular domain of CD137, particularly within the cysteine-rich domains CRD1 and/or CRD2, more particularly within amino acid residues 24-86 of SEQ ID NO: 32, provided that amino acid residue Asn42 of CD137 is not a critical residue for binding.
17. The antibody of any one of the previous items, wherein the antibody is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, a Fab, an Fv, an scFv, a dsFv, an scAb, an STAB, and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody Technology™), preferably an Fv or an scFv.
18. The antibody of any one of the preceding items, which is a single-chain variable fragment (scFv).
19. The antibody of item 18, wherein said scFv has the amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, preferably SEQ ID NO: 29 or SEQ ID NO: 31, more preferably SEQ ID NO: 29.
20. The isolated antibody of item 17, wherein the antibody is an IgG selected from the group consisting of an IgGl, an IgG2, an IgG3 and an IgG4, preferably IgG4.
21. The isolated antibody of any one of the previous items, wherein the antibody is humanized.
22. The antibody of any one of the preceding items which is a multispecific molecule, in particular a multispecific molecule having at least a second functional molecule.
23. The antibody of item 22, wherein said antibody is in a format selected from the group consisting of a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a bispecific T-cell engager (BiTE; tandem di-scFv), a tandem tri-scFv, a tribody (Fab-(scFv)2) or bibody (Fab-(scFv)1), Fab, Fab-Fv2, Morrison (IgG CH_3-scFv fusion (Morrison L) or IgG CL-scFv fusion (Morrison H)), triabody, scDb-scFv, bispecific Fab2, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, scFv-HSA-scFv fusion, di-diabody, DVD-Ig, COVD, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusions, such as bsAb (scFv linked to C-terminus of light chain), Bs1Ab (scFv linked to N-terminus of light chain), Bs2Ab

(scFv linked to N-terminus of heavy chain), Bs3Ab (scFv linked to C-terminus of heavy chain), Ts1Ab (scFv linked to N-terminus of both heavy chain and light chain), Ts2Ab (dsscFv linked to C-terminus of heavy chain), bispecific antibodies based on heterodimeric Fc domains, such as Knob-into-Hole antibodies (KiHs); an Fv, scFv, scDb, tandem-di-scFv, tandem tri-scFv, Fab-(scFv)2, Fab-(scFv)1, Fab, Fab-Fv2, COVD fused to the N- and/or the C-terminus of either chain of a heterodimeric Fc domain or any other heterodimerization domain, a MATCH and DuoBodies.

24. A pharmaceutical composition comprising the antibody of any one of items 1-23, and a pharmaceutically acceptable carrier.
25. The antibody of any one of items 1-23, or the composition of item 24 for use as a medicament.
26. The antibody of any one of items 1-23, or the composition of item 24 for use in a manufacture of a medicament for use in the treatment of a cancer.
27. The antibody of any one of items 1-23, or the composition of item 24 for use in the treatment of a cancer.
28. Use of the antibody of any one of items 1-23, or the composition of item 24 for the treatment of a cancer in a subject in need thereof.
29. A method of treating a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of any one of items 1-23, or the composition of item 24.
30. A nucleic acid encoding the antibody of any one of items 1-23.
31. A vector comprising the nucleic acid of item 30.
32. A host cell comprising the nucleic acid of item 30 or the vector of item 31.
33. A method of producing the antibody of any one of items 1-23, the method comprising the step of culturing a host cell comprising the nucleic acid of item 30 or the vector of item 31.
34. A kit comprising the antibody of any one of items 1 to 23, or the composition of item 24.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 Heatmap of epitope binning results of rabbit IgG clone 38-27-A11 and urelumab and utomilumab. Binding level normalized to theoretical Rmax in percent (%) of analyte molecules (column) to immobilized molecules (row). No binding (dark grey) means same

epitope, bright grey means the secondary molecule (analyte) can bind and has another epitope than the immobilized molecule.

FIG. 2 No inhibition of CD137 binding to CD137L in competition ELISA. The absorbances measured in the competitive ELISA assessing the binding of CD137L to CD137 are represented in function of increasing concentrations of PRO1359 or PRO1360, respectively. The inhibitory antibody goat anti-human CD137 served as a reference.

FIG. 3 Binding of PRO1359 and PRO1360 to human CD137 expressing Jurkat cells as assessed by flow cytometry (FC). The geometric mean of the fluorescence signal obtained is represented in function of the molecules' concentrations in ng/ml. Urelumab was used as a reference.

FIG. 4 (A) Structure of CD137. CRD1 to 4 represent cysteine rich domains 1 to 4. (B) Designed constructs of CD137 ECD.

FIG. 5 Tri-specific scDb-scFv molecules PRO1480 and PRO1481 were tested in CD137 activity assay in the presence of HCC827 cells stimulated with IFNy (10 ng/ml) for 6 h and 24 h. In this experiment, PRO885 served as reference molecule to assess the relative activation of CD137 signaling. Tri-specific scDb-scFv molecule PRO1186 was taken along on each plate to compare its activity with the other scDb-scFv molecules. Luminescence was read 6 h or 24 h after addition of Jurkat reporter cells and concentrations of tested molecules with increasing RLU values only were fitted using sigmoidal 4PL fit (GraphPad Prism).

FIG. 6 *Ex vivo* T cell activation assay. PBMC were stimulated with 10 ng/ml SEA and treated with serial dilutions of the scDb-scFv constructs PRO1480 for 96 h. Activation of T-cells was assessed by quantification of IL-2 in harvested supernatants by ELISA. Treatment with PRO1480 resulted in pronounced IL-2 secretion when compared to the cocktail of the reference molecules. Data were fitted using sigmoidal 4PL fit (GraphPad Prism).

FIG. 7 IL-2 secretion of human PBMCs stimulated with or without Staphylococcal Enterotoxin A (SEA) (96 h, 1 mg/ml HSA). PBMC were stimulated with 10 ng/ml SEA and treated with serial dilutions of a cocktail of the reference molecules avelumab and urelumab and the scDb-scFv PRO1480 and PRO1186 for 96 h. Activation of T-cells was assessed by quantification of IL-2 in harvested supernatants by ELISA. Treatment with PRO1480 resulted in pronounced IL-2 secretion when compared to treatment with PRO1186. Data were fitted using sigmoidal 4PL fit (GraphPad Prism).

FIG. 8 Potency and maximum of T cell activation by different anti-CD137 antibody fragments. The potency and maximum of T cell activation by antibody constructs scDb-scFv

PRO1480 and PRO1186 is represented by the area under the curve (AUC) of IL-2 secretion as shown in FIG. 7 and maximum stimulation of Il-2 secretion, respectively.

FIG. 9 Schematic view of CD137 binding of different anti-CD137 antibody fragments. The extracellular domain (ECD) of CD137 is comprised of four cysteine rich domains (CRD1 to 4). (A) Anti-CD137 antibody PRO1480 binds to an epitope on CD137 that is comprised in the distal part of the ECD in CRD1 and/or CRD2; (B) in contrast, anti-CD137 antibody PRO1186 binds to an epitope on CD137 that is comprised in the part of the ECD that is proximal to the cell in CRD4.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides antibodies that specifically bind to human CD137 protein, and pharmaceutical compositions, production methods, and methods of use of such antibodies and compositions.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains.

The terms “comprising” and “including” are used herein in their open-ended and non-limiting sense unless otherwise noted. With respect to such latter embodiments, the term "comprising" thus includes the narrower term “consisting of”.

The terms “a” and “an” and “the” and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. For example, the term “a cell” includes a plurality of cells, including mixtures thereof. Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

In a first aspect, the present invention relates to antibodies that specifically bind to human CD137.

In one aspect, the disclosure provides an isolated antibody having a binding specificity for human CD137, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 10 or fewer amino acid substitutions, e.g., 9 or fewer amino acid substitutions, 8 or fewer amino acid substitutions, 7 or fewer amino acid substitutions, 6 or fewer amino acid substitutions, 5 or fewer amino acid substitutions, 4 or fewer amino acid substitutions, 3 or fewer amino acid substitutions, 2 or fewer amino acid substitutions, 1 or 0 amino acid substitutions, preferably 0 amino acid substitutions, from a

set of CDRs in which HCDR1′ is an amino acid sequence selected from any one of SEQ ID Nos: 1, 4, 7, and 10, preferably SEQ ID NO: 1; HCDR2′ is an amino acid sequence selected from any one of SEQ ID Nos: 2, 5, 8, and 11, preferably SEQ ID NO: 2; HCDR3′ is an amino acid sequence selected from any one of SEQ ID Nos: 3, 6, 9, and 12, preferably SEQ ID NO: 3; LCDR1′ is an amino acid sequence selected from any one of SEQ ID Nos: 16, 19, and 22, preferably SEQ ID NO: 16; LCDR2′ is an amino acid sequence selected from any one of SEQ ID Nos: 17, 20, and 23, preferably SEQ ID NO: 17; and LCDR3′ is an amino acid sequence selected from any one of SEQ ID Nos: 18, 21, and 24, preferably SEQ ID NO: 18.

In a particular embodiment, the present invention relates to an isolated antibody having a binding specificity for human CD137, which comprises: HCDR1, HCDR2, and HCDR3 sequences having at least 90 percent identity to the sequences of SEQ ID NOs: 1, 2, and 3, respectively, LCDR1 and LCDR3 sequences having at least 90 percent identity to the sequences of SEQ ID NOs: 16 and 18, respectively, and an LCDR2 sequence having at least 85 percent identity to the sequence of SEQ ID NO: 17.

The term “antibody” and the like, as used herein, includes: whole antibodies or single chains thereof; and any antigen-binding fragment (i.e., “antigen-binding portion”) or single chains thereof; and molecules comprising antibody CDRs, VH regions or VL regions (including without limitation multispecific antibodies). A naturally occurring “whole antibody” is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. 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.

The terms "antigen-binding fragment", "antigen-binding fragment thereof", "antigen binding portion", and the like, as used herein, refer to one or more fragments of an intact whole antibody that retain the ability to specifically bind to a given antigen (e.g., CD137). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F (ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody Technology™).

The term "Complementarity Determining Regions" ("CDRs") are amino acid sequences with boundaries determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering scheme), ImMunoGenTics (IMGT) numbering (Lefranc, M.-P., The Immunologist, 7, 132-136 (1999); Lefranc, M.-P. et al., Dev. Comp. Immunol., 27, 55-77 (2003) ("IMGT" numbering scheme) and numbering scheme described in Honegger & Plückthun, J. Mol. Biol. 309 (2001) 657-670 ("AHo" numbering). For example, for classic formats, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in human VH and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in human VL. Under IMGT the CDR amino acid residues in the VH are numbered approximately 26-35 (HCDR1), 51-57 (HCDR2) and 93-102 (HCDR3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (LCDR1), 50-52 (LCDR2), and 89-97 (LCDR3) (numbering according

to "Kabat"). Under IMGT, the CDRs of an antibody can be determined using the program IMGT/DomainGap Align.

In the context of the present invention, the numbering system suggested by Honegger & Plückthun ("AHo") is used (Honegger & Plückthun, J. Mol. Biol. 309 (2001) 657-670), unless specifically mentioned otherwise. Furthermore, the following residues are defined as CDRs according to AHo numbering scheme: LCDR1 (also referred to as CDR-L1): L24-L42; LCDR2 (also referred to as CDR-L2): L58-L72; LCDR3 (also referred to as CDR-L3): L107-L138; HCDR1 (also referred to as CDR-H1): H27-H42; HCDR2 (also referred to as CDR-H2): H57-H76; HCDR3 (also referred to as CDR-H3): H108-H138. For the sake of clarity, the numbering system according to Honegger & Plückthun takes the length diversity into account that is found in naturally occurring antibodies, both in the different VH and VL subfamilies and, in particular, in the CDRs, and provides for gaps in the sequences. Thus, in a given antibody variable domain usually not all positions 1 to 149 will be occupied by an amino acid residue.

Preferably, the "antigen-binding region" comprises at least amino acid residues 4 to 138 of the variable light (VL) chain and 5 to 138 of the variable heavy (VH) chain (in each case numbering according to Honegger & Plückthun), more preferably amino acid residues 3 to 144 of VL and 4 to 144 of VH, and particularly preferred are the complete VL and VH chains (amino acid positions 1 to 149 of VL and 1 to 149 of VH). Antigen binding portions can also be incorporated into maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, scDb-scFv, v-NAR and bis-scFv (see, e.g., Holliger and Hudson, 2005, Nature Biotechnology, 23, 1126, 36). Antigen binding portions of antibodies can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies). Antigen binding portions can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al., 1995 Protein Eng. 8 (10): 1057-1062; and U.S. Pat. No. 5,641,870).

The term "binding specificity" as used herein refers to the ability of an individual antibody combining site to react with one antigenic determinant and not with a different antigenic determinant. As use herein, the term "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an

antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In its most general form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between the target of interest and an unrelated molecule, as determined, for example, in accordance with a specificity assay methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA, RIA, ECL, IRMA, SPR (Surface plasmon resonance) tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard colour development (e.g. secondary antibody with horseradish peroxide and tetramethyl benzidine with hydrogen peroxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (= negative reaction) may be about 0.1 OD; typical positive reaction may be about 1 OD. This means the ratio between a positive and a negative score can be 10-fold or higher. In a further example, an SPR assay can be carried out, wherein at least 10-fold, preferably at least 100-fold difference between a background and signal indicates specific binding. Typically, determination of binding specificity is performed by using not a single reference molecule, but a set of about three to five unrelated molecules, such as milk powder, transferrin or the like. The antibody of the invention has a binding specificity for human CD137. In a specific embodiment, the antibody of the invention has a binding specificity for human CD137 and does not bind to human CD40 and/or does not bind to human OX40, in particular as determined by SPR.

Suitably, the antibody of the invention is an isolated antibody. The term "isolated antibody", as used herein, refers to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds CD137 is substantially free of antibodies that specifically bind antigens other than CD137). An isolated antibody that specifically binds CD137 may, however, have cross-reactivity to other antigens, such as CD137 molecules from other species. Thus, in one embodiment, the antibody of the invention has a binding specificity for human CD137 and Macaca fascicularis (also known as Cynomolgus monkey or "Cynomolgus") CD137. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

Suitably, the antibody of the invention is a monoclonal antibody. The term "monoclonal antibody" or "monoclonal antibody composition" as used herein refers to antibodies that are substantially identical in their amino acid sequence or are derived from the

same genetic source. A monoclonal antibody composition displays a binding specificity and affinity for a particular epitope, or binding specificities and affinities for specific epitopes.

Antibodies of the invention include, but are not limited to, the chimeric, and humanized.

The term "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. For example, a mouse antibody can be modified by replacing its constant region with the constant region from a human immunoglobulin. Due to the replacement with a human constant region, the chimeric antibody can retain its specificity in recognizing the antigen while having reduced antigenicity in human as compared to the original mouse antibody.

A "humanized" antibody, as used herein, is an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts (i.e., the constant region as well as the framework portions of the variable region). Additional framework region modifications may be made within the human framework sequences as well as within the CDR sequences derived from the germline of another mammalian species. The humanized antibodies of the invention may include amino acid residues not encoded by human sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing). See, e.g., Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855, 1984; Morrison and Oi, Adv. Immunol., 44:65-92, 1988; Verhoeyen et al., Science, 239: 1534-1536, 1988; Padlan, Molec. Immun., 28:489-498, 1991; and Padlan, Molec. Immun., 31: 169-217, 1994. Other examples of human engineering technology include, but are not limited to the Xoma technology disclosed in U.S. Pat. No. 5,766,886.

The term "recombinant humanized antibody" as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell transformed to express the humanized antibody, e.g., from a transfectoma, and antibodies prepared, expressed, created or isolated by any other means

that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences.

Suitably, the antibody of the invention is humanized. Suitably, the antibody of the invention is humanized and comprises rabbit-derived CDRs.

The term "CD137" refers in particular to human CD137 with UniProt ID number Q07011 reproduced herein as SEQ ID NO: 32. Suitably, the antibodies of the invention target CD137, in particular human CD137 as shown in UniProt ID number Q07011, reproduced herein as SEQ ID NO: 32. Suitably, the antibodies of the invention target human and cynomolgus (Macaca fascicularis) CD137. The antibodies of the invention specifically bind CD137. In a specific embodiment, the antibody of the invention has a binding specificity for human CD137 and does not bind to human CD40 and/or does not bind to human OX40, in particular as determined by SPR. Preferably, the antibodies of the invention do not block CD137/CD137L interaction.

Suitably, the antibody of the present invention is a CD137 agonist. An "activator" or "activating antibody" or "agonist" or "agonist antibody" is one that enhances or initiates signaling by the antigen to which it binds. In the context of the present invention, the term "CD137 agonist" encompasses the antibody of the present invention that is capable to activate CD137 signaling upon clustering of CD137-antigen-binding fragments thereof, e.g., wherein binding of at least two of said CD137-antigen-binding fragments allow for multimerization of the bound CD137 molecules and their activation. In some embodiments, agonist antibodies activate signaling without the presence of the natural ligand.

Antibodies of the invention include, but are not limited to, the humanized monoclonal antibodies isolated as described herein, including in the Examples. Examples of such anti-human CD137 antibodies are antibodies whose sequences are listed in Table 1. Additional details regarding the generation and characterization of the antibodies described herein are provided in the Examples.

The isolated antibody of the disclosure having a binding specificity for human CD137 comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein: (a) said VH comprises, in sequence, the three complementary determining regions HCDR1, HCDR2 and HCDR3, and (b) said VL comprises, in sequence, the three complementary determining regions LCDR1, LCDR2 and LCDR3. For the sake of clarity, the CDR regions are not linked to each other, but are flanked by framework regions FR1 to FR4.

The present invention provides antibodies that specifically bind to CD137 protein, said antibodies comprising a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 1. In particular, the invention provides antibodies that specifically bind to CD137 protein, said antibodies comprising one, two or three VH CDRs having an amino acid sequence of any of the corresponding VH CDRs listed in Table 1.

The present invention provides an isolated antibody having a binding specificity for human CD137 which comprises a heavy chain variable region (VH), wherein said VH comprises, in sequence, the three complementary determining regions HCDR1, HCDR2 and HCDR3, said HCDR1 having an amino acid sequence selected from any one of SEQ ID Nos: 1, 4, 7, and 10, preferably SEQ ID NO: 1, said HCDR2 having an amino acid sequence selected from any one of SEQ ID Nos: 2, 5, 8 and 11, preferably SEQ ID NO: 2, said HCDR3 having an amino acid sequence selected from any one of SEQ ID Nos: 3, 6, 9 and 12, preferably SEQ ID NO: 3. In particular, the invention provides antibodies that have a binding specificity for human CD137 and comprise HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively.

The present invention also provides antibodies that specifically bind to CD137 protein, said antibodies comprising a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 1. In particular, the invention provides antibodies that specifically bind to CD137 protein, said antibodies comprising one, two or three VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 1.

The present invention provides an isolated antibody having a binding specificity for human CD137, which comprises a light chain variable region (VL), wherein said VL comprises, in sequence, the three complementary determining regions LCDR1, LCDR2 and LCDR3, said LCDR1 having an amino acid sequence selected from any one of SEQ ID Nos: 16, 19 and 22, preferably SEQ ID NO: 16, said LCDR2 having an amino acid sequence selected from any one of SEQ ID Nos: 17, 20 and 23, preferably SEQ ID NO: 17, said LCDR3 having an amino acid sequence selected from any one of SEQ ID Nos: 18, 21 and 24, preferably SEQ ID NO: 18. In particular, the invention provides antibodies that have a binding specificity for human CD137 and comprise LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively.

Suitably, the present invention provides an isolated antibody having a binding specificity for human CD137, which comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(a) said VH comprises, in sequence, the three complementary determining regions HCDR1, HCDR2 and HCDR3, said HCDR1 having an amino acid sequence selected from any one of SEQ ID Nos: 1, 4, 7, and 10, said HCDR2 having an amino acid sequence selected from any one of SEQ ID Nos: 2, 5, 8 and 11, said HCDR3 having an amino acid sequence selected from any one of SEQ ID Nos: 3, 6, 9 and 12; and

(b) said VL comprises, in sequence, the three complementary determining regions LCDR1, LCDR2 and LCDR3, said LCDR1 having an amino acid sequence selected from any one of SEQ ID Nos: 16, 19 and 22, said LCDR2 having an amino acid sequence selected from any one of SEQ ID Nos: 17, 20 and 23, said LCDR3 having an amino acid sequence selected from any one of SEQ ID Nos: 18, 21 and 24.

In particular, the invention provides antibodies that have a binding specificity for human CD137 and comprise (a) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and (b) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively.

Other antibodies of the invention include amino acids that have been mutated, yet have at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity in the CDR regions with the CDR regions depicted in the sequences described in Table 1. In one aspect, other antibodies of the invention includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions depicted in the sequence described in Table 1.

The terms “identical” or “identity”, in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. “Percent (%) identity” and “homology” with respect to nucleic acid, a peptide, polypeptide or antibody sequence are defined as the percentage of nucleic acid/amino acid residues in a candidate sequence that are identical with the nucleic acid/amino acid residues in the specific nucleic acid, peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2 or ALIGN software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977; and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4: 11-17, 1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol, Biol. 48:444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar 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 terms “polypeptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

Suitably, the isolated antibody of the invention having a binding specificity for human CD137 comprises: a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

(a) said VH comprises, in sequence, the three complementary determining regions HCDR1, HCDR2 and HCDR3,

said HCDR1 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 1, 4, 7, and 10, preferably SEQ ID NO: 1;

said HCDR2 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 2, 5, 8 and 11, preferably SEQ ID NO: 2;

said HCDR3 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 3, 6, 9 and 12, preferably SEQ ID NO: 3; and/or

(b) said VL comprises, in sequence, the three complementary determining regions LCDR1, LCDR2 and LCDR3,

said LCDR1 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 16, 19 and 22, preferably SEQ ID NO: 16;

said LCDR2 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 17, 20 and 23, preferably SEQ ID NO: 17;

said LCDR3 having an amino acid sequence having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to any one of SEQ ID NOs: 18, 21 and 24, preferably SEQ ID NO: 18.

In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises: (a) HCDR1, HCDR2, and HCDR3 having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to the sequences of SEQ ID NOs: 1, 2, and 3, respectively, and/or LCDR1, LCDR2, and LCDR3 having at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity to the sequences of SEQ ID NOs: 16, 17, and 18, respectively. In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises: (a) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and/or LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively.

In a further embodiment, the present invention provides an isolated antibody that specifically binds CD137 (e.g., human CD137 protein), wherein said antibody comprises a VH domain and a VL domain. In the context of the present invention the terms "VH" (variable heavy chain), "VL" (variable light chain), "Vκ" and "Vλ" refer to families of antibody heavy and light chain sequences that are grouped according to sequence identity and homology. Methods for the determination of sequence homologies, for example by using a homology search matrix such as BLOSUM (Henikoff, S. & Henikoff, J. G., Proc. Natl. Acad. Sci. USA 89 (1992) 10915-10919), and methods for the grouping of sequences according to homologies are well known to one of ordinary skill in the art. For VH, Vκ and Vλ different subfamilies can be identified, as shown, for example, in Knappik et al., J. Mol. Biol. 296 (2000) 57-86, which groups VH in VH1A, VH1B and VH2 to VH6, Vκ in Vκ1 to Vκ4 and Vλ in Vλ1 to Vλ3. *In vivo*, antibody Vκ chains, Vλ chains, and VH chains are the result of the random rearrangement of germline κ chain V and J segments, germline λ chain V and J segments, and heavy chain V, D and J segments, respectively. To which subfamily a given antibody variable chain belongs is determined by the corresponding V segment, and in particular by the framework regions FR1 to FR3. Thus, any VH sequence that is characterized in the present application by a particular set of framework regions HFR1 to HFR3 only, may be combined with any HFR4 sequence, for example a HFR4 sequence taken from one of the heavy chain germline J segments, or a HFR4 sequence taken from a rearranged VH sequence.

Suitably, the present invention provides an isolated antibody that specifically binds CD137 (e.g., human CD137 protein), wherein said antibody comprises a VH3 domain. A specific example of a VH belonging to VH3 family is represented under SEQ ID NO: 13 or SEQ ID NO: 14. In particular, framework regions FR1 to FR3 taken from SEQ ID NO: 13 or SEQ ID NO: 14 belong to VH3 family (Table 1, first three regions marked in non-bold). Suitably, a VH belonging to VH3 family, as used herein, is a VH comprising FR1 to FR3 having at least 85%, preferably at least 90%, more preferably at least 95% sequence identity to FR1 to FR3 of SEQ ID NO: 13 or SEQ ID NO: 14. More particularly, framework regions FR1 to FR4 taken from SEQ ID NO: 13 or SEQ ID NO: 14 belong to VH3 family (Table 1, regions marked in non-bold). Suitably, a VH belonging to VH3 family, as used herein, is a VH comprising FR1 to FR4 having at least 85%, preferably at least 90%, more preferably at least 95% sequence identity to FR1 to FR4 of SEQ ID NO: 13 or SEQ ID NO: 14.

Suitably, the present invention provides an isolated antibody that specifically binds CD137 (e.g., human CD137 protein), wherein said antibody comprises Vκ frameworks FR1,

FR2 and FR3, particularly Vκ1 or Vκ3 frameworks, preferably Vκ1 frameworks FR1 to 3, and a framework FR4, which is selected from a Vκ FR4, particularly Vκ1 FR4 or Vκ3 FR4, and a Vλ FR4. Suitable Vκ1 frameworks FR1 to FR3 are set forth in SEQ ID NO: 25 or SEQ ID NO: 26 (Table 1, FR regions are marked in non-bold). Suitable Vκ1 frameworks FR1 to FR3 comprise the amino acid sequences having at least 60, 70, 80, 90 percent identity to amino acid sequences corresponding to FR1 to FR3 and taken from SEQ ID NO: 25 or SEQ ID NO: 26 (Table 1, FR regions are marked in non-bold).

Suitable Vλ FR4 are as set forth in SEQ ID NO: 33 to SEQ ID NO: 39. In one embodiment the present invention provides an isolated antibody that specifically binds CD137 (e.g., human CD137 protein), wherein said antibody comprises Vλ FR4 comprising the amino acid sequence having at least 60, 70, 80, 90 percent identity to an amino acid sequence selected from any of SEQ ID NO: 33 to SEQ ID NO: 39, preferably to SEQ ID NO: 33 or SEQ ID NO: 34, more preferably SEQ ID NO: 33.

In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises:

(i) the HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and the LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively;

(ii) VH3 domain framework sequences FR1 to FR4; and

(iii) a VL domain comprising a VL framework comprising Vκ frameworks FR1, FR2 and FR3, particularly Vκ1 or Vκ3 FR1 to FR3, preferably Vκ1 FR1 to FR3, and a framework FR4, which is selected from a Vκ FR4, particularly Vκ1 FR4 or Vκ3 FR4, and a Vλ FR4, particularly Vλ FR4 comprising the amino acid sequence having at least 60, 70, 80, 90 percent identity to comprising an amino acid sequence selected from any of SEQ ID NO: 33 to SEQ ID NO: 39, preferably to SEQ ID NO: 33 or SEQ ID NO: 34, more preferably to SEQ ID NO: 33, more particularly Vλ FR4 comprising an amino acid sequence selected from any one of SEQ ID NO: 33 to SEQ ID NO: 39, preferably Vλ FR4 comprising an amino acid sequence SEQ ID NO: 33 or SEQ ID NO: 34, more preferably SEQ ID NO:33.

In one embodiment, the present invention thus provides an antibody having a binding specificity for human CD137 and comprising a VL comprising:

(i) CDR domains CDR1, CDR2 and CDR3;

(ii) human Vκ framework regions FR1 to FR3, particularly human Vκ1 framework regions FR1 to FR3;

(iii) FR4, which is selected from (a) a human Vλ germ line sequence for FR4, particularly a Vλ germ line sequence selected from the list of: SEQ ID NO: 33 to 39, preferably SEQ ID NO: 33 or SEQ ID NO: 34, more preferably SEQ ID NO: 33; and (b) a Vλ-based sequence, which has one or two mutations, particularly one mutation, compared to the closest human Vλ germ line sequence for FR4 comprising an amino acid sequence selected from any one of SEQ ID NO: 33 to SEQ ID NO: 39, preferably SEQ ID NO: 33 or SEQ ID NO: 34, more preferably SEQ ID NO:33.

In a more preferred embodiment, the antibody of the invention having a binding specificity for human CD137 comprises:

(i) the HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and the LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively;

(ii) VH3 domain framework sequences FR1 to FR4; and

(iii) a VL domain comprising a VL framework comprising Vκ1 frameworks FR1, FR2 and FR3, and a Vλ FR4 comprising the amino acid sequence having at least 60, 70, 80, 90 percent identity to comprising an amino acid sequence selected from any one of SEQ ID NO: 33 to SEQ ID NO: 39, particularly Vλ FR4 as set forth in SEQ ID NO: 33 to SEQ ID NO: 39, preferably SEQ ID NO: 33 or SEQ ID NO: 34, more preferably SEQ ID NO:33.

The present invention provides an isolated antibody that specifically binds CD137 (e.g., human CD137 protein), wherein said antibody comprises a VH domain listed in Table 1.

The invention also provides an isolated antibody that specifically binds to CD137, wherein said antibody comprises (or alternatively, consisting of) a VH amino acid sequence listed in Table 1, wherein no more than about 10 amino acids in a framework sequence (for example, a sequence which is not a CDR) have been mutated (wherein a mutation is, as various non- limiting examples, an addition, substitution or deletion).

The invention also provides an isolated antibody that specifically binds to CD137, wherein said antibody comprises a VH amino acid sequence listed in Table 1, wherein no more than about 20 amino acids in a framework sequence (for example, a sequence which is not a CDR) have been mutated (wherein a mutation is, as various non-limiting examples, an addition, substitution or deletion).

Other antibodies of the invention include amino acids that have been mutated, yet have at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity in the VH regions with the VH regions depicted in the sequences described in Table 1.

The present invention provides an isolated antibody that specifically binds to CD137 protein, said antibody comprises a VL domain listed in Table 1.

The invention also provides an isolated antibody that specifically binds to CD137, wherein said antibody comprises a VL amino acid sequence listed in Table 1, wherein no more than about 10 amino acids in a framework sequence (for example, a sequence which is not a CDR) have been mutated (wherein a mutation is, as various non- limiting examples, an addition, substitution or deletion).

The invention also provides an isolated antibody that specifically binds to CD137, wherein said antibody comprises a VL amino acid sequence listed in Table 1, wherein no more than about 20 amino acids in a framework sequence (for example, a sequence which is not a CDR) have been mutated (wherein a mutation is, as various non- limiting examples, an addition, substitution or deletion).

Other antibodies of the invention include amino acids that have been mutated, yet have at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identity in the VL regions with the VL regions depicted in the sequences described in Table 1.

Suitably, the invention provides an isolated antibody that specifically binds human CD137, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence SEQ ID NO: 13 or SEQ ID NO: 14 or SEQ ID NO: 15, preferably SEQ ID NO: 13, and in particular wherein said antibody comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively. In one embodiment, the invention provides an isolated antibody or that specifically binds human CD137, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence SEQ ID NO: 13 and wherein said heavy chain variable region comprises G51C (AHo numbering). In a further embodiment, the invention provides an isolated antibody that specifically binds human CD137, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence SEQ ID NO: 14 and wherein said heavy chain variable region comprises V2S, Y105F and Q141P (AHo numbering).

In another embodiment, the invention provides an isolated antibody that specifically binds human CD137, wherein said antibody comprises a light chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence SEQ ID NO: 25 or SEQ ID NO: 26 or SEQ ID NO: 27, preferably SEQ ID NO: 25, and in particular wherein said antibody comprises LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively. In one embodiment, the invention provides an isolated antibody that specifically binds human CD137 wherein said antibody comprises a light chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence SEQ ID NO: 25 and wherein said light chain variable region comprises T141C (AHo numbering). In a further embodiment, the invention provides an isolated antibody that specifically binds human CD137 wherein said antibody comprises a light chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence SEQ ID NO: 26 and wherein said light chain variable region comprises I2F, M4L and A51P (AHo numbering).

The invention also provides an isolated antibody that specifically binds to CD137, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13; and a light chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25.

In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises: a heavy chain variable region comprising an amino acid sequence selected from any of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13; and a light chain variable region comprising an amino acid sequence selected from any of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25.

Thus, the present invention provides an isolated antibody that specifically binds human CD137, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence SEQ ID NO: 13; and a light chain variable region comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the

amino acid sequence SEQ ID NO: 25, and wherein the antibody comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and/or LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, preferably wherein the antibody comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively.

In a further embodiment, the isolated antibody of the invention having a binding specificity for human CD137 comprises: (a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 25; (b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 26; or (c) a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 27. In a preferred embodiment, the isolated antibody of the invention having a binding specificity for human CD137 comprises a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 25.

In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises:

(a) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, a VH sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 13, and a VL sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 25;

(b) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, a VH sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 14, and a VL sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 26, preferably wherein said VH comprises V2S, Y105F and Q141P (AHo numbering) and said VL comprises I2F, M4L and A51P (AHo numbering); or

(c) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, a VH sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 15, and a VL sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 27, preferably wherein said VH comprises G51C (AHo numbering) and said VL comprises T141C (AHo numbering).

In a preferred embodiment, the antibody of the invention having a binding specificity for human CD137 comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, a VH sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 15, and a VL sequence at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence SEQ ID NO: 27, preferably wherein said VH comprises a G51C mutation (AHo numbering) and said VL comprises T141C mutation (AHo numbering). Suitably, said antibody of the present invention is mutated to form an artificial interdomain disulfide bridge within the framework region, in particular wherein the pair of cysteines replaces Gly 51 (AHo numbering) on said VH and Thr 141 (AHo numbering) on said VL. It was surprisingly found that such antibody of the present invention comprising an interdomain disulfide bridge has a significantly increased thermostability.

The term "artificial" with reference to a disulfide bridge ("S-S bridge" or "diS") means that the S-S bridge is not naturally formed by the wild-type antibody, but is formed by an engineered mutant of a parent molecule, wherein at least one foreign amino acid contributes to the disulfide bonding. The site-directed engineering of artificial disulfide bridges clearly differentiates from those naturally available in native immunoglobulins or in modular antibodies, such as those described in WO 2009/000006, because at least one of the sites of bridge piers of an artificial disulfide bridge is typically located aside from the positions of Cys residues in the wild-type antibody, thus, providing for an alternative or additional disulfide bridge within the framework region. The artificial disulfide bridge of the present invention may be engineered within an antibody domain ("intradomain bridge"), which would stabilize the beta-sheet structure or bridging the domains ("interdomain bridge") or chains of domains ("interchain bridge"), to constrain the structure of a multispecific antibody according to the invention and support its interaction with potential binding partners.

In one embodiment, the antibody of the invention having a binding specificity for human CD137 comprises:

(a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 25;

(b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 26; or

(c) a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 27.

In a preferred embodiment, the antibody of the invention having a binding specificity for human CD137 comprises a VH sequence of SEQ ID NO: 13, and a VL of SEQ ID NO: 25.

In one embodiment, an antibody that specifically binds to CD137 is an antibody that is described in Table 1. In one embodiment, an antibody that specifically binds to CD137 is as set forth in SEQ ID NO: 29, SEQ ID NO: 30, or SEQ ID NO: 31. In one embodiment, an antibody that specifically binds to CD137 is as set forth in SEQ ID NO: 30. In one embodiment, an antibody that specifically binds to CD137 is as set forth in SEQ ID NO: 29 or SEQ ID NO: 31. In a preferred embodiment, an antibody that specifically binds to CD137 is as set forth in SEQ ID NO: 29.

Other antibodies of the invention include those wherein the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity to the sequences described in Table 1. In one embodiment, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in Table 1, while retaining substantially the same therapeutic activity. The term "substantially the same activity" as used herein refers to the activity as indicated by substantially the same activity being at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98% or even at least 100% or at least 110%, or at least 120%, or at least 130%, or at least 140%, or at least 150%, or at least 160%, or at least 170%, or at least 180%, or at least 190%, e.g. up to 200% of the activity as determined for the parent antibody, e.g., the antibody of the disclosure, in particular the antibody of the disclosure described in Table 1.

Given that each of these antibodies can bind to CD137 and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the VH CDR1, 2 and 3 sequences and VL CDR1, 2 and 3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and match, although each antibody must contain a VH CDR1, 2 and 3 and a VL CDR1, 2 and 3 to create other CD137-binding binding molecules of the invention. Such "mixed and matched" CD137-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (e.g., ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by mutating one or more VH and/or VL CDR region sequences

with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies of the present invention.

In yet another embodiment, the present invention provides an antibody comprising amino acid sequences that are homologous to the sequences described in Table 1, and said antibody binds to CD137, and retains the desired functional properties of those antibodies described in Table 1.

For example, the invention provides an isolated monoclonal antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence that is at least 80 percent, at least 90 percent, or at least 95 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13; the light chain variable region comprises an amino acid sequence that is at least 80 percent, at least 90 percent, or at least 95 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25; wherein the antibody specifically binds to human CD137 protein.

In one embodiment, the VH and/or VL amino acid sequences may be 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, 95 percent, 96 percent, 97 percent, 98 percent or 99 percent identical to the sequences set forth in Table 1. In one embodiment, the VH and/or VL amino acid sequences may be identical except an amino acid substitution in no more than 1, 2, 3, 4 or 5 amino acid positions.

In one embodiment, an antibody of the invention has a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein one or more of these CDR sequences have specified amino acid sequences based on the antibodies described herein or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the CD137-binding antibodies of the invention.

The term “conservatively modified variant” or “conservative variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to

any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations", which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

For polypeptide sequences, "conservatively modified variants" or "conservative variants" include individual substitutions, deletions or additions to a polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). In one embodiment, the term "conservative sequence modifications" are used to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence.

Accordingly, the invention provides an isolated monoclonal antibody consisting of a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein: the heavy chain variable region CDR1 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 1, 4, 7, 10, preferably SEQ ID NO: 1, or conservative variants thereof; the heavy chain variable region CDR2 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 2, 5, 8, 11, preferably SEQ ID NO: 2, or conservative variants thereof; the heavy chain variable region CDR3 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 3, 6, 9, 12, preferably SEQ ID NO: 3, or conservative variants thereof; the light chain variable region CDR1 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 16, 19, 22, preferably SEQ ID NO: 16, or conservative variants thereof; the light

chain variable region CDR2 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 17, 20, 23, preferably SEQ ID NO: 17, or conservative variants thereof; and the light chain variable region CDR3 comprises, preferably consists of, an amino acid sequence selected from any of SEQ ID NOs: 18, 21, 24, preferably SEQ ID NO: 18, or conservative variants thereof; wherein the antibody specifically binds to CD137 and is capable of activating CD137 signaling with or without additional cross-linking.

In one embodiment, an antibody of the invention is optimized for expression in a mammalian cell and has a heavy chain variable region and a light chain variable region, wherein one or more of these sequences have specified amino acid sequences based on the antibodies described herein or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the CD137-binding antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody optimized for expression in a mammalian cell comprising a heavy chain variable region and a light chain variable region wherein: the heavy chain variable region comprises an amino acid sequence selected from any of SEQ ID NOs: 13, 14, and 15, preferably SEQ ID NO: 13, and conservative modifications thereof; and the light chain variable region comprises an amino acid sequence selected from any of SEQ ID NOs: 25, 26, and 27, preferably SEQ ID NO: 25, and conservative modifications thereof; wherein the antibody specifically binds to CD137 and is capable of activating CD137 signaling with or without additional cross-linking.

In one embodiment, an antibody of the invention is optimized for expression in a mammalian cell has a full length heavy chain sequence and a full length light chain sequence, wherein one or more of these sequences have specified amino acid sequences based on the antibodies described herein or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the CD137-binding antibodies of the invention.

As used herein, the term, “optimized” means that a nucleotide sequence has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a cell of Pichia, a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the “parental” sequence. The optimized sequences herein have been engineered to have codons that are preferred in mammalian cells. However, optimized expression of these sequences in other eukaryotic cells or prokaryotic cells is also envisioned herein. The amino acid sequences encoded by optimized nucleotide sequences are also referred to as optimized.

Another type of variable region modification is to mutate amino acid residues within the VH and/or VL CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (e.g., affinity) of the antibody of interest, known as "affinity maturation." 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 as described herein and provided in the Examples. Conservative modifications (as discussed above) can be introduced. The mutations may be amino acid substitutions, additions or deletions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

An “affinity-matured” antibody is one with one or more alterations in one or more variable domains thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al, Bio/Technology 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of hypervariable region (HVR) and/or framework residues is described by, for example: Barbas et al. Proc. Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Jackson et al, J. Immunol. 154(7):3310- 9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

An antibody of the invention further can be prepared using an antibody having one or more of the VH and/or VL sequences shown herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (i.e., VH and/or VL), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression

vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. et al., 1998 Nature 332:323-327; Jones, P. et al., 1986 Nature 321:522- 525; Queen, C. et al., 1989 Proc. Natl. Acad. Sci. U.S.A. 86: 10029-10033; U.S. Pat. No. 5,225,539 to Winter, and U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al.).

Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences or rearranged antibody sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., et al., 1991 Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., et al., 1992 J. Mol. Biol. 227:776-798; and Cox, J. P. L. et al., 1994 Eur. J Immunol. 24:827-836; the contents of each of which are expressly incorporated herein by reference. For example, germline DNA sequences for human heavy and light chain variable region genes and rearranged antibody sequences can be found in "IMGT" database (available on the Internet at www.imgt.org; see Lefranc, M.P. et al., 1999 Nucleic Acids Res. 27:209-212; the contents of each of which are expressly incorporated herein by reference).

An example of framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, e.g., consensus sequences and/or framework sequences used by monoclonal antibodies of the invention. The VH CDR1, 2 and 3 sequences, and the VL CDR1, 2 and 3 sequences, 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 CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see e.g., U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al).

A wide variety of antibody /immunoglobulin frameworks or scaffolds can be employed so long as the resulting polypeptide includes at least one binding region which specifically binds to CD137. Such frameworks or scaffolds include the five main idiotypes of human

immunoglobulins, antigen-binding fragments thereof, and include immunoglobulins of other animal species, preferably having humanized aspects.

In one aspect, the invention pertains to a method of generating non-immunoglobulin based antibodies using non-immunoglobulin scaffolds onto which CDRs of the invention can be grafted. Known or future non-immunoglobulin frameworks and scaffolds may be employed, as long as they comprise a binding region specific for the target CD137 protein. Known non-immunoglobulin frameworks or scaffolds include, but are not limited to, fibronectin (Compound Therapeutics, Inc., Waltham, Mass.), ankyrin (Molecular Partners AG, Zurich, Switzerland), lipocalin (Pieris Proteolab AG, Freising, Germany), small modular immuno-pharmaceuticals (Trubion Pharmaceuticals Inc., Seattle, Wash.), maxybodies (Avidia, Inc., Mountain View, Calif), Protein A (Affibody AG, Sweden), and affilin (gamma-crystallin or ubiquitin) (Scil Proteins GmbH, Halle, Germany).

Suitably, the antibody of the invention specifically binds to CD137 and is characterized by one or more of the following parameters:

(i) binds to human CD137 with a dissociation constant (KD) of less than 50 nM, particularly less than10 nM, more particularly less than 5 nM as measured by surface plasmon resonance;

(ii) binds to human CD137 with a K_{off} rate of $5x10^{-3}$ s^{-1} or less, or 10^{-3} s^{-1} or less or $5x10^{-4}$ s^{-1} or less, or 10^{-4} s^{-1} or less as measured by SPR;

(iii) binds to human CD137 with a K_{on} rate of at least 10^4 $M^{-1}s^{-1}$ or greater, at least 10^5 $M^{-1}s^{-1}$ or greater, at least $5x10^5$ $M^{-1}s^{-1}$ or greater, at least 10^6 $M^{-1}s^{-1}$ or greater, as measured by SPR;

(iv) optionally, cross-competes with urelumab; and

(v) optionally, is cross-reactive with Macaca fascicularis (Cynomolgus) CD137;

(vi) optionally, does not inhibit the interaction between CD137 and CD137L, in particular as measured by a competition ELISA.

As used herein, the term "affinity" refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

"Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., of an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity", "bind to", "binds to" or "binding to" refers to intrinsic binding affinity that reflects a 1:1

interaction between members of a binding pair (e.g., an antibody fragment and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity, i.e. binding strength are described in the following.

The term "K_{assoc}", "Ka" or "K_{on}", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}", "Kd" or "K_{off}", as used herein, is intended to refer to the dissociation rate of a particular antibody- antigen interaction. In one embodiment, the term "KD", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e. Kd/Ka) and is expressed as a molar concentration (M). The "KD" or "KD value" or "K_D" or "K_D value" according to this invention is in one embodiment measured by using surface-plasmon resonance assays using a MASS-1 SPR instrument (Sierra Sensors). To measure affinity, an antibody specific for the Fc region of rabbit IgGs (Bethyl Laboratories, Cat. No. A120-111A) is immobilized on a sensor chip (SPR-2 Affinity Sensor, High Capacity Amine, Sierra Sensors) using a standard amine-coupling procedure. Rabbit monoclonal antibodies in B-cell supernatants are captured by the immobilized anti-rabbit IgG antibody. A minimal IgG concentration in the B-cell supernatants is required to allow sufficient capture. After capturing of the monoclonal antibodies, human CD137 ECD (Peprotech, cat. 310-15-1MG) is injected into the flow cells for 3 min at a concentration of 90 nM, and dissociation of the protein from the IgG captured on the sensor chip is allowed to proceed for 5 min. After each injection cycle, surfaces are regenerated with two injections of 10 mM Glycine-HCl. The apparent dissociation (kd) and association (ka) rate constants and the apparent dissociation equilibrium constant (KD) are calculated with the MASS-1 analysis software (Analyzer, Sierra Sensors) using one-to-one Langmuir binding model and quality of the fits being monitored based on relative Chi^2 (Chi^2 normalized to the extrapolated maximal binding level of the analyte), which is a measure for the quality of the curve fitting. The smaller the value for the Chi^2 the more accurate is the fitting to the one-to-one Langmuir binding model. Results are deemed valid if the response units (RU) for ligand binding are at least 2% of the RUs for antibody capturing. Samples with RUs for ligand binding with less than 2% of the

RUs for antibody capturing are considered to show no specific binding of CD137 to the captured antibody. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al, J. Mol. Biol. 293:865-881 (1999).

Suitably, the affinity of the antibody of the invention to CD137 may be comparable to or higher than the affinity of CD137L to CD137. Suitably, the affinity of the antibody of the invention to CD137 may be comparable to or higher than the affinity of urelumab to CD137. It will be appreciated that the higher affinity of the CD137 binding domain may be particularly suitable for use in an antibody, wherein said antibody is monovalent for CD137. The binding affinity of an antibody may be determined, for example, by the dissociation constant (KD). A stronger affinity is represented by a lower KD, while a weaker affinity is represented by a higher KD.

Thus, in a suitable embodiment, the antibody of the invention may have a KD of between 5 to 50,000 pM, 5 to 40,000 pM, 5 to 30,000 pM, 5 to 20,000 pM, 5 to 10,000 pM, 5 to 9,000 pM, 5 to 8,000 pM, 5 to 7,000 pM, 5 to 6,000 pM, 5 to 5,000 pM, in particular as measured by SPR. In a further embodiment, the antibody of the invention binds to human CD137 with a KD of between 10 nM and 10 pM, preferably between 10 nM and 0.1 nM, more preferably between 5 nM and 1 nM, in particular as measured by SPR.

In a suitable embodiment, the antibody of the invention may have a KD of less than approximately 50 nM, less than approximately 45 nM, less than approximately 40 nM, less than approximately 35 nM, less than approximately 30 nM, less than approximately 25 nM, less than 20 nM, less than approximately 15 nM, less than approximately 10 nM, less than approximately 9 nM, less than approximately 8 nM, less than approximately 7 nM, less than approximately 6 nM, less than approximately 5 nM, in particular as measured by SPR. Suitably, the antibody of the invention has a KD of less than 10 nM, in particular as measured by SPR. Preferably, the antibody of the invention binds to human CD137 with a KD of less than 5 nM, in particular as measured by SPR.

Suitably, the antibody of the invention binds to human CD137 with a K_{on} rate of at least 10^3 $M^{-1}s^{-1}$ or greater, at least 10^4 $M^{-1}s^{-1}$ or greater, at least $5x10^4$ $M^{-1}s^{-1}$ or greater, at least 10^5 $M^{-1}s^{-1}$ or greater, at least $5x10^5$ $M^{-1}s^{-1}$ or greater, at least 10^6 $M^{-1}s^{-1}$ or greater as measured by surface plasmon resonance (SPR). Suitably, the antibody of the invention has a K_{on} rate of at least 10^5 $M^{-1}s^{-1}$ or greater, in particular at least $5x10^5$ $M^{-1}s^{-1}$ or greater, as measured by SPR.

Suitably, the antibody of the invention binds to human CD137 with a K_{off} rate of 10^{-3} s^{-1} or less, $3x10^{-3}$ s^{-1} or less, $5x10^{-3}$ s^{-1} or less, 10^{-4} s^{-1} or less, $5x10^{-4}$ s^{-1} or less as

measured by surface plasmon resonance (SPR). Suitably, the antibody of the invention has a K_{off} rate of $5x10^{-3}$ s^{-1} or less as measured by SPR.

Suitably, the antibody of the invention has beneficial biophysical properties.

Suitably, the antibodies of the invention, when in scFv format, has a melting temperature (Tm), as determined by differential scanning fluorimetry, of at least 50°C, e.g., at least 55°C, preferably at least 60°C, more preferably at least 65°C in particular wherein said antibody is formulated in 50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl. DSF is described earlier (Egan, et al., MAbs, 9(1) (2017), 68-84; Niesen, et al., Nature Protocols, 2(9) (2007) 2212-2221). The midpoint of transition for the thermal unfolding of the scFv constructs is determined by Differential Scanning Fluorimetry using the fluorescence dye SYPRO® Orange (see Wong & Raleigh, Protein Science 25 (2016) 1834-1840). Samples in phosphate-citrate buffer at pH 6.4 are prepared at a final protein concentration of 50 µg/mL and containing a final concentration of 5x SYPRO® Orange in a total volume of 100 µl. Twenty-five microliters of prepared samples are added in triplicate to white-walled AB gene PCR plates. The assay is performed in a qPCR machine used as a thermal cycler, and the fluorescence emission is detected using the software's custom dye calibration routine. The PCR plate containing the test samples is subjected to a temperature ramp from 25°C to 96°C in increments of 1°C with 30 s pauses after each temperature increment. The total assay time is about two hours. The Tm is calculated by the software GraphPad Prism using a mathematical second derivative method to calculate the inflection point of the curve. The reported Tm is an average of three measurements.

The antibody of the invention, in particular when expressed in the scFv (single chain variable fragment) antibody format, is characterized by a loss in monomer content, after storage for at least two weeks, particularly for at least four weeks, at 4°C, of less than 7%, e.g., less than 6%, less than 5%, less than 4%, less than 3%, preferably less than 2%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein the antibody of the invention is formulated 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4. The loss in monomer content is as determined by area under the curve calculation of SE-HPLC chromatograms. SE-HPLC is a separation technique based on a solid stationary phase and a liquid mobile phase as outlined by the USP chapter 621. This method separates molecules based on their size and shape utilizing a hydrophobic stationary phase and aqueous mobile phase. The separation of molecules is occurring between the void volume (V0) and the total permeation volume (VT) of a specific column. Measurements by SE-HPLC are performed on a Chromaster HPLC system (Hitachi High-Technologies

Corporation) equipped with automated sample injection and a UV detector set to the detection wavelength of 280 nm. The equipment is controlled by the software EZChrom Elite (Agilent Technologies, Version 3.3.2 SP2) which also supports analysis of resulting chromatograms. Protein samples are cleared by centrifugation and kept at a temperature of 4-6°C in the autosampler prior to injection. For the analysis of scFv samples the column Shodex KW403-4F (Showa Denko Inc., #F6989202) is employed with a standardized buffered saline mobile phase (50 mM sodium-phosphate pH 6.5, 300 mM sodium chloride) at the recommended flow rate of 0.35 mL/min. The target sample load per injection is 5 µg. Samples are detected by an UV detector at a wavelength of 280 nm and the data recorded by a suitable software suite. The resulting chromatograms are analyzed in the range of V0 to VT thereby excluding matrix associated peaks with >10 min elution time.

Furthermore, the antibody of the invention, in particular when expressed in the scFv (single chain variable fragment) antibody format, is characterized by a loss in monomer content, after five consecutive freeze-thaw cycles, of less than 5%, preferably less than 3%, more preferably less than 1%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein the antibody of the invention is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

The term “epitope” refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide, or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides.

In one embodiment, an antibody of the invention cross-competes for binding to CD137 with urelumab. Urelumab, also referred to as BMS-663513, is a fully humanized IgG4 mAb from Bristol-Myers Squibb, and is described in WO 2004/010947, US 6,887,673 and US 7,214,493, which are hereby incorporated into the present application by reference in their entirety. Suitably, the antibody of the invention may, according to non-limiting theory, bind to the same or an overlapping (e.g., a structurally similar or spatially proximal) epitope on CD137 as urelumab.

In one embodiment, an antibody of the invention does not cross-compete for binding with utomilumab. The present invention provides an antibody that binds to a different epitope than utomilumab. Utomilumab, also referred to as PF-05082566, is a fully human IgG2 mAb from Pfizer, and is described in WO 2012/032433 and US 8,821,867, which are hereby incorporated into the present application by reference in their entirety. Suitably, the antibody of the invention may, according to a non-limiting theory, binds to a different (e.g., a

structurally different or a spatially remote) epitope on CD137 than utomilumab. The term "recognize" as used herein refers to an antibody that finds and interacts (e.g., binds) with its conformational epitope.

The antibody of the present invention binds human CD137 extracellular domain at an epitope located in the distal part of the extracellular domain of CD137, particularly within the cysteine rich domains 1 to 2 (CRD1 to 2), in particular located within the amino acid residues 24-86 of SEQ ID NO: 32, provided that amino acid residue Asn42 of CD137 is not a critical residue for binding.

Thus, in a further aspect, the present disclosure also provides antibodies that bind to the same epitope as do any of the illustrative antibodies of the disclosure, in particular any of the illustrative antibodies listed in Table 1. The present disclosure provides an isolated antibody wherein said antibody binds human CD137 extracellular domain at an epitope comprising, in particular located within, the distal part of the extracellular domain of CD137, particularly within the cysteine-rich domains , provided that amino acid residue Asn42 of CD137 is not a critical residue for binding CRD1 and/or CRD2, more particularly within amino acid residues 24-86 of SEQ ID NO: 32, provided that amino acid residue Asn42 of CD137 is not a critical residue for binding.

In particular embodiments, said antibody binds human CD137 extracellular domain at an epitope characterized by a set of critical residues, determined in accordance with Example 13, comprising the residues Arg 41, Gln43, Cys45, Pro49, Ser52, and Ser80.

In the context of the present invention, the term "critical residue" relates to a residue of an antigen, that is part of the epitope an antibody-based molecule is binding to, and that is critical for the interaction between the antibody-based molecule and its epitope. In particular, a critical residue is characterized by fulfilling one of more of the following criteria: (i) being buried to more than 50 % upon binding of the antibody-based molecule to the epitope, (ii) having a specific side chain hydrogen bond interaction, (iii) being involved in the hydrogen bond network, and/or (iv) exhibiting a critical interaction with important binding residue of antibody-based molecule. In particular embodiments, these criteria are determined in accordance with the methods disclosed in Example 13.

In particular embodiments, said antibody binds human CD137 extracellular domain at an epitope characterized by a set of critical residues, determined in accordance with Example 13, further comprising the residues Ala33, Asn40, and Cys48.

In particular embodiments, said antibody binds human CD137 extracellular domain at an epitope characterized by a set of critical residues, determined in accordance with Example 13, further comprising the residues Pro32, Gly34, Thr35, Ser46, Pro47, Pro50, Cys78, and Ser79.

In some embodiments, the isolated antibody comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and/or LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, preferably wherein the antibody comprises HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively. Suitably, the antibodies have one or more biological properties described hereinabove.

Additional antibodies can therefore be identified based on their ability to cross-compete (e.g., to competitively inhibit the binding of, in a statistically significant manner) with other antibodies of the invention in CD137 binding assays. In a particular embodiment, the disclosure provides isolated antibodies that compete or cross-compete for binding to the same epitope on the human CD137 with any of the illustrative antibodies of the disclosure, in particular with any of the illustrative antibodies listed in Table 1.

The terms “compete” or “cross-compete” and related terms are used interchangeably herein to mean the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to CD137 in a standard competitive binding assay.

The ability or extent to which an antibody or other binding agent is able to interfere with the binding of another antibody or binding molecule to CD137, and therefore whether it can be said to cross-compete according to the invention, can be determined using standard competition binding assays. One particularly suitable quantitative cross-competition assay uses a FACS- or an AlphaScreen-based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) an antibody or fragment thereof and the other an antibody or fragment thereof in terms of their binding to the target. In general, a cross-competing antibody or fragment thereof is for example one which will bind to the target in the cross-competition assay such that, during the assay and in the presence of a second antibody or fragment thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is up to 100% (e.g. in FACS based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (e.g. unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the to be tested potentially cross-blocking antibody or fragment thereof that is present in a given

amount. Preferably, cross-competing antibodies or fragments thereof have a recorded displacement that is between 10% and 100%, more preferred between 50% and 100%.

Suitably, the isolated antibody of the present invention is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, an IgG antibody, a Fab, an Fv, a scFv, dsFv, a scAb, STAB, and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody Technology™).

Suitably, the isolated antibody of the invention is an Fv fragment. Suitably, the isolated antibody of the invention is an scFv fragment. "Single-chain Fv" or "scFv" or "sFv" antibody comprise the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptides further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding (see, for example, Plückthun, The pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315). In particular embodiments, said functional fragment is in an scFv format comprising a polypeptide linker between the VH and VL domains, wherein said linker comprises one or more units of four (4) glycine amino acid residues and one (1) serine amino acid residue $(GGGGS)_n$, wherein n=1, 2, 3, 4, 5, 6, 8 or 8, preferably n=4. In particular embodiments, said functional fragment is in an scFv format comprising the linker according to SEQ ID NO: 28. In one embodiment, the isolated antibody of the invention that specifically binds to human CD137 comprises an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to the amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, preferably SEQ ID NO: 29. In a further embodiment, the isolated antibody of the invention is a single-chain variable fragment (scFv) as shown in SEQ ID NO: 29, SEQ ID NO: 30, or SEQ ID NO: 31, preferably SEQ ID NO: 29. In one embodiment, the isolated antibody of the invention is a single-chain variable fragment (scFv) as shown in SEQ ID NO: 30. In one embodiment, the isolated antibody of the invention is a single-chain variable fragment (scFv) as shown in SEQ ID NO: 31. In a preferred embodiment, the isolated antibody of the invention is a single-chain variable fragment (scFv) as shown in SEQ ID NO: 35.

Suitably, the isolated antibody of the invention is an IgG antibody isotype. The term "isotype" refers to the antibody class (e.g., IgM, IgE, IgG such as IgG1 or IgG4) that is

provided by the heavy chain constant region genes. Isotype also includes modified versions of one of these classes, where modifications have been made to alter the Fc function, for example, to enhance or reduce effector functions or binding to Fc receptors. In one embodiment, the isolated antibody of the invention is an IgG selected from the group consisting of an IgGl, an IgG2, an IgG3 and an IgG4, preferably IgG4. Suitably, the isolated antibody of the invention is IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, a VH sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 13, and a VL sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 25. In a more specific embodiment, the antibody of the invention is an IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, a heavy chain sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 14, and a light chain sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 26. Suitably, the isolated antibody of the invention is an IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18, respectively, a VH sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 15, and a VL sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 27.

In another particular embodiment, the isolated antibody of the invention is a multispecific molecule, in particular a multispecific molecule having at least a second functional molecule, e.g., bispecific molecule, trispecific molecule, tetraspecific, pentaspecific, or hexaspecific molecule.

The term “multispecific molecule” or “multispecific antibody” as used herein, refers to an antibody that binds to two or more different epitopes on at least two or more different targets (e.g., CD137 and another target different from CD137), or binds to two or more different epitopes of the same target. The term “multispecific molecule” includes bispecific,

trispecific, tetraspecific, pentaspecific and hexaspecific antibodies. The term "bispecific antibody" as used herein, refers to an antibody that binds to two different epitopes on two different targets or on the same target. The term "trispecific antibody" as used herein, refers to an antibody that binds to three different epitopes on three different targets or on the same target.

An antibody of the invention, or antigen-binding regions thereof, can be derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g., another antibody or ligand for a receptor) to generate a multispecific molecule that binds to at least two binding sites and/or different target molecules. The antibody of the invention may in fact be derivatized or linked to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules. To create a multispecific molecule of the invention, an antibody of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a multispecific molecule results.

Accordingly, the present invention includes multispecific molecules comprising at least one first binding specificity for CD137 and a second binding specificity for a second target epitope. For example, the second target epitope is present on another target molecule different from CD137.

Bivalent CD137 antibodies were shown to be generally weak in their ability to induce the signaling in the absence of an exogenous clustering. To illustrate, anti-CD137 antibody utomilumab is only capable to activate CD137 signaling when either cross-linked to anti-human F(ab')2 secondary antibody or immobilized to tissue culture plastic (Fisher at al., Cancer Immunol Immunother 61:1721-1733 (2012)). Studies in rodent agonistic antibodies to CD40 (TNFRSF5), another member of TNFRSF, have suggested that the exogenous clustering can be partially achieved through the interaction with Fcγ-receptor (Li F, Ravetch JV, Science 333(6045):1030–10 (2011); White AL, et al., J Immunol 187(4):1754–1763 (2011)). The interaction with Fcγ-receptor can however deplete the CD137-expressing cells through effector mechanisms. The current bivalent antibodies targeting CD137 are thus either ineffective agonists or lead to the depletion of CD137-positive cells. Suitably, second binding specificity of the multispecific molecule is capable of providing additional cross-linking of the CD137-binding antibody of the present invention. Accordingly, the present invention includes multispecific molecules comprising at least one first binding specificity for CD137 and a second binding specificity for a second target epitope. For example, the second target

epitope is another epitope of CD137 different from the first target epitope. The multispecific molecule can further include a third binding specificity, in addition to the first and second target epitope.

In a further embodiment, the present invention includes multispecific molecules monovalent, bivalent or multivalent for CD137 specificity, preferably monovalent.

In another particular embodiment of the present invention, the isolated antibody of the present invention is a molecule that is monovalent or multivalent for CD137 specificity, e.g., bivalent, trivalent, tetravalent, pentavalent, or hexavalent.

The term "monovalent molecule" or "monovalent antibody", as used herein, refers to an antibody that binds to a single epitope on a target molecule, such as CD137.

The term "multivalent molecule" or "multivalent antibody" refers to a single binding molecule with more than one valency, where "valency" is described as the number of antigen-binding moieties that binds to epitopes on identical target molecules. As such, the single binding molecule can bind to more than one target molecule, or more than one binding site on a target molecule that contains multiple copies of the epitope. Examples of multivalent antibodies include, but are not limited to bivalent antibodies, trivalent antibodies, tetravalent antibodies, pentavalent antibodies, and the like. The term "bivalent antibody" as used herein, refers to an antibody that has two antigen binding moieties, each of which binds to an identical epitope.

Suitably, the isolated antibody of the present invention is a multispecific molecule, e.g., bispecific molecule, and / or a multivalent molecule, e.g., monovalent for CD137 specificity molecule, bivalent for CD137 specificity molecule, which is an antibody format selected from any suitable multispecific, e.g. bispecific, format known in the art, including, by way of non-limiting example, formats based on a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a bispecific T-cell engager (BiTE; tandem di-scFv), a tandem tri-scFv, a tribody (Fab-(scFv)2) or bibody (Fab-(scFv)1), Fab, Fab-Fv2, Morrison (IgG CH_3-scFv fusion (Morrison L) or IgG CL-scFv fusion (Morrison H)), triabody, scDb-scFv, bispecific Fab2, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, scFv-HSA-scFv fusion, di-diabody, DVD-Ig, COVD, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusions, such as bsAb (scFv linked to C-terminus of light chain), Bs1Ab (scFv linked to N-terminus of light chain), Bs2Ab (scFv linked to N-terminus of heavy chain), Bs3Ab (scFv linked to C-terminus of heavy chain), Ts1Ab (scFv linked to N-terminus of both heavy chain and light chain), Ts2Ab (dsscFv linked to C-terminus of heavy chain), Bispecific antibodies based on heterodimeric Fc domains, such as Knob-into-

Hole antibodies (KiHs) (bispecific IgGs prepared by the KiH technology); an Fv, scFv, scDb, tandem-di-scFv, tandem tri-scFv, Fab-(scFv)2, Fab-(scFv)1, Fab, Fab-Fv2, COVD fused to the N- and/or the C-terminus of either chain of a heterodimeric Fc domain or any other heterodimerization domain, a MATCH (described in WO 2016/0202457; Egan T., et al., mAbs 9 (2017) 68-84) and DuoBodies (bispecific IgGs prepared by the Duobody technology) (MAbs. 2017 Feb/Mar;9(2):182-212. doi: 10.1080/19420862.2016.1268307).

The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a VH connected to VL in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain to create two antigen-binding sites. In particular embodiments, said polypeptide linker comprises units of four (4) glycine amino acid residues and one (1) serine amino acid residue $(GGGGS)_n$, wherein n=1 or 2, preferably 1. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404097, WO 93/01161, Hudson et al., Nat. Med. 9:129-134 (2003), and Holliger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

The bispecific scDb, in particular the bispecific monomeric scDb, particularly comprises two variable heavy chain domains (VH) or fragments thereof and two variable light chain domains (VL) or fragments thereof connected by linkers L1, L2 and L3 in the order VHA-L1-VLB-L2-VHB-L3-VLA, VHA-L1-VHB-L2-VLB-L3-VLA, VLA-L1-VLB-L2-VHB-L3-VHA, VLA-L1-VHB-L2-VLB-L3-VHA, VHB-L1-VLA-L2-VHA-L3-VLB, VHB-L1-VHA-L2-VLA-L3-VLB, VLB-L1-VLA-L2-VHA-L3-VHB or VLB-L1-VHA-L2-VLA-L3-VHB, wherein the VLA and VHA domains jointly form the antigen binding site for the first antigen, and VLB and VHB jointly form the antigen binding site for the second antigen.

The linker L1 particularly is a peptide of 2-10 amino acids, more particularly 3-7 amino acids, and most particularly 5 amino acids, and linker L3 particularly is a peptide of 1-10 amino acids, more particularly 2-7 amino acids, and most particularly 5 amino acids. In particular embodiments, the linker L1 and/or L3 comprises units of four (4) glycine amino acid residues and one (1) serine amino acid residue $(GGGGS)_n$, wherein n=1 or 2, preferably n=1.

The middle linker L2 particularly is a peptide of 10-40 amino acids, more particularly 15-30 amino acids, and most particularly 20-25 amino acids. In particular embodiments, said

linker L2 comprises one or more units of four (4) glycine amino acid residues and one (1) serine amino acid residue $(GGGGS)_n$, wherein n=1, 2, 3, 4, 5, 6, 7 or 8, preferably n=4.

In one embodiment of the present invention, the isolated antibody is a multispecific and/or multivalent antibody in a scDb-scFv format. The term "scDb-scFv" refers to an antibody format, wherein a single-chain Fv (scFv) fragment is fused by a flexible Gly-Ser linker to a single-chain diabody (scDb). In one embodiment, said flexible Gly-Ser linker is a peptide of 2-40 amino acids, e.g., 2-35, 2-30, 2-25, 2-20, 2-15, 2-10 amino acids, particularly 10 amino acids. In particular embodiments, said linker comprises one or more units of four (4) glycine amino acid residues and one (1) serine amino acid residue $(GGGGS)_n$, wherein n=1, 2, 3, 4, 5, 6, 7 or 8, preferably n=2.

In one embodiment of the present invention, the isolated antibody is a multispecific and/or multivalent antibody in a MATCH format described in WO 2016/0202457; Egan T., et al., mAbs 9 (2017) 68-84.

Multispecific and/or multivalent molecules of the present invention can be produced using any convenient antibody manufacturing method known in the art (see, e.g., Fischer, N. & Leger, O., Pathobiology 74 (2007) 3-14 with regard to the production of bispecific constructs; Hornig, N. & Färber-Schwarz, A., Methods Mol. Biol. 907 (2012)713-727, and WO 99/57150 with regard to bispecific diabodies and tandem scFvs). Specific examples of suitable methods for the preparation of the bispecific construct of the present invention further include, inter alia, the Genmab (see Labrijn et al., Proc. Natl. Acad. Sci. USA 110 (2013) 5145-5150) and Merus (see de Kruif et al., Biotechnol. Bioeng. 106 (2010) 741-750) technologies. Methods for production of bispecific antibodies comprising a functional antibody Fc part are also known in the art (see, e.g., Zhu et al., Cancer Lett. 86 (1994) 127-134); and Suresh et al., Methods Enzymol. 121 (1986) 210-228).

Other antibodies which can be employed in the multispecific and in the multivalent molecules of the invention are murine, chimeric and humanized monoclonal antibodies.

The multispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-5-acetyl-thioacetate (SATA), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3- (2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4- (N-

maleimidomethyl)cyclohexane-l-carboxylate (sulfo-SMCC) (see e.g., Karpovsky et al., 1984 J. Exp. Med. 160: 1686; Liu, M A et al., 1985 Proc. Natl. Acad. Sci. USA 82:8648). Other methods include those described in Paulus, 1985 Behring Ins. Mitt. No. 78, 118-132; Brennan et al., 1985 Science 229:81-83), and Glennie et al., 1987 J. Immunol. 139: 2367-2375). Conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, 111).

When the binding specificities are antibodies, they can be conjugated by sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, for example one, prior to conjugation.

Alternatively, two or more binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb X mAb, mAb X Fab, Fab X F (ab')2 or ligand X Fab fusion protein. A multispecific molecule of the invention can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain multispecific molecule comprising two binding determinants. Multispecific molecules may comprise at least two single chain molecules. Methods for preparing multispecific molecules are described for example in U.S. Pat. No. 5,260,203; U.S. Pat. No. 5,455,030; U.S. Pat. No. 4,881,175; U.S. Pat. No. 5,132,405; U.S. Pat. No. 5,091,513; U.S. Pat. No. 5,476,786; U.S. Pat. No. 5,013,653; U.S. Pat. No. 5,258,498; and U.S. Pat. No. 5,482,858.

Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (REA), FACS analysis, bioassay (e.g., growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody) specific for the complex of interest.

In a further aspect, the invention provides a nucleic acid encoding the antibody of the invention. The present invention also provides nucleic acid sequences that encode CDRs, VH, VL, the full length heavy chain, and the full length light chain of the antibodies that specifically bind to CD137 protein. Such nucleic acid sequences can be optimized for expression in mammalian cells.

The term “nucleic acid” is used herein interchangeably with the term “polynucleotide(s)” and refers to one or more deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which

are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081, 1991; Ohtsuka et al., J. Biol. Chem. 260:2605-2608, 1985; and Rossolini et al., Mol. Cell. Probes 8:91-98, 1994).

The invention provides substantially purified nucleic acid molecules which encode polypeptides comprising segments or domains of the CD137-binding antibody chains described above. When expressed from appropriate expression vectors, polypeptides encoded by these nucleic acid molecules are capable of exhibiting CD137 antigen binding capacity.

Also provided in the invention are polynucleotides which encode at least one CDR region and usually all three CDR regions from the heavy or light chain of the CD137-binding antibody set forth in Table 1. Some other polynucleotides encode all or substantially all of the variable region sequence of the heavy chain and/or the light chain of the CD137-binding antibody set forth in Table 1. Because of the degeneracy of the code, a variety of nucleic acid sequences will encode each of the immunoglobulin amino acid sequences.

The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (e.g., sequences as described in the Examples below) encoding a CD137-binding antibody. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., 1979, Meth. Enzymol. 68:90; the phosphodiester method of Brown et al., Meth. Enzymol. 68: 109, 1979; the diethylphosphoramidite method of Beaucage et al., Tetra. Lett., 22: 1859, 1981; and the solid support method of U.S. Pat. No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, e.g., PCR Technology: Principles and Applications for DNA Amplification, H. A. Erlich (Ed.), Freeman Press, NY, N.Y., 1992; PCR Protocols: A Guide to Methods and Applications, Innis et al. (Ed.), Academic Press, San Diego, Calif, 1990; Mattila et al., Nucleic Acids Res. 19:967, 1991; and Eckert et al., PCR Methods and Applications 1:17, 1991.

Also provided in the invention are expression vectors and host cells for producing the CD137-binding antibodies described above.

The term "vector" is intended to refer to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian 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.

Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a functional relationship between two or more polynucleotide (e.g., DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

Various expression vectors can be employed to express the polynucleotides encoding the CD137-binding antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, e.g., Harrington et

al., Nat Genet. 15:345, 1997). For example, nonviral vectors useful for expression of the CD137-binding polynucleotides and polypeptides in mammalian (e.g., human) cells include pThioHis A, B and C, pcDNA3.1/His, pEBVHis A, B and C, (Invitrogen, San Diego, Calif.), MPS V vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent et al., supra; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld et al., Cell 68: 143, 1992.

The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (e.g., enhancers) that are operably linked to the polynucleotides encoding a CD137-binding antibody. In one embodiment, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, e.g., arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under non-inducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of a CD137-binding antibody. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., Results Probl. Cell Differ. 20: 125, 1994; and Bittner et al., Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted CD137-binding antibody sequences. More often, the inserted CD137-binding antibody sequences are linked to signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding CD137-binding antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies and antigen-binding fragments thereof. Typically, such constant regions are human.

The term “recombinant host cell” (or simply “host cell”) refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to 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, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The host cells for harboring and expressing the CD137-binding antibody chains can be either prokaryotic or eukaryotic. E. coli is one prokaryotic host useful for cloning and expressing the polynucleotides of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilis, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express CD137-binding polypeptides of the invention. Insect cells in combination with baculovirus vectors can also be used.

In one embodiment, mammalian host cells are used to express and produce the CD137-binding polypeptides of the present invention. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes or a mammalian cell line harboring an exogenous expression vector. These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed including the CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, e.g., Winnacker, FROM GENES TO CLONES, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, e.g., Queen, et al., Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the

dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPS V promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See generally Sambrook, et al., Molecular Cloning: A Laboratory Manual. 4th edition, Cold Spring Harbor 2012). Other methods include, e.g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation-nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express CD137-binding antibody chains or binding fragments can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type. The present invention thus provides a method of producing the antibody of the invention, wherein said method comprises the step of culturing a host cell comprising a nucleic acid or a vector encoding the antibody of the invention, whereby said antibody of the invention or a fragment thereof is expressed.

In a further aspect, the present invention relates to a pharmaceutical composition comprising the antibody of the present invention, and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers enhance or stabilize the composition, or facilitate preparation of the composition. Pharmaceutically acceptable carriers include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

A pharmaceutical composition of the present invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon

the desired results. Administration can be intravenous, intramuscular, intraperitoneal, or subcutaneous, or administered proximal to the site of the target. The pharmaceutically acceptable carrier should be suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., antibody, and multispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

Pharmaceutical compositions of the invention can be prepared in accordance with methods well known and routinely practiced in the art. See, e.g., Remington: The Science and Practice of Pharmacy, Mack Publishing Co., 20th ed., 2000; and Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically, a therapeutically effective dose or efficacious dose of the CD137-binding antibody is employed in the pharmaceutical compositions of the invention. The CD137-binding antibodies are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors.

Antibodies are usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of CD137-binding antibody in the patient. Alternatively, an antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, humanized antibodies show longer half-life than that of chimeric antibodies and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

In one aspect, the invention provides a pharmaceutical combination comprising the anti-CD137 antibody of the invention, as defined herein, with one or more additional therapeutic agents, e.g., one or more anti-cancer agents, cytotoxic or cytostatic agents, hormone treatment, vaccines, and/or other immunotherapies. Suitably, the anti-CD137 antibody of the invention can be used in combination with an inhibitor of an inhibitory (or immune checkpoint) molecule chosen from PD-1, PDL1, PDL2, CTLA-4, TIM-3, LAG-3, CEACAM (e.g., CEACAM-1, CEACAM-3, and/or CEACAM-5), VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, TGFR beta, and IDO (indoleamine-2,3 dioxygenase). Inhibition of an inhibitory molecule can be performed by inhibition at the DNA, RNA or protein level.

It has been surprisingly found that the anti-CD137 antibodies of the invention have a strong beneficial synergistic interaction and improved anti-proliferative activity when used in combination with PDL1 inhibitors. Thus, the invention provides a pharmaceutical combination comprising the anti-CD137 antibody of the invention, as defined herein, and a PDL1 inhibitor, particularly for use in the treatment or prevention of a proliferative disease. The present invention further relates to a pharmaceutical combination comprising the anti-CD137 antibody of the invention, as defined herein, and a PDL1 inhibitor, particularly for simultaneous, separate or sequential use in the treatment or prevention of a proliferative disease.

The term "combination" or "pharmaceutical combination" is defined herein to refer to either a fixed combination in one dosage unit form, a non-fixed combination or a kit of parts for the combined administration where the therapeutic agents, e.g., the anti-CD137 antibody of the invention and the PDL1 inhibitor, may be administered together, independently at the same time or separately within time intervals that allow that the combination partners show a cooperative, e.g., synergistic, effect.

The term "fixed combination" means that the therapeutic agents, e.g. the anti-CD137 antibody of the invention and the PDL1 inhibitor, are administered to a patient simultaneously in the form of a single entity or dosage form.

The term "non-fixed combination" means that the therapeutic agents, e.g. an anti-CD137 antibody of the invention and the PDL1 inhibitor, are both administered to a patient as separate entities or dosage forms either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two therapeutic agents in the body of the subject, e.g., a mammal or human, in need thereof.

The term "PDL1" refers in particular to human PDL1 with UniProt ID number Q9NZQ7.

The term "blocker" or "inhibitor" or "antagonist" refers to an agent that inhibits or reduces a biological activity of the target molecule it binds to. In some embodiments, an inhibitor substantially or completely inhibits the biological activity of the target molecule. Suitable PDL1 inhibitors target, decrease, and/or inhibit the binding ability of PDL1 to its binding partners, thereby interfering with the PDL1 function. In particular, a suitable PDL1 inhibitor blocks the interaction of PDL1 with PD-1. In some embodiments, a suitable PDL1 inhibitor blocks the interaction of PDL1 with PD-1 and B7-1. Suitably, the PDL1 inhibitor utilized in a pharmaceutical combination of the present invention is an anti-PDL1 antibody.

The term "synergistic effect" as used herein refers to action of two therapeutic agents such as, for example, (a) an anti-CD137 antibody of the invention, and (b) PDL1 inhibitor, producing an effect, for example, slowing the symptomatic progression of a proliferative disease, particularly a cancer, or symptoms thereof, which is greater than the simple addition of the effects of each therapeutic agent administered by themselves. A synergistic effect can be calculated, for example, using suitable methods such as the Sigmoid-Emax equation (Holford, N. H. G. and Scheiner, L. B., Clin. Pharmacokinet. 6: 429-453 (1981)), the equation of Loewe additivity (Loewe, S. and Muischnek, H., Arch. Exp. Pathol Pharmacol. 114: 313-326 (1926)) and the median-effect equation (Chou, T. C. and Talalay, P., Adv.

Enzyme Regul. 22: 27-55 (1984)). Each equation referred to above can be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively. Synergy may be further shown by calculating the synergy score of the combination according to methods known by one of ordinary skill.

The term "combined administration" as used herein is defined to encompass the administration of the selected therapeutic agents to a single patient, and is intended to include treatment regimens in which the therapeutic agents are not necessarily administered by the same route of administration or at the same time.

The term "a combined preparation" is defined herein to refer to especially a "kit of parts" in the sense that the therapeutic agents (a) and (b) as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the therapeutic agents (a) and (b) simultaneously or at different time points. The parts of the kit of parts can then, e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the therapeutic agent (a) to the therapeutic agent (b) to be administered in the combined preparation can be varied, e.g., in order to cope with the needs of a patient sub-population to be treated or the needs of the single patient.

The term "jointly therapeutically active" or "joint therapeutic effect" as used herein means that the therapeutic agents may be given separately (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that they prefer, in the warm-blooded animal, especially human, to be treated, still show a beneficial (preferably synergistic) interaction (joint therapeutic effect). Whether this is the case can, *inter alia*, be determined by following the blood levels, showing that both therapeutic agents are present in the blood of the human to be treated at least during certain time intervals.

Pharmaceutical combinations of the present invention comprise the anti-CD137 antibody of the invention, as defined herein, particularly for use in the treatment or prevention of a proliferative disease. In a preferred embodiment, the pharmaceutical combinations of the present invention comprise the antibody of the invention, wherein said antibody is an IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1,2 and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, a VH sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to

SEQ ID NO: 13, and a VL sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 25. In another embodiment, the pharmaceutical combinations of the present invention comprise the antibody of the invention, wherein said antibody is an IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, a heavy chain sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 14, and a light chain sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 26. In another embodiment, the pharmaceutical combinations of the present invention comprise the antibody of the invention, wherein said antibody is an IgG4 comprising HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 16, 17, and 18 respectively, a VH sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 15, and a VL sequence comprising an amino acid sequence that is at least 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent, preferably at least 90 percent, identical to SEQ ID NO: 27.

In one aspect, the present invention relates to an antibody of the present invention, or the composition of the present invention, or the combination of the present invention for use as a medicament.

In another aspect, the present invention relates to an antibody of the present invention, or the composition of the present invention, or the combination of the present invention for use in a manufacture of a medicament for use in the treatment of a proliferative disease, in particular a cancer.

In one aspect, the present invention relates to an antibody of the present invention, or the composition of the present invention, or the combination of the present invention for use in the treatment a proliferative disease, in particular a cancer.

In another aspect, the present invention relates to use of an antibody of the present invention, or the composition of the present invention, or the combination of the present invention for the treatment of a proliferative disease, in particular a cancer, in a subject in need thereof.

In one aspect, the present invention provides a method of treating a proliferative disease, in particular a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an antibody of the invention, or the composition of the invention, or the combination of the present invention.

The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

The terms "treatment", "treating", "treat", "treated", and the like, as used herein, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease or delaying the disease progression. "Treatment", as used herein, covers any treatment of a disease in a mammal, e.g., in a human, and includes: (a) inhibiting the disease, i.e., arresting its development; and (b) relieving the disease, i.e., causing regression of the disease.

The term "therapeutically effective amount" or "efficacious amount" refers to the amount of an agent that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the agent, the disease and its severity and the age, weight, etc., of the subject to be treated.

In one embodiment, the proliferative disease is a cancer. The term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. The terms "tumor" and "cancer" are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors. The term "cancer" is used herein to mean a broad spectrum of tumors, including all solid and haematological malignancies. Examples of such tumors include, but are not limited to: a benign or especially malignant tumor, solid tumors, brain cancer, kidney cancer, liver cancer, adrenal gland cancer, bladder cancer, breast cancer, stomach cancer (e.g., gastric tumors), oesophageal cancer, ovarian cancer, cervical cancer, colon cancer, rectum cancer, prostate cancer, pancreatic cancer, lung cancer (e.g. non-small cell lung cancer and small cell lung cancer), vaginal cancer, thyroid cancer, melanoma (e.g., unresectable or metastatic melanoma), renal cell carcinoma, sarcoma, glioblastoma, multiple myeloma or gastrointestinal cancer,

especially colon carcinoma or colorectal adenoma, a tumor of the neck and head, endometrial cancer, Cowden syndrome, Lhermitte-Duclos disease, Bannayan-Zonana syndrome, prostate hyperplasia, a neoplasia, especially of epithelial character, preferably mammary carcinoma or squamous cell carcinoma, chronic lymphocytic leukemia, chronic myelogenous leukemia (e.g., Philadelphia chromosome-positive chronic myelogenous leukemia), acute lymphoblastic leukemia (e.g., Philadelphia chromosome-positive acute lymphoblastic leukemia), non-Hodgkin's lymphoma, plasma cell myeloma, Hodgkin's lymphoma, a leukemia, and any combination thereof. In a preferred embodiment, the cancer is a lung cancer, preferably non-small cell lung cancer (NSCLC). In another embodiment, said cancer is a colorectal cancer.

The antibody of the present invention, or the multispecific molecule of the present invention, or the composition of the present invention, or the combination of the present invention inhibits the growth of solid tumors, but also liquid tumors. In a further embodiment, the proliferative disease is a solid tumor. The term "solid tumor" especially means a breast cancer, ovarian cancer, colon cancer, rectum cancer, prostate cancer, stomach cancer (especially gastric cancer), cervical cancer, lung cancer (e.g., non-small cell lung cancer and small cell lung cancer), and a tumor of the head and neck. Further, depending on the tumor type and the particular combination used, a decrease of the tumor volume can be obtained. The antibody of the present invention, or the multispecific molecule of the present invention, or the composition of the present invention, or the combination of the present invention is also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases in a subject having a cancer.

The term "prevent" or "prevention" refers to a complete inhibition of development of a disease, or any secondary effects of disease. The term "prevent" or "prevention" as used herein covers prevention of a disease or condition from occurring in an individual who may be predisposed to the disease but has not yet been diagnosed as having it.

In a further aspect, the present invention relates to a kit comprising the antibody of the invention described herein. Also within this disclosure is a kit comprising the multispecific molecule of the invention. Also within this disclosure is a kit comprising the pharmaceutical composition of the invention. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody molecule for administration; pharmaceutically acceptable carriers; and devices or other materials for

administration to a subject. In a specific embodiment, the kit comprises the antibody of the invention in a pharmaceutically effective amount. In a further embodiment, the kit comprises a pharmaceutically effective amount of the antibody of the invention in lyophilized form and a diluent and, optionally, instructions for use. Said kit may further comprise a filter needle for reconstitution and a needle for injecting.

TABLE 1. Examples of CD137 antibodies of the present invention (CDR residues shown in bold and italic letters).

SEQ ID NUMBER	Ab region	Sequence
38-27-A11		
SEQ ID NO: 1	**HCDR1** (H27-H42; AHo numbering)	GFSFSANYYPC
SEQ ID NO: 2	**HCDR2** (H57-H76; AHo numbering)	CIYGGSSDITYDANWTK
SEQ ID NO: 3	**HCDR3** (H108-H138; AHo numbering)	RSAWYSGWGGDL
SEQ ID NO: 4	**HCDR1** (AHo definition)	ASGFSFSANYY
SEQ ID NO: 5	**HCDR2** (AHo definition)	IYGGSSDITYDANWTKG
SEQ ID NO: 6	**HCDR3** (AHo definition)	SAWYSGWGGD
SEQ ID NO: 7	**HCDR1** (Kabat definition)	ANYYPC
SEQ ID NO: 8	**HCDR2** (Kabat definition)	CIYGGSSDITYDANWTK
SEQ ID NO: 9	**HCDR3** (Kabat definition)	SAWYSGWGGDL
SEQ ID NO: 10	**HCDR1** (Chothia definition)	GFSFSANY
SEQ ID NO: 11	**HCDR2** (Chothia definition)	GGSS
SEQ ID NO: 12	**HCDR3** (Chothia definition)	AWYSGWGGD
SEQ ID NO: 13	**VH**	EVQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQAPGKGLEWIG***CIYGGSS***

	(VH3) (38-27-A11 sc02)	***DITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYYCA***RSAWYSGWGGDL***W GQGTLVTVSS
SEQ ID NO: 14	**VH** (VH3) (38-27-A11 sc03)	ESQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQAPGKGLEWIG***CIYGGSS*** ***DITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYFCA***RSAWYSGWGGDL***W GPGTLVTVSS
SEQ ID NO: 15	**VH** (VH3) (38-27-A11 sc07) (G51C)	EVQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQAPGKCLEWIG***CIYGGSS*** ***DITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYYCA***RSAWYSGWGGDL***W GQGTLVTVSS
SEQ ID NO: 16	**LCDR1** (L24-L42; AHo numbering) (Kabat definition)	QASQSISNRLA
SEQ ID NO: 17	**LCDR2** (L58-L72; AHo numbering) (Kabat definition)	SASTLAS
SEQ ID NO: 18	**LCDR3** (L107-L138; AHo numbering) (Kabat definition)	QSTYYGNDGNA
SEQ ID NO: 19	**LCDR1** (AHo definition)	ASQSISNR
SEQ ID NO: 20	**LCDR2** (AHo definition)	SASTLASGVPSR
SEQ ID NO: 21	**LCDR3** (AHo definition)	TYYGNDGN
SEQ ID NO: 22	**LCDR1** (Chothia definition)	SQSISNR
SEQ ID NO: 23	**LCDR2** (Chothia definition)	SAS
SEQ ID NO: 24	**LCDR3**	TYYGNDGN

	(Chothia definition)	
SEQ ID NO: 25	**VL** (Vk1-sk17) (38-27-A11 sc02)	DIQMTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKAPKLLIY***SASTLAS***GV PSRFSGSGSGTDFTLTISSLQPEDFATYYC***QSTYYGNDGNA***FGTGTKVTVLG
SEQ ID NO: 26	**VL** (Vk1-sk17) (38-27-A11 sc03)	DFQLTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKPPKLLIY***SASTLAS***GVP SRFSGSGSGTDFTLTISSLQPEDFATYYC***QSTYYGNDGNA***FGTGTKVTVLG
SEQ ID NO: 27	**VL** (Vk1-sk17) (38-27-A11 sc07) (T141C)	DIQMTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKAPKLLIY***SASTLAS***GV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQSTYYGNDGNAFGCGTKVTVLG
SEQ ID NO: 28	**Linker**	GGGGSGGGGSGGGGSGGGGS
SEQ ID NO: 29	**scFv** (VL-linker-VH) (38-27-A11 sc02) (PRO1359)	DIQMTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKAPKLLIY***SASTLAS***GV PSRFSGSGSGTDFTLTISSLQPEDFATYYC***QSTYYGNDGNA***FGTGTKVTVLGGGGGSG GGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQAP GKGLEWIG***CIYGGSSDITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYYC A***RSAWYSGWGGDL***WGQGTLVTVSS
SEQ ID NO: 30	**scFv** (VL-linker-VH) (38-27-A11 sc03) (PRO1360)	DFQLTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKPPKLLIY***SASTLAS***GVP SRFSGSGSGTDFTLTISSLQPEDFATYYC***QSTYYGNDGNA***FGTGTKVTVLGGGGGSG GGGSGGGGSGGGGSESQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQAP GKGLEWIG***CIYGGSSDITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYFCA ***RSAWYSGWGGDL***WGPGTLVTVSS
SEQ ID NO: 31	**scFv** (VL-linker-VH) (38-27-A11 sc07) (VL-T141C; VH-G51C) (PRO1704)	DIQMTQSPSSLSASVGDRVTITC***QASQSISNRLA***WYQQKPGKAPKLLIY***SASTLAS***GV PSRFSGSGSGTDFTLTISSLQPEDFATYYC***QSTYYGNDGNA***FGCGTKVTVLGGGGGS GGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAAS***GFSFSANYYPC***WVRQ APGKCLEWIG***CIYGGSSDITYDANWTK***GRFTISRDNSKNTVYLQMNSLRAEDTAVYY CA***RSAWYSGWGGDL***WGQGTLVTVSS

TABLE 2. Other sequences related to the present invention.

SEQ ID NUMBER	Ab region	Sequence
SEQ ID NO: 32	Human CD137	MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGG QRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQELTK KGCKDCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPADLSPGAS SVTPPAPAREPGHSPQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCEL
SEQ ID NO: 33	Vλ germline-based FR4 (Sk17)	FGTGTKVTVLG
SEQ ID NO: 34	Vλ germline-based FR4 (Sk12)	FGGGTKLTVLG
SEQ ID NO: 35	Vλ germline-based FR4	FGGGTQLIILG
SEQ ID NO: 36	Vλ germline-based FR4	FGEGTELTVLG
SEQ ID NO: 37	Vλ germline-based FR4	FGSGTKVTVLG
SEQ ID NO: 38	Vλ germline-based FR4	FGGGTQLTVLG
SEQ ID NO: 39	Vλ germline-based FR4	FGGGTQLTALG

TABLE 3. Examples of molecules comprising an antibody of the invention.

SEQ ID NUMBER	Ab Format	Sequence
PRO1480		
SEQ ID NO: 40	scDb-scFv	DIQMTQSPASLSASVGDRVTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGTGTKVTVLG**GG GGS**EVQLVESGGGLVQPGGSLRLSCAASGFSFSANYYPCWVRQAPGKGLEWIGCIY GGSSDITYDANWTKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARSAWYSGWG GDLWGQGTLVTVSS**GGGGSGGGGSGGGGSGGGGS**IQMTQSPSSLSASVGDRVTIT CQASQSISNRLAWYQQKPGKAPKLLIYSASTLASGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQSTYYGNDGNAFGTGTKVTVLG**GGGGS**EVQLVESGGGLVQPGGSLRLS CAASGFSFNSDYWIYWVRQAPGKGLEWIASIYGGSSGNTQYASWAQGRFTISRDNS

		KNTVYLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQGTLVTVSS**GGGGSGGG** **GS**IQMTQSPSSLSASVGDRVTITCQSSESVYSNNQLSWYQQKPGQPPKLLIYDASDLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSSSSDTAFGGGTKLTVL**GGGG** **GSGGGGSGGGGSGGGGS**EVQLVESGGGLVQPGGSLRLSCAASGFSLSSNAMGWV RQAPGKGLEYIGIISVGGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYF CARDRHGGDSSGAFYLWGQGTLVTVSS
PRO1481		
SEQ ID NO: 41	scDb-scFv	DIQMTQSPASLSASVGDRVTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGTGTKVTVLG**GG** **GGS**ESQLVESGGGLVQPGGSLRLSCAASGFSFSANYYPCWVRQAPGKGLEWIGCIY GGSSDITYDANWTKGRFTISRDNSKNTVYLQMNSLRAEDTAVYFCARSAWYSGWG GDLWGPGTLVTVSS**GGGGSGGGGSGGGGS**FQLTQSPSSLSASVGDRVTIT CQASQSISNRLAWYQQKPGKPPKLLIYSASTLASGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQSTYYGNDGNAFGTGTKVTVLG**GGGGS**EVQLVESGGGLVQPGGSLRLS CAASGFSFNSDYWIYWVRQAPGKGLEWIASIYGGSSGNTQYASWAQGRFTISRDNS KNTVYLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQGTLVTVSS**GGGGSGGG** **GS**IQMTQSPSSLSASVGDRVTITCQSSESVYSNNQLSWYQQKPGQPPKLLIYDASDLA SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSSSSDTAFGGGTKLTVL**GGGG** **GSGGGGSGGGGSGGGGS**EVQLVESGGGLVQPGGSLRLSCAASGFSLSSNAMGWV RQAPGKGLEYIGIISVGGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYF CARDRHGGDSSGAFYLWGQGTLVTVSS
PRO1480diS		
SEQ ID NO: 42	scDb-scFv	DIQMTQSPASLSASVGDRVTITCQASQSIGTYLAWYQQKPGKPPKLLIYRAFILASGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGTGTKVTVLG**GG** **GGS**EVQLVESGGGLVQPGGSLRLSCAASGFSFSANYYPCWVRQAPGKCLEWIGCIY GGSSDITYDANWTKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARSAWYSGWG GDLWGQGTLVTVSS**GGGGSGGGGSGGGGS**IQMTQSPSSLSASVGDRVTIT CQASQSISNRLAWYQQKPGKAPKLLIYSASTLASGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQSTYYGNDGNAFGCGTKVTVLG**GGGGS**EVQLVESGGGLVQPGGSLRLS CAASGFSFNSDYWIYWVRQAPGKGLEWIASIYGGSSGNTQYASWAQGRFTISRDNS KNTVYLQMNSLRAEDTAVYFCARGYVDYGGATDLWGQGTLVTVSS**GGGGSGGG** **GS**IQMTQSPSSLSASVGDRVTITCQSSESVYSNNQLSWYQQKPGQPPKLLIYDASDLA

		SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCAGGFSSSSDTAFGGGTKLTVLG**GGG GSGGGGSGGGGSGGGGS**EVQLVESGGGLVQPGGSLRLSCAASGFSLSSNAMGWV RQAPGKGLEYIGIISVGGFTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYF CARDRHGGDSSGAFYLWGQGTLVTVSS
PRO1186		
SEQ ID NO: 43	scDb-scFv	DIQMTQSPSSLSASVGDRVTITCQASQSIGTYLAWYQQKPGKAPKLLIYRAFILASGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQSNFYSDSTTIGPNAFGTGTKVTVLG**GG GGS**QVQLQESGPGLVKPSETLSLTCKVSGFSFSNSYWICWIRQPPGKGLEWIGCTFV GSSDSTYYANWAKGRVTISVDSSKNQFSLKLSSVTAADTAVYYCARHPSDAVYGYA NNLWGQGTLVTVSS**GGGGSGGGGSGGGGSGGGGS**DIQMTQSPSSLSASVGDRVTI TCQASQSINNVLAWYQQKPGKAPKLLIYRASTLASGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQSSYGNYGDFGTGTKVTVLG**GGGGS**QVQLQESGPGLVKPSETLSLTC KVSGFSFNSDYWIYWIRQPPGKGLEWIGSIYGGSSGNTQYASWAQGRVTISVDSSKN QFSLKLSSVTAADTAVYYCARGYVDYGGATDLWGQGTLVTVSS**GGGGSGGGGS**V VMTQSPSSLSASVGDRVTITCQASQIISSRSAWYQQKPGQPPKLLIYQASKLASGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQCTYIDSNFGAFGGGTKLTVLG**GGGGSGG GGSGGGGSGGGGS**EVQLVESGGGLVQPGGSLRLSCAASGFSFSSSYWICWVRQAP GKGLEWVGCVFTGDGTTYYASWAKGRFTISRDNSKNTVYLQMNSLRAEDTATYFC ARPVSVYYYGMDLWGQGTLVTVSS
PRO885		
SEQ ID NO: 44	scDb	DIQMTQSPSSLSASVGDRVTITCQASQSINDYLAWYQQKPGKAPKLLIYKASTLASG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGYIITDIDNVFGTGTKVTVLG**GGGG S**QVQLQESGPGLVKPSETLSLTCKVSGFSFSNSYWICWIRQPPGKGLEWIGCTFVGSS DSTYYANWAKGRVTISVDSSKNQFSLKLSSVTAADTAVYYCARHPSDAVYGYANN LWGQGTLVTVSS**GGGGSGGGGSGGGGSGGGGS**IQMTQSPSSLSASVGDRVTITCQ ASQSINNVLAWYQQKPGKAPKLLIYRASTLASGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQSSYGNYGDFGTGTKVTVLG**GGGGS**QVQLQESGPGLVKPSETLSLTCKVS GFSFSSGYDMCWIRQPPGKGLEWIGCVVAGSVDITYYASWAKGRVTISVDSSKNQFS LKLSSVTAADTAVYYCARKDAYSDAFNLWGQGTLVTVSS

Throughout the text of this application, should there be a discrepancy between the text of the specification (e.g., Tables 1 to 3) and the sequence listing, the text of the specification shall prevail.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

To the extent possible under the respective patent law, all patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference.

The following Examples illustrates the invention described above, but is not, however, intended to limit the scope of the invention in any way. Other test models known as such to the person skilled in the pertinent art can also determine the beneficial effects of the claimed invention.

Examples

NOVEL ANTIBODIES DIRECTED AGAINST HUMAN CD137

Example 1: Generation of rabbit antibodies directed against human CD137.

Rabbits have been immunized with recombinantly produced and purified human CD137 extracellular domain (Peprotech, cat. 310-15-1MG). During the immunization, the strength of the humoral immune response against the antigen was qualitatively assessed by determining the maximal dilution (titer) for the serum of each rabbit that still produced detectable binding of the polyclonal serum antibodies to the antigen. Serum antibody titers

against the immobilized antigen (recombinant human CD137 ECD) were assessed using an enzyme-linked immunosorbent assay (ELISA).

Example 2: Hit Identification and selection.

Within the Hit identification procedure, a flow-cytometry-based sorting procedure was developed that specifically detects and allows for the isolation of high-affinity human CD137 ECD binding B-cells. To identify CD137 binding B-cells, CD137 ECD was labeled with the fluorescent dye R-Phycoerythrin (RPE). Since the CD137L binding site as well as the binding site of an anti-CD137 antibody on the labeled CD137 could potentially be blocked by the bulky RPE label, accessibility of the epitopes was confirmed by flow-cytometry. CD137L ECD fused to the Fc part of a human IgG1, urelumab, rabbit polyclonal anti-human CD137 or goat polyclonal anti-human CD137 were captured on protein G beads, and binding of R-PE labeled CD137 was confirmed by flow-cytometry. The fluorescence intensity is proportional to the amount of labeled CD137 bound to CD137L immobilized on the beads. Binding of CD137 to CD137L and anti-CD137 antibodies was found while no binding of RPE-labeled CD137 to Infliximab was detected.

Screening:

B-cells expressing CD137-specific antibodies (IgG) were isolated in the sorting campaign. The results obtained during the screening phase are based on assays performed with non-purified antibodies from culture supernatants of antibody secreting cells (ASC). The rabbit monoclonal antibodies in each cell culture supernatant were characterized in a high-throughput ELISA for binding to recombinant human CD137 ECD. CD137-binding supernatants were further characterized for binding kinetics to human and cynomolgus CD137. In addition, neutralization potential of the CD137 interaction to CD137L as well as to urelumab was determined by competition ELISA. Binding to membranous CD137 expressed on stable transduced Jurkat cells was also assessed. Mouse CD137 binding potential of the supernatants was analyzed by direct ELISA.

Direct ELISA

ELISA plates were coated by adding 50 µl of PBS containing 250 ng/ml human CD137 (Peprotech, cat. 310-15-1MG) overnight at 4°C. Next day, plates were washed three times in overflow mode with 300 µl wash buffer (PBS, 0.005% Tween 20) per wells and

270 µl of blocking buffer (PBS, 1% BSA, 0.2% Tween 20) were added to each well for 1h at RT without shaking. Then, plates were washed three times in overflow mode with 300 µl wash buffer and 50 µl of each supernatant was added, plates were incubated 1.5 h at RT under gentle agitation. After 3 washes in overflow mode with 300 µl wash buffer, 50 µl of a HRP-coupled goat-anti-rabbit IgG antibody 1:5'000 diluted in blocking buffer were added to each well. After 1h incubation at RT on a nutating mixer, plates were washed three times in overflow mode with 300 µl wash buffer per well prior to the addition of 50 µl TMB (3,3',5,5'-tetramethylbenzidine). After 5 to 10 minutes development the enzymatic reaction was stopped by addition of 50 µl of 1M HCl per well and plate was read at 450 nm using 690 nm as a reference wavelength.

Affinity to hCD137 by SPR

Binding affinities of antibodies towards human CD137 were measured by SPR using a MASS-1 SPR instrument (Sierra Sensors). For affinity screening, an antibody specific for the Fc region of rabbit IgGs (Bethyl Laboratories, Cat. No. A120-111A) was immobilized on a sensor chip (MASS-1 Affinity Sensor, High Capacity Amine, Sierra Sensors) using a standard amine-coupling procedure. Rabbit monoclonal antibodies in B-cell supernatants were captured by the immobilized anti-rabbit IgG antibody. A minimal IgG concentration in the B-cell supernatants is required to allow sufficient capture. After capturing of the monoclonal antibodies, human CD137 ECD (Peprotech, cat. 310-15-1MG) was injected into the flow cells for 3 min at a concentration of 90 nM and dissociation of the protein from the IgG captured on the sensor chip was allowed to proceed for 5 min. After each injection cycle, surfaces were regenerated with two injections of 10 mM Glycine-HCl. The apparent dissociation (k_d) and association (k_a) rate constants and the apparent dissociation equilibrium constant (K_D) were calculated with the MASS-1 analysis software (Analyzer, Sierra Sensors) using one-to-one Langmuir binding model and quality of the fits was monitored based on relative Chi^2 (Chi^2 normalized to the extrapolated maximal binding level of the analyte), which is a measure for the quality of the curve fitting. The smaller the value for the Chi^2 the more accurate is the fitting to the one-to-one Langmuir binding model. For most of the hits the relative Chi^2 value was below 15%. Results were deemed valid if the response units (RU) for ligand binding were at least 2% of the RUs for antibody capturing. Samples with RUs for ligand binding with less than 2% of the RUs for antibody capturing were considered to show no specific binding of CD137 to the captured antibody.

CD137 / CD137L competition ELISA

ELISA plates were coated by adding 50 µl of PBS containing 50 ng/ml CD137 Fc chimera (R&D Systems, cat. 838-4B-100) overnight at 4°C. Next day, plates were washed three times in overflow mode with 450 µl wash buffer (PBS, 0.005% Tween 20) per wells and 300 µl of blocking buffer (PBS with 1% BSA and 0.2 Tween 20) were added to each well for 1h at RT on a nutating mixer. Then, the positive control (neutralizing goat anti-CD137 antibody) was diluted in 100% negative supernatant and 50 µl of the neutralizing antibody were added to the corresponding wells of the binding plate. In addition, 50 µl of the supernatant of the positive hits were transferred to the binding plate and incubated for 1h at RT with shaking. Next, ELISA plates were washed 3 times in overflow mode with 450 µl wash buffer per well and 50 µl of 20 ng/ml biotinylated recombinant human CD137 ligand (Acro Biosystem, cat. 41L-H5257) diluted in blocking buffer were added to the wells. After 1h of incubation at RT with shaking, the ELISA plates were washed 3 times in overflow mode with 450 µl wash buffer per well. Then, 50 µl of 10 ng/ml streptavidin-poly-HRP diluted in blocking buffer was added to each wells of the ELISA plates. After 1h incubation at RT, plates were washed three times with 450 µl wash buffer and developed for 5 to 10 minutes after addition of 50 µl TMB. Finally, the enzymatic reaction was stopped by addition of 50 µl of 1M HCl and plate was read at 450 nm using 690 nm as a reference wavelength.

Species specificity by SPR: cynomolgus CD137

Binding kinetics to cynomolgus CD137 were also determined for the hits identified in the initial screening ELISA using the same SPR setup as described for the binding to human CD137, but replacing human CD137 ECD by cynomolgus monkey CD137 ECD (Acro Biosystem, cat. 41B-C52H4).

Urelumab competition ELISA

ELISA plates were coated by adding 50 µl of PBS containing 2 µg/ml urelumab (produced by Evitria, Schlieren, Switzerland) overnight at 4°C. Next day, plates were washed three times in overflow mode with 450 µl wash buffer (PBS, 0.005% Tween 20) per wells and 300 µl of blocking buffer (PBS with 1% BSA and 0.2 Tween 20) were added to each well for 1h at RT on a nutating mixer. Then, urelumab was diluted in 95% negative supernatant spiked and pre-incubated for 1 h with 5% biotinylated CD137 ECD (Peprotech, cat. 310-15-1MG) at 7.5 ng/ml and was added to the corresponding wells of the binding plate. In addition, 55 µl of the supernatant of the positive hits were also spiked and pre-incubated

for 1h with 5% biotinylated CD137 ECD at 7.5 ng/ml and transferred to the binding plate and incubated for 1 h at RT with shaking. Next, the ELISA plates were washed 3 times in overflow mode with 450 µl wash buffer per well. Then, 50 µl of 10 ng/ml streptavidin-poly-HRP diluted in blocking buffer was added to each well of the ELISA plate. After 1 h incubation at RT, plates were washed three times with 450 µl wash buffer and developed for 5 to 10 minutes after addition of 50 µl TMB. Finally, the enzymatic reaction was stopped by addition of 50 µl of 1M HCl, and the plate was read at 450 nm using 690 nm as a reference wavelength.

Cell-based binding assay by FC: human CD137

Jurkat wild type (control cells that do not express CD137) and Jurkat CD137 cells (clone C6, 1) were harvested and cell numbers were determined. Cell suspensions were centrifuged for 5 min at 400xg and 40 µl of cell suspensions (40,000 cells) diluted in PBS-EB (1x DPBS, 2% BCS H.I., 2 mM EDTA) were added to designated wells in a non-binding plate. Supernatant from positive hits were directly transferred into 96 well plate according to plate layout. Positive control samples (urelumab) were diluted in PBS-EB and transferred to the plate, final samples were of 95% negative supernatant. After incubation at 4°C for 1 h, plates were washed 3 times using 100 µl of PBS-EB. Then, cell pellets were re-suspended with 50 µl secondary antibody solution at a concentration of 2 µg/ml (for B-cell clones: goat-anti-rabbit IgG labeled with AF647; for urelumab: goat-anti-human IgG labeled with PE) and incubated for 1 h at 4°C. Next, cells were washed again three times using 100 µl of PBS-EB. The cell pellets were then re-suspended with 50 µl PBS-EB and analyzed with NovoCyte 2060 flow cytometer device. Fluorescence intensity of PE and AF647 for 20,000 events was recorded for each sample and the geometric mean of fluorescence intensity MFI was calculated. The data were first corrected for unspecific antibody binding (blank and Jurkat wild type cell binding) and then normalized to binding levels obtained for urelumab.

Direct ELISA mouse CD137

As a first step to identify mouse cross-reactive CD137 binder, a direct ELISA against the mouse CD137 was performed. For this purpose, cell culture supernatants of B-cell clones were screened for the presence of antibodies to mouse CD137 by ELISA. ELISA plates were coated by adding 50 µl of PBS containing 250 ng/ml mouse CD137 (Acro Biosystem, cat. 41B-M52H7) overnight at 4°C. Next day, plates were washed three times in overflow mode with 300 µl wash buffer (PBS, 0.005% Tween 20) per wells and 270 µl of blocking buffer

(PBS, 1% BSA, 0.2% Tween 20) were added to each well for 1 h at RT without shaking. Then, plates were washed three times in overflow mode with 300 µl wash buffer, 50 µl of each supernatant were added, and the plates were incubated 1.5 h at RT under gentle agitation. After 3 washes in overflow mode with 300 µl wash buffer, 50 µl of a HRP-coupled goat-anti-rabbit IgG antibody 1:5,000 diluted in blocking buffer was added to each well. After 1 h incubation at RT on a nutating mixer, plates were washed three times in overflow mode with 300 µl wash buffer per well prior to the addition of 50 µl TMB. After 5 to 10 minutes development the enzymatic reaction was stopped by addition of 50 µl of 1M HCl per well and plate was read at 450 nm using 690 nm as a reference wavelength.
In this assay, supernatants from 85 B-cell clones produced a signal that was clearly above background (>0.1 OD).

Species specificity by SPR: mouse

Binding kinetics to mouse CD137 were also determined using the same setup as described for the binding to the human CD137, but in this case human CD137 ECD was replaced by mouse CD137 ECD (Acro Biosystem, cat. 41B-M52H7).

Selection of Screening Hits

Based on pharmacologic properties of monoclonal antibodies in B-cell supernatant a clone 38-27-A11 was selected for hit confirmation analysis. Pharmacologic properties of the monoclonal antibody of the clone 38-27-A11 in B-cell supernatant are presented in Table 4. In a next step, the selected clone was used for RNA isolation and RT-PCR to amplify the sequence of rabbit antibody light and heavy chain variable regions (38-27-A11).

TABLE 4. Pharmacodynamic properties of monoclonal antibodies in B-cell supernatants: 38-27-A11.

Clone ID	Affinity to hCD137 (SPR)	Affinity to cynomolgus CD137 (SPR)		Neutralization in CD137/CD137L inhibition ELISA	Urelumab competitive ELISA	IgG concentration	Binding to CD137 expressing cell line	
	K_D [M]	K_D [M]	Ratio of K_D cyno CD137 / K_D human CD137	% inhibition at 20 ng/ml CD137L	% inhibition at 7.5 ng/mL CD137	conc. [ng/ml]	relative binding to urelumab	normalized to urelumab and rabbit IgG concentration
38-27-A11	9.56E-11	5.21E-11	0.5	26.9	15.4	2547.6	148%	0.6

TABLE 5. Summary of affinity measurement to human CD137 for 38-27-A11 rabbit IgG.

clone ID	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax
38-27-A11	1.46E+06	1.43E-04	9.78E-11	109%

TABLE 6. Summary of affinity measurement to cynomolgus monkey CD137 for 38-27-A11 rabbit IgG.

clone ID	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax	Ratio of K_D cynomolgus monkey CD137 / K_D human CD137
38-27-A11	1.06E+06	2.23E-05	2.11E-11	157%	0.2

Example 4: Pharmacological Characterization of Monoclonal Antibody 38-27-A11

4.1 Affinities to human and cynomolgus CD137

Binding kinetics of the purified monoclonal rabbit antibody 38-27-A11 to human and cynomolgus CD137 were determined by SPR measurements (Tables 5 and 6). Binding affinities of antibodies towards human CD137 were measured by SPR using a Biacore T200 SPR instrument (GE Healthcare). Regarding the experimental setup, an antibody specific for the Fc region of rabbit IgGs (Bethyl Laboratories, Cat. No. A120-111A) was immobilized on a sensor chip (CM5 chip, GE Healthcare) using a standard amine-coupling procedure. Rabbit monoclonal antibodies were captured by the immobilized anti-rabbit IgG antibody. After capturing of the monoclonal antibodies, human CD137 (PeproTech, cat. 310-15) or cynomolgus monkey CD137 (Acro Biosystem, cat. 41B-C52H4) was injected into the flow cells for 3 min at concentrations ranging from 90 to 0.35 nM and dissociation of the protein from the IgG captured on the sensor chip was allowed to proceed for 12 min. The apparent dissociation (kd) and association (ka) rate constants and the apparent dissociation equilibrium constant (KD) were calculated with the Biacore T200 software evaluation tool (GE Healthcare) using one-to-one Langmuir binding model.

4.2 Epitope binning of recombinant rabbit IgG 38-27-A11

To characterize the binding epitopes of recombinant rabbit IgG 38-27-A11, epitope binning was performed by SPR using a MASS-1 instrument (Sierra Sensors). By using this approach, the binding epitopes of the rabbit IgG 38-27-A11 on CD137 were mapped against urelumab and utomilumab. A sandwich setup was utilized to examine if the antibodies block one another's binding to human CD137. The rabbit IgG 38-27-A11 plus the competitor IgGs were immobilized on high capacity amine sensor chips (HCA, Sierra Sensors). Then, 90 nM of the antigen CD137 (PeproTech, cat. 310-15) were injected and captured on the rabbit IgG 38-27-A11, followed immediately by an injection of 22.5 nM of the second antibody (competitor IgGs). The capture levels of human CD137 on each rabbit IgG and the second binder response levels were determined (response units, RU). By calculating the theoretical maximum response (Rmax), which depends on the molecular weights of the involved proteins and the capture levels, the relative binding level (in %) of the proteins on the captured antigen was determined. If the molecules bind the same epitope or overlapping (e.g., a structurally similar or spatially proximal) epitopes on CD137, no binding of the antibody injected over the captured CD137 should be observed. Consequently, when binding of the

antibody is observed the two antibody pairs bind non-overlapping epitopes. The relative binding levels (in %) were determined for each antibody pair. By definition, a binding level below 10% indicates the same or an overlapping (e.g., a structurally similar or spatially proximal) on CD137 and above 30% refers to non-overlapping epitopes. The IgG 38-27-A11 did not compete with utomilumab for binding to CD137 suggesting non-overlapping epitopes, while competed with urelumab for binding to CD137 suggesting either the same or overlapping epitopes (FIG. 1).

4.3 Activation of CD137 signaling by NF-kB reporter gene assay

Potency to activate CD137 clustering and subsequently CD137 signaling was assessed in the NF-kB reporter gene assay. In this assay, the activation of CD137 signaling in a NF-kB Jurkat reporter cells was assessed. The activity of CD137 signaling is reported by measurement of Luciferase expression which is driven by CD137 induced NF-kB activation in the Jurkat reporter cell line. Moreover, clustering of CD137, which is required for activation of the signaling pathway, is facilitated via binding of the bivalent anti-CD137 rabbit IgGs.

In detail, 50,000 cells of the CD137 expressing NF-kB reporter gene Jurkat cells (Promega) were seeded in a 96-well white cell culture plate. Serial dilutions of three fold steps of the rabbit IgGs ranging from 9,000 to 1.37 ng/ml were prepared in assay buffer. To each dilution, an excess of 2.5 fold of the crosslinking antibody (goat anti-rabbit IgG Fc specific antibody, Bethyl, cat. A120-111A) was added. The highest concentration of each rabbit IgG was measured also without cross-linker, in order to determine whether antibody binding without further clustering is sufficient to induce CD137 signaling. Urelumab, as a positive control, was included on each plate and crosslinking of urelumab was achieved by the addition of 1.25 excess of cross-linker (rabbit anti-human IgG Fc specific antibody, Bethyl, cat. A80-304A) to each dilution. The highest concentration of urelumab, as it was done for the rabbit IgGs, was measured also without the addition of cross-linker. Prepared serial dilutions of the recombinant IgGs and urelumab were added to the reporter gene cells and incubated for 6 h at 37°C in a humidified cell culture incubator. Luciferase expression was detected by addition of Luciferase reagent and was read by a luminescence reader 6 h after addition of the anti-CD137 IgGs. Data were analyzed by normalization the relative luminescence units (RLU) of the test samples to the RLU measured for urelumab at its highest concentration with cross-linker. Normalized data were plotted as a function of rabbit IgG concentration and fitted by using a sigmoidal 4-parameter fit. Maximum activation of

NF-kB signaling (relative to urelumab), EC_{50} values, and relative EC_{50} values (relative to urelumab) were reported (Table 7).

TABLE 7. Potency to activate CD137 signaling in the NF-kB reporter gene assay of the 38-27-A11 rabbit IgG.

clone ID	Potency in CD137 NF-kB reporter gene assay			
	EC_{50} [ng/ml]	rel. EC_{50} ($EC_{50,\ urelumab}$/ $EC_{50,\ rabbit\ IgG}$)	Maximum activation (relative to urelumab in %)	Comment
38-27-A11	158.7	0.33	86.8	no activation without crosslinker

Example 5: Humanization of the rabbit IgG 38-27-A11

Based on data obtained during hit screening, a CD137 binder 38-27-A11 was humanized by grafting the CDRs on VH3 based framework (Table 8). Full graft designates CDR graft plus framework residues following AHo humanization protocol (CDR graft plus grafting of all rabbit residues potentially in contact with antigen (according to AHo) were limited to residues with >20% change in solvent accessibility upon interface formation in order to reduce total number of mutations (rabbit framework residues)).

TABLE 8. Humanization of the rabbit IgG 38-27-A11.

Clone ID	Protein ID	Grafting Strategy	Mutations VL	Mutations VH	Framework
38-27-A11 sc02	PRO1359	CDR	-	-	Vk1/VH3
38-27-A11 sc03	PRO1360	FULL	I2F;M4L;A51P	V2S;Y105F;Q141P	Vk1/VH3
38-27-A11 sc07	PRO1704	CDR	T141C	G51C	Vk1/VH3

Codon optimized nucleotide sequences were designed and the corresponding genes were synthesized and cloned into mammalian expression vectors. Table 9 summarizes manufacture of scFv molecules. Expression of mammalian constructs was performed in CHO-S cells using CHOgro transient transfection kit (Mirus). Cultures were harvested after 5-7 days (cell viability <70%) of expression at 37°C by centrifugation, and proteins were purified from clarified culture supernatants by Protein L affinity chromatography followed, if needed, by a polishing step by size-exclusion chromatography. For the quality control of the manufactured material standard analytical methods, such as SE-HPLC, UV280 and SDS-PAGE were used.

Example 6: Pharmacodynamics Characterization of humanized scFvs

6.1 Affinity to human CD137

Affinity of the humanized scFvs PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) to human CD137 was determined by SPR analysis on a T200 device (Biacore, GE Healthcare). In this experiment, Fc tagged human CD137 (R&D Systems, cat. 838-4B-100) was captured using the Human Antibody Capture kit from GE healthcare (cat. BR-1008-39). After each analyte injection cycle, the anti-human Fc-specific IgG was regenerated and new antigen was captured. The scFvs were injected as analyte using a dose response multi-cycle kinetic assay over the captured CD137 with concentrations of the analyte ranging from 0.19 to 45 nM (three-fold dilutions steps) diluted in running buffer. Association and dissociation time were set to 300 s and 720 s, respectively. The obtained sensorgrams were fitted using the 1:1 binding model. Data are shown in Table 10.

6.2 Species cross-reactivity (binding to cynomolgus monkey CD137 by SPR)

Cross-reactivity to cynomolgus CD137 was measured with cynomolgus monkey Fc-tagged CD137 (R&D Systems, cat. 9324-4B-100) in a similar assay as used to measure binding to human CD137. Table 11 summarizes the affinities obtained for the scFvs PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03).

6.3 Neutralization of CD137/CD137L interaction by competition ELISA

To show that anti-human CD137 scFvs PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) did not interfere with the binding of CD137L to CD137, a competitive ELISA was employed. The commercial inhibitory polyclonal anti-CD137 goat antibody (Antibodies online, Cat# ABIN636609) served as a reference. Regarding the experimental setup, 50 ng/ml of human CD137 (tagged with Fc, R&D Systems, cat. 838-4B-100) were coated on ELISA plates overnight and serial dilutions of three fold steps of the scFvs starting at 50 µg/ml were added to the ELISA plates. Afterwards, biotinylated CD137L (in-house biotinylation of CD137L, Acro Biosystem, cat. 41L-H5257) was added and bound ligand was detected by addition of Streptavidin-HRP. Finally, the HRP substrate TMB was added. After development for 5 min, the reaction was stopped with 1 M HCl solution. The absorbance was measured at 450 nm and 690 nm as reference wave-length. The data are shown in Table 12.

Table 9. ScFv production summary table.

Clone ID	Protein ID	Grafting Strategy	Expression volume [mL]	Expression system	Protein amount post protein L [mg]	Yield post protein L [mg/L]	SEC polishing Y/N?	Final yield [mg]	Yield per L expression [mg/L]	Purity SE-HPLC [% monomer]
38-27-A11 sc02	PRO1359	CDR	200	CHO	13.7	68.5	Y	6.8	34.2	99.0
38-27-A11 sc03	PRO1360	FULL	200	CHO	12.9	64.7	Y	6.2	30.8	99.1

Table 10. Affinities of scFv to human CD137.

				Affinity to human CD137 (SPR)			
Clone ID	Protein ID	Framework	Grafting Strategy	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax
38-27-A11 sc02	PRO1359	Vk1/VH3	CDR	8.66E+05	2.81E-03	3.24E-09	84.3%
38-27-A11 sc03	PRO1360	Vk1/VH3	FULL	1.28E+06	3.00E-04	2.34E-10	82.1%
38-27-A11 sc07	PRO1704	Vk1/VH3	CDR	1.15E+06	3.53E-03	3.07E-09	83.3%

Table 11. Affinities of scFv to cynomolgus monkey CD137.

				Affinity to cynomolgus monkey CD137 (SPR)			
Clone ID	Protein ID	Framework	Grafting Strategy	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax
38-27-A11 sc02	PRO1359	Vk1/VH3	CDR	9.27E+05	2.67E-03	2.88E-09	84.8%
38-27-A11 sc03	PRO1360	Vk1/VH3	FULL	1.05E+06	2.52E-04	2.40E-10	89.2%

6.4 Binding to human CD137 expressing cells by Flow cytometry

Binding potency to human CD137 expressing cells was determined for PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03). 50,000 CD137 expressing Jurkat cells (or as reference cell line Jurkat cells lacking CD137 expression) were distributed to round bottom non-tissue culture treated 96 well plates. Cells were washed twice with 100 µl PBS by centrifugation at 400 x g for 5 min. Cells were resuspended in 100 µl of serial dilutions of five-fold steps prepared in staining buffer (PBS, 2% BCS heat inactivated, 2 mM EDTA) of the tested scFvs as well as of the control IgG urelumab and ranging from 10,000 to 0.64 ng/ml (for scFvs: from 381.19 to 0.02 nM). After 1 h incubation at 4°C on a nutating mixer, cells were washed 3 times with 100 µl staining buffer and centrifugation steps of 5 min at 400 x g. Then, cells treated with scFvs were resuspended in 100 µl of staining buffer containing 0.5 µg/ml of APC labelled protein-L and cells treated with urelumab (human IgG4) were resuspended in 100 µl of staining buffer containing 2 µg/ml of goat anti-human IgG labelled with APC. Plates were incubated 1 h at 4°C on a nutating mixer, then washed 3 times with 100 µl of staining buffer and resuspended in a final volume of 50 µl of staining buffer. Finally, APC signal of 20,000 events per well was analyzed by flow cytometry using a Novocyte flow cytometer system (ACEA Bioscience). Individual EC_{50} values on each plate were calibrated against the EC_{50} of the reference molecule urelumab that was taken along on each plate (relative EC_{50}: EC_{50}, urelumab / EC_{50}, test scFv). Data are summarized in Table 13 and FIG. 3.

6.5 Selectivity for CD137 versus CD40 and OX40 by SPR

In addition to cross-reactivity to cynomolgus monkey CD137, selectivity of binding of the anti-human CD137 scFvs PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) to human CD137 and not to other members of the TNFR superfamily, such as CD40 and OX40, is desired. Therefore, binding of PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) to human CD40 and OX40 was tested. Binding of scFvs to human Fc-tagged CD40 (AcroBiosystems, cat. CD0-H5253) and human Fc-tagged OX40 (Acro-Biosystems, cat. OX0-H5255) was determined by SPR analysis on a T200 device (Biacore, GE Healthcare). In this experiment, Fc-tagged CD40 and OX40 were captured using the Human Antibody Capture kit from GE healthcare (cat. BR-1008-39). After each analyte injection cycle, the

anti-human Fc-specific IgG was regenerated and new antigen was captured. The scFvs were injected as analyte using a high concentration of 180 nM of the analyte diluted in running buffer. Association and dissociation time were set to 300 s and 720 s, respectively. Obtained sensorgrams were fitted using the 1:1 binding model. Data are summarized in Table 14.

Table 12. Potencies of selected scFvs to inhibit the interaction between CD137 and CD137L.

Clone ID	Protein ID	Framework	Grafting Strategy	Potency in CD137/CD137L competition ELISA		
				IC_{50} [ng/ml]	rel. IC_{50} ($IC_{50,\ reference}$/ $IC_{50,\ scFv}$)	Maximum activation (relative to reference in %)
38-27-A11 sc02	PRO1359	Vk1/VH3	CDR		no inhibition	
38-27-A11 sc03	PRO1360	Vk1/VH3	FULL		no inhibition	

Table 13. Summary of binding potency to cellular CD137 of the tested scFvs.

Clone ID	Protein ID	Framework	Grafting Strategy	Binding to cellular human CD137		
				EC_{50} [ng/ml]	rel. EC_{50} ($EC_{50,\ urelumab}$ / $EC_{50,\ scFv}$)	Maximum binding (relative to urelumab)
38-27-A11 sc02	PRO1359	Vk1/VH3	CDR	3986	0.016	18%
38-27-A11 sc03	PRO1360	Vk1/VH3	FULL	148.2	0.424	29%

Table 14. ScFvs tested for binding to human CD40 and OX40 by SPR.

Clone ID	Protein ID	Framework	Grafting Strategy	Affinity to human CD40 (SPR)				Affinity to human OX40 (SPR)			
				k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	K_D [M]	Binding level normalized to theoretical Rmax
38-27-A11 sc02	PRO1359	Vk1/VH3	CDR	no binding				no binding			
38-27-A11 sc03	PRO1360	Vk1/VH3	FULL	no binding				no binding			

Example 7: Biophysical Characterization of the humanized scFvs

The scFvs PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) were concentrated to >10 mg/mL using centrifugal concentration tubes after purification (Table 15).

ScFvs were subjected to stability studies such as a four-week stability study, in which the scFvs were formulated in an aqueous buffer (50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4) at 10 mg/ml and stored at -80°C, 4°C and 40°C for four weeks. At the minimum, the fraction of monomers and oligomers in the formulation were evaluated by integration of SE-HPLC peak areas after one week, two weeks and at the end of each study. Table 16 compares d7 and endpoint measurements obtained at d28 of the study. In addition, the compatibility of the scFv molecules was assessed with respect to freeze-thawing (F/T) cycles (colloidal stability). For the F/T stability assessment the same analytical methods (SE-HPLC, UV-Vis) and parameters (% monomer content and % monomer loss) as for the storage stability study were applied to monitor the quality of the molecules over five F/T cycles. Table 17 shows the course of monomer content in % over five repeated F/T cycles. None of the molecules lost >2% monomeric content after repeated F/T cycles.

Thermal unfolding of the molecules was assessed by using the fluorescence dye SYPRO orange. Samples in relevant excipient conditions were prepared and the assay was performed in a qPCR machine. Fluorescence emission was detected using the software's custom dye calibration routine. The PCR plate containing the test samples was subjected to a temperature ramp from 25°C to 96°C in increments of 1°C. The midpoint of the unfolding transition (Tm) was calculated by the software GraphPad Prism using a mathematical second derivative method to calculate the inflection point of the curve. The reported Tm is an average of three measurements. Table 18 shows melting temperatures of the molecules formulated in generic buffer (50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl).

PRO1359 (38-27-A11 sc02) and PRO1360 (38-27-A11 sc03) were subjected to a short-term pH stress stability study, in which the scFv molecules were formulated at 1 mg/ml in a set of aqueous (phosphate-citrate) buffer systems with pH values between 3.5 and 7.5. Monomeric content in % and % monomer loss was analyzed after storage for 2 weeks at 4°C and 40°C in the respective buffer systems. A tabulated summary of monomeric content, monomeric loss, concentration and concentration loss over the course of the study is shown in Table 19.

Table 15. Manufacture of domains for stability study.

Clone ID	Protein ID	Grafting Strategy	Expression volume [mL]	Expression system	Protein amount post protein L [mg]	Yield post protein L [mg/L]	SEC polishing Y/N?	Final yield [mg]	Yield per L expression [mg/L]	Purity SE-HPLC [% monomer]	Monomeric content at 10 mg/mL [%]	Monomeric loss upon concentratio to 10 mg/mL
38-27-A11 sc02	PRO1359	CDR	200	CHO	13.7	68.5	Y	6.8	34.2	99	99.0	0.0
38-27-A11 sc03	PRO1360	FULL	200	CHO	12.9	64.7	Y	6.2	30.8	99.1	98.7	0.4

Table 16. Four week stability study of the scFv domains.

Description	Protein ID	Temp. [°C]	Initial monomeric content [%]	Monomeric content [%]			Monomeric content loss [%]		Protein concentration [mg/mL]			Protein content loss [%]	
				d0	d7	d28	d7	d28	d0	d7	d28	d7	d28
38-27-A11 sc02	PRO1359	-80	99.0	99.0	98.8	98.8	0.2	0.2	10.5	11.2	11.6	-7.0	-11.0
		4		99.0	98.8	97.9	0.2	1.2	10.5	11.2	11.1	-6.6	-5.5
		40		99.0	80.1	79.1	19.1	20.1	10.5	10.8	11.1	-3.2	-6.3
38-27-A11 sc03	PRO1360	-80	98.7	98.7	98.6	97.4	0.1	1.3	10.9	9.7	10.3	11.1	5.6
		4		98.7	97.7	93.1	1.0	5.7	10.9	10.6	9.3	2.8	14.6
		40		98.7	79.0	77.4	20.0	21.6	10.9	12.0	11.2	-9.9	-2.7

Table 17. Assessment of F/T stability over time course of 28d.

Clone ID	PRO ID	F/T-1*	F/T-2*	F/T-3*	F/T-4*	F/T-5*
38-27-A11 sc02	PRO1359	0.0	0.2	0.1	0.1	0.2
38-27-A11 sc03	PRO1360	0.1	0.1	0.3	0.2	1.3

*% monomeric loss upon F/T

Table 18. Differential Scanning Fluorimetry of the scFv domains.

Clone ID	Protein ID	Grafting Strategy	Tm [°C]
38-27-A11 sc02	PRO1359	CDR	64.4

Table 19. Tabulated summary of pH stability assessment.

Clone ID	Protein ID	Temperature	Final buffer	monomeric content [%]			monomeric loss [%]		protein concentration [mg/mL]			content loss [%]	
				d0	d7	d14	d7	d14	d0	d7	d14	d7	d14
38-27-A11 sc02	PRO1359	4°C	pH 3.5	NA	96.9	96.8	NA	-0.1	1.0	1.0	1.0	-8.6	0.8
			pH 4.5	96.9	97.0	97.0	0.1	0.0	1.0	1.0	1.0	-7.3	3.8
			pH 5.5	96.7	97.0	96.8	0.3	-0.2	1.0	0.9	1.0	-6.7	7.7
			pH 6.5	96.7	97.0	96.9	0.3	-0.1	1.0	1.0	1.0	4.0	-3.2
			pH 7.5	96.6	97.0	96.7	0.4	-0.3	1.0	1.0	1.0	4.0	-2.4
		40°C	pH 3.5	NA	97.2	97.3	NA	0.1	1.0	1.0	1.0	-5.8	4.1
			pH 4.5	96.9	97.0	97.3	0.1	0.3	1.0	0.9	1.0	-9.0	8.9
			pH 5.5	96.7	97.0	97.4	0.3	0.4	1.0	0.9	1.0	-7.9	10.8
			pH 6.5	96.7	97.5	97.5	0.9	0.0	1.0	0.9	1.0	-4.6	7.0
			pH 7.5	96.6	98.0	97.6	1.4	-0.4	1.0	1.0	1.0	-0.2	4.5
38-27-A11 sc03	PRO1360	4°C	pH 3.5	94.1	94.0	93.9	-0.1	-0.1	1.1	1.0	1.2	-11.1	21.1
			pH 4.5	93.8	94.0	93.7	0.3	-0.3	1.0	1.0	1.1	2.5	7.7
			pH 5.5	93.7	94.0	93.7	0.4	-0.3	0.9	1.0	1.1	10.5	4.8
			pH 6.5	NA	94.0	93.7	NA	-0.3	1.0	1.0	1.0	5.8	2.8
			pH 7.5	NA	94.0	93.9	NA	-0.1	1.0	1.0	1.1	3.1	4.2
		40°C	pH 3.5	94.1	97.0	97.3	3.1	0.3	1.1	1.0	1.0	-8.7	1.6
			pH 4.5	93.8	97.0	96.9	3.5	-0.1	1.0	1.0	1.1	-0.5	6.6
			pH 5.5	93.7	97.0	96.9	3.6	-0.1	0.9	1.0	1.1	8.1	9.4
			pH 6.5	NA	97.0	96.9	NA	-0.1	1.0	1.0	1.1	2.1	8.1
			pH 7.5	NA	NA	97.0	NA	NA	1.0	NA	1.1	NA	NA

Example 8: Epitope mapping by complex formation analysis of CD137 ECD variants in solution.

In this study, various CD137 ECD variants were designed based on their annotated structural motifs that are cysteine rich domains (CRD) and a stalk proximal to the membrane (UniProtKB, Q07011; FIG. 4A):

>sp|Q07011|CD137 ECD|aa 24-186 extracellular domain*

LQDPCSN CPAGTFCDNN RNQICS	**CRD1:** amino acid 24 to 46
PCPP NSFSSAGGQR TCDICRQCKG VFRTRKECSS TSNAEC	**CRD2**: amino acid 47 to 86
DCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCK	**CRD3:** amino acid 87 to 118
DC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP	**CRD4:** amino acid 119 to 160
SPADLSPGAS SVTPPAPARE PGHSPQ	**stalk:** amino acid 161 to 186

* The extra cellular domain of CD137 (UniProt accession number: Q07011; full sequence of CD137 see SEQ ID NO: 32), spanning the amino acids 24 to 186, consists of 4 cysteine rich domains (CRD1 to 4) and a stalk proximal to the membrane. The underlined residues indicate the amino acid sequence within which epitope of utomilumab is located (WO 2012/032433).

The motif combinations (FIG. 4B) were N-terminally attached to a PreScission protease site (3C site) and a human hinge-Fc domain. The membrane proximal motifs CRD4 and stalk were always exclusively built in together. In total eight CD137 ECD variants and PRO1480 were included in the binding analysis (FIG. 4, Table 20). Expression of the proteins was performed in FreeStyle CHO-S cells using the transient CHOgro expression system (Mirus). The genes of interest were optimized for mammalian expression, synthesized and cloned into a standard pcDNA3.1 vector. The signal sequences originate from mouse heavy chain IgG. Expression cultures were cultivated in batch for 6 to 7 days (cell viability < 70%) either at 37 °C or for one day at 37 °C followed by a temperature shift to 32 °C for five to six days. The culture supernatants were separated by centrifugation followed by 0.45 µm filtration. The target proteins were captured from the clarified culture supernatants by Protein L affinity chromatography for PRO1480 or by Protein A affinity chromatography for the CD137 ECD variants followed by polishing size-exclusion chromatography. During the binding experiment, PRO1480 was incubated with each of the CD137 ECD variants at equimolar ratios at a concentration at least 500 fold above KD. The evaluation of binding, yes or no, was conducted with retention time shift analysis by SE-HPLC of the complex relative to the individual proteins. The results are summarized in Table 21.

Table 20. Expression construct sequences.

Name	Sequence
>Q07011|ECD|Full-Fc (SEQ ID NO: 45)	mgwslillflvavatgvhslqdpcsncpagtfcdnnrnqicspcppnsfssaggqrtcdicrqckgv frtrkecsstsnaecdctpgfhclgagcsmceqdckqgqeltkkgckdccfgtfndqkrgicrpwt ncsldgksvlvngtkerdvvcgpspadlspgassvtppaparepghspqlevlfqgplevlfqgpd kthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvev hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvy tlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksr wqqgnvfscsvmhealhnhytqkslslspgk
>Q07011|ECD|CRD1-Fc (SEQ ID NO: 46)	Mgwslillflvavatgvhslqdpcsncpagtfcdnnrnqicslevlfqgpdkthtcppcpapeaag gpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynsty rvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlppsreemtknqvslt clvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhea lhnhytqkslslspgk
>Q07011|ECD|CRD1-2-Fc (SEQ ID NO: 47)	mgwslillflvavatgvhslqdpcsncpagtfcdnnrnqicspcppnsfssaggqrtcdicrqckgv frtrkecsstsnaeclevlfqgpdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvv dvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnka lgapiektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennyk ttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk
>Q07011|ECD|CRD1-2-3-Fc (SEQ ID NO: 48)	mgwslillflvavatgvhslqdpcsncpagtfcdnnrnqicspcppnsfssaggqrtcdicrqckgv frtrkecsstsnaecdctpgfhclgagcsmceqdckqgqeltkkgcklevlfqgpdkthtcppcpa peaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree qynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlppsreemt knqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk
>Q07011|ECD|CRD2-3-4-Fc (SEQ ID NO: 49)	mgwslillflvavatgvhspcppnsfssaggqrtcdicrqckgvfrtrkecsstsnaecdctpgfhcl gagcsmceqdckqgqeltkkgckdccfgtfndqkrgicrpwtncsldgksvlvngtkerdvvcgp levlfqgpdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfn wyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakg qprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsffl yskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk
>Q07011|ECD|CRD3-4-Fc (SEQ ID NO: 50)	mgwslillflvavatgvhsdctpgfhclgagcsmceqdckqgqeltkkgckdccfgtfndqkrgicr pwtncsldgksvlvngtkerdvvcgplevlfqgpdkthtcppcpapeaaggpsvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwl ngkeykckvsnkalgapiektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiave wesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp gk
>Q07011|ECD|CRD4-Fc (SEQ ID NO: 51)	mgwslillflvavatgvhsdccfgtfndqkrgicrpwtncsldgksvlvngtkerdvvcgplevlfqg pdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepq vytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvd ksrwqqgnvfscsvmhealhnhytqkslslspgk
>Q07011|ECD|CRD2-3-Fc (SEQ ID NO: 52)	mgwslillflvavatgvhspcppnsfssaggqrtcdicrqckgvfrtrkecsstsnaecdctpgfhcl gagcsmceqdckqgqeltkkgcklevlfqgpdkthtcppcpapeaaggpsvflfppkpkdtlmi srtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngk eykckvsnkalgapiektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewes ngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

Table 21. Summary of binding of PRO1480 to the various CD137 ECD variants.

CD137 ECD variant name	CD137 ECD variant architecture	PRO1480 (38-27-A11)
Q07011|ECD|Full-Fc	CRD1-2-3-4-stalk-3C site-Fc	**Yes**
Q07011|ECD|CRD1-Fc	CRD1-3C site-Fc	No
Q07011|ECD|CRD1-2-Fc	CRD1-2-3C site-Fc	**Yes**
Q07011|ECD|CRD1-2-3-Fc	CRD1-2-3-3C site-Fc	**Yes**
Q07011|ECD|CRD2-3-4-Fc	CRD2-3-4-3C site-Fc	No
Q07011|ECD|CRD3-4-Fc	CRD3-4-3C site-Fc	No
Q07011|ECD|CRD4-Fc	CRD4-3C site-Fc	No
Q07011|ECD|CRD2-3-Fc	CRD2-3-3C site-Fc	No

THE MULTISPECIFIC MOLECULES COMPRISING THE ANTIBODY OF THE INVENTION

The exemplary multispecific molecules comprising the antibody of the invention are included in Table 3. PRO1480 and PRO1481 are derived from 38-27-A11 sc02 and 38-27-A11 sc03, respectively.

Example 9: Affinities to PDL1, CD137, HSA and MSA.

Affinity to PDL1 was determined by SPR measurements using a Biacore T200 device (GE Healthcare). In this experiment, Fc tagged PDL1 from different species was captured using the Human Antibody Capture kit from GE Healthcare (cat. BR-1008-39). After each analyte injection cycle, the anti-human Fc-specific IgG was regenerated and new antigen was captured. For all formats, the multispecific molecules were injected as analyte using a dose response multi-cycle kinetic assay with concentrations of the analyte ranging from 0.18 to 45 nM (two-fold dilutions steps) diluted in running buffer. Association and dissociation time were set to 300 s and 720 s, respectively. The apparent dissociation (k_d) and association (k_a) rate constants and the apparent dissociation equilibrium constant (KD) were calculated using one-to-one Langmuir binding model. Affinity to CD137 of the different species was determined using the identical setup as for PDL1 with the exception that CD137-Fc chimeric proteins from different species were captured by the immobilized antibody.

The Fc containing formats were directly captured by the antibody specific for the Fc region of human IgGs. Two-fold serial dilutions of PDL1 extracellular domain or CD137 extracellular domain ranging from 90 to 0.35 nM were tested for binding to the IgG captured on the biosensor chip. After each injection cycle, surfaces were regenerated with one injection of a 3 M $MgCl_2$ solution.

Affinity of molecules to serum albumin (SA) of the different species was determined by SPR measurements using a Biacore T200 device (GE Healthcare). SA was directly coupled to a CM5 sensor chip (GE Healthcare) using amine coupling chemistry. After performing a regeneration scouting and surface performance test to find best assay conditions, a dose response was measured and obtained binding curves were double-referenced (empty reference channel and zero analyte injection) and fitted using the 1:1 Langmuir model to retrieve kinetic parameters. The assay was run in a 1 X PBS-Tween buffer at pH 5.5.

The data obtained are summarized in Table 22. The measurement of binding kinetics of the CD137 specific humanized constructs derived from clone 38-27-A11 show nearly identical affinities (compare CDR graft of clone 38-27-A11, PRO1480, and STR graft PRO1481 in Table 22).

Example 10: Assessment of the CD137 agonistic effect of anti-PDL1xCD137 molecules by using a cell-based assay of transgenic NF-kB Jurkat reporter cell line expressing CD137.

In this assay the activation of CD137 signaling in Jurkat cells was assessed. The activity of CD137 signaling is reported by measurement of Luciferase expression which is driven by CD137 induced NF-kB activation in a Jurkat reporter cell line. The expression of Luciferase directly correlates with the activity of CD137. Moreover, clustering of CD137 which is required for activation of the signal pathway is facilitated via then formation of an immunological synapse between the Jurkat cells and a PDL1 expressing cell line. Therefore, PDL1 expression is needed for clustering and activation of CD137 on the reporter cell line.

HCC827 cells stimulated for 24h with 10 ng/ml IFNy to increase PDL1 expression were seeded at 25,000 cells per well on 96-well culture plates. Then, serial dilutions of the anti-PDL1xCD137 molecules as well as the competitor urelumab were prepared and added to the cells. Next, Jurkat reporter cells were prepared in assay medium containing HSA at 25 mg/ml or without and added at a cell density of 40,000 cells per well. Luciferase expression was detected by addition of Luciferase reagent and was read by a luminescence reader 6 or 24 h after addition of Jurkat cells. Data were analyzed by normalization the relative luminescence units (RLU) of the test samples to the RLU measured for PRO885 (FIG. 5) yielding values of the relative activation of CD137 signaling. As shown in FIG. 5 and Table 23, scDb-scFvs PRO1480 and PRO1481 derived from clone 38-27-A11 were able to stimulate CD137 signaling.

TABLE 22. Affinities of different formats to PDL1, CD137 and serum albumin from different species.

		Affinity to human PD-L1			Affinity to cynomolgus PD-L1			Affinity to human CD137			Affinity to cynomolgus CD137			Affinity to human SA		
PRO ID	Format	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)
PRO885	scDb	2.1E+06	1.4E-04	6.5E-11	ND	ND	ND	2.4E+05	7.6E-04	3.2E-09	3.4E+05	7.0E-04	2.1E-09	NA	NA	NA
PRO1186	scDb-scFv	6.2E+06	2.3E-05	3.7E-12	TBD	TBD	TBD	1.9E+05	5.0E-04	2.6E-09	TBD	TBD	TBD	2.5E+05	7.2E-04	2.9E-09
PRO1480	scDb-scFv	3.5E+06	5.1E-05	1.5E-11	4.0E+06	3.4E-05	8.5E-12	5.8E+05	2.2E-04	3.7E-10	6.4E+05	3.0E-04	4.7E-10	3.27E+05	3.37E-04	1.03E-09
PRO1481	scDb-scFv	4.7E+06	4.3E-05	9.2E-12	ND	ND	ND	6.3E+05	2.0E-04	3.3E-10	ND	ND	ND	ND	ND	ND

NA: not applicable
TBD: to be determined
NB: no significant binding
ND: not determined

TABLE 23. NF-kB reporter gene activation by PDL1 x CD137 multispecific constructs.

		Activation of NF-kB reporter gene HCC827 +IFNg				
PRO ID	Format	Timepoint (h)	IC_{50} (ng/ml)	rel. IC_{50} [&]	max. activation (%)	HSA
PRO1480	scDb-scFv	6	6.44	1.93	114.3	yes
		24	4.67	1.00	120.9	yes
PRO1481	scDb-scFv	6	7.22	1.04	147.3	yes
		24	5.51	0.58	116.8	yes

NA: not applicable
ND: not determined

[&]: $IC_{50,\ PRO1186}$ (ng/ml)/$IC_{50,\ test\ molecule}$ (ng/ml)

Example 11: Assessment of the stimulatory effect of concomitant PDL1 blockade and CD137 stimulation in a cell-based assay using human PBMC stimulated with superantigen SEA.

In this experiment, the synergistic effect of PD-1/ PDL1 inhibition and CD137 agonism was assessed. The assay used peripheral blood mononuclear cells (PBMC) that were stimulated with the superantigen Staphylococcal Enterotoxin A (SEA) in order to induce expression of PDL1 on antigen-presenting cells (APC) and T cells respectively and CD137 on T-cells. By applying anti-PDL1xCD137 molecules two T-cell regulatory signaling pathways were targeted concomitantly: inhibition of the inhibitory PD-1/PDL1 pathway as well as activation of the CD137 pathway via formation of an immunological synapse mediated by the trispecific anti-PDL1xCD137xHSA molecule (PRO1480). The activation of T-cells was assessed by the secretion of Interleukin-2 (IL-2) and compared to the effect mediated by PDL1 inhibition mediated by a cocktail of the reference molecules avelumab and urelumab.

Peripheral blood mononuclear cells (PBMC) were isolated from fresh human whole blood by means of density gradient centrifugation. Then, PBMC were depleted for NK cells using anti-CD56 antibody and the MACS cell separation kit (Miltenyi Biotec). Next, 100,000 PBMCs per well were added to the 96-well plate, followed by the addition of serial dilutions of PRO1480 or of the combination of urelumab and avelumab in assay buffer containing SEA at a concentration of 10 ng/ml. After 96 h of incubation at 37°C and 5% CO_2, cell supernatants were harvested and human Interleukin-2 (IL-2) levels in the culture supernatants were quantified using the IL-2 human ELISA MAX assay from BioLegend according to kit instructions. IL-2 concentrations were interpolated from an IL-2 standard curve, back-calculated and plotted against avelumab/urelumab combination and PRO1480 concentrations for calculation of EC_{50} values.

As shown in FIG. 6, IL-2 was secreted by T-cells following concomitant blockade of PD-1/PDL1 interaction and stimulation of CD137 by the addition of PRO1480. When compared to the combination of avelumab and urelumab, PRO1480 showed higher T cell activation and better potency. This finding demonstrates that the trispecific anti-PDL1xCD137xHSA scDb-scFv PRO1480 is able to induce stronger T cell stimulation when compared to the cocktail of the reference molecules, avelumab and urelumab.

Example 12: Epitope-dependence of the stimulatory effect of concomitant PDL1 blockade and CD137 stimulation.

Example 11 was repeated by comparing the stimulatory effect of two constructs with different anti-CD137 antibody fragments. As shown in FIG. 7, which shows data from a single representative example out of a series of three to four individual experiments, IL-2 was secreted by T-cells following concomitant blockade of PD-1/PDL1 interaction and stimulation of CD137 by the addition of PRO1480 and PRO1186 (see Example 10). When compared to PRO1186 that targets a membrane proximal epitope on CRD4 of CD137 ECD (see FIG. 9(B)), PRO1480, that targets a membrane distal tip epitope on CRD1 and CRD2 on CD137 ECD (see FIG. 9(A)), showed higher maximum T cell activation as shown by higher IL-2 secretion and superior potency (FIG. 8, which shows the pooled results of said series of three to four individual experiments). This finding demonstrates that the trispecific anti-PDL1xCD137xHSA scDb-scFv PRO1480 targeting a membrane distal tip epitope is able to induce stronger T cell co-stimulation when compared to molecules, that target non-distal epitopes.

Example 13: Epitope determination

Structure determination of PRO1359 alone and in complex with the ECD of 4-1BB.

The scFv (PRO1359) was transiently expressed in CHO cells and purified from the harvest using Capto-L resin (GE-Healthcare). The protein was polished to a high monomeric content using a Superdex 75 size exclusion chromatography column (GE-Healthcare).

Residues 24-160 of 4-1BB (Uniprot: Q07011) were expressed with an N-terminal secretion sequence and C-terminal hingeless Fc-tag including a IdeS cleavage site [Novarra, S., et al., A hingeless Fc fusion system for site-specific cleavage by IdeS. mAbs, 2016. 8(6): p. 1118-1125]. The fusion protein was transiently expressed in CHO cells and captured from the cell supernatant using protein A resin (GE Healthcare). The Fc-tag was cleaved by IdeS at 37°C and removed using protein A beads.

Crystallization of the anti-4-1BB scFv PRO1359

The anti-4-1BB scFv is concentrated to 10 mg/ml in 25 mM Hepes, 100 mM NaCl, pH 6.7. Crystallization conditions were screened by the sitting drop vapor diffusion technique with a mother liquor to protein ratio of 1:1. Crystals were grown in 0.1 M Tris pH 8.5, 2 M $NH_4H_2PO_4$ and cryo-protected by adding 100 % ethylene glycol to the mother liquor to a final concentration of 20%.

Crystallization of the complex of anti-4-1BB scFv PRO1359 and the ECD of 4-1BB.

The complex was formed by mixing an equimolar ratio of both proteins followed by complex purification with a Superdex 75 size exclusion chromatography column in 50 mM Hepes, 100 mM NaCl, pH 6.7.

Fractions corresponding to the complex were concentrated to 10 mg/ml. Initial crystals were grown by sitting drop vapor diffusion at 20°C in 0.1 M sodium acetate, pH 5.5, 22 % PEG2000 MME, 0.17 M to 0.23 M calcium acetate and an equal volume of mother liquor and complex. Crystals were crushed using SeedBeads (Hampton Research) and used as crystals seeds in subsequent crystallization screens.

Final crystals were grown at 20°C in 0.1 M Tris acetate pH 8.5, 1 M sodium formate, 25 % PEG2000 MME with a mother liquor to protein to seed ratio of 1:1:0.125. Crystals were cryoprotected in 80 mM Tris acetate pH 8.5, 0.8 M sodium formate, 22.4 % PEG2000 MME, 20% ethylene glycol and frozen in liquid nitrogen.

Diffraction experiment and structure solution

A native dataset of the scFv alone and in complex with 4-1BB was collected at the Swiss Light Source at the Paul-Scherrer Institute, Villigen, Switzerland. The scFv crystallized in space group $P6_5$ and was processed using XDS to a resolution of 1.6 Å. The complex crystallized in space group I222 and was processed using XDS to a resolution of 2.2 Å [Kabsch, W., XDS. Acta Crystallographica Section D, 2010. 66(2): p. 125-132.].

The structure of the scFv was solved by molecular replacement with Phaser using an in-house scFv model [McCoy, A.J., et al., Phaser crystallographic software. Journal of Applied Crystallography, 2007. 40(4): p. 658-674]. The complex structure was solved by molecular replacement using the scFv apo structure and a reduced model of 4-1BB (pdb code: 6BWV, chain D).

Refinement

Structures were refined using Refmac [Murshudov, G.N., A.A. Vagin, and E.J. Dodson, Refinement of Macromolecular Structures by the Maximum-Likelihood Method. Acta Crystallographica Section D, 1997. 53(3): p. 240-255.]. Manual model building was executed in Coot [Emsley, P., et al., Features and development of Coot. Acta Crystallographica Section D, 2010. 66(4): p. 486-501.].

The apo structure was refined to a resolution of 1.6 Å with residues 1-110 and 128-251 being well defined by the electron density and final Rwork/Rfree values of 15.8 %/18 %.

The complex structure was similarly refined to a resolution of 2.2 Å. For CD137, residues 24-158 are visible in the electron density with residues 139-149 and 156-158 being only weakly defined. The scFv is well defined for residues 3-109 and 131-252. The complex was refined to Rwork/Rfree values of 19.2 %/23.5 %.

5

Interface description

CD137 epitope

The binding interface is analyzed using the PISA service of the European Bioinformatics Institute [Krissinel, E. and K. Henrick, Inference of Macromolecular Assemblies from

10 Crystalline State. Journal of Molecular Biology, 2007. 372(3): p. 774-797.]. The epitope of CD137 is located within the first and second cysteine rich domain (CRD). The accessible surface area that is buried upon binding of the scFv is 770 $Å^2$ (see Table 26). Critical residues are stated in Table 24. The hydrogen bond network is summarized in **Table** 25.

Table 24. Residues of CD137 involved in the binding to the scFv

CD137	Priority	>50% buried	Specific side chain hydrogen bond interaction	Involved in Hydrogen bond network	Critical interaction with important binding residues of antibody fragment	% of accessible surface buried upon binding
ASN30						18.5%
PRO32	3	X				76.1%
ALA33	2			X		34.1%
GLY34	3	X				100.0%
THR35	3	X				98.9%
PHE36						3.2%
ASN40	2			X		6.3%
ARG41	1	X	X	X	X	78.2%
ASN42				X		25.6%
GLN43	1	X	X	X		54.9%
CYS45	1	X		X	X	92.1%
SER46	3	X				84.3%
PRO47	3	X				100.0%
CYS48	2	X		X		100.0%
PRO49	1			X	X	34.7%
PRO50	3	X				76.6%
ASN51						12.8%
SER52	1			X	X	25.4%
CYS65						16.7%
GLU77						15.8%
CYS78	3	X				57.8%
SER79	3	X				72.1%
SER80	1	X	X	X		100.0%
THR81						31.4%

Table 25. Overview of hydrogen bonds between CD137 and PRO1359

#	CD137	Distance [Å]	PRO1359
1	ARG 41[NH1]	3.23	TYR 92[O]
2	ARG 41[NH2]	3.26	TYR 92[O]
3	ARG 41[NH1]	2.83	TYR 93[O]*
4	ASN 42[N]	2.70	ASN 95[OD1]
5	SER 80[OG]	3.17	ALA 161[O]
6	SER 80[OG]	2.81	ASN 162[OD1]
7	SER 80[N]	2.82	ASN 162[OD1]
8	ASN 40[O]	3.87	ASN 95[ND2]
9	ALA 33[O]	2.87	TYR 183[OH]
10	GLN 43[OE1]	3.54	THR 190[N]
11	CYS 45[O]	2.89	TYR 191[OH]*
12	SER 52[O]	3.08	TRP 233[NE1]*
13	PRO 49[O]	3.23	TRP 233[NE1]*
14	CYS 48[O]	2.94	TRP 237[NE1]

* If mutated to alanine the affinity was reduced by >1000 fold

Table 26. Summary of binding interface

	CD137		PRO1359	
Number of residues				
interface	24	17.80%	20	8.70%
surface	133	98.50%	215	93.90%
total	135	100.00%	229	100.00%
Solvent-accessible area, Å^2				
interface	770.5	8.40%	750.8	7.20%
total	9133.6	100.00%	10466.4	100.00%
Solvation energy, kcal/mol				
isolated structure	-82.2	100.00%	-209.8	100.00%
gain on complex formation	-3.1	3.70%	-1.6	0.80%

CLAIMS

1. An isolated antibody having a binding specificity for human CD137, which comprises: HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2, and 3, respectively, and LCDR1, LCDR 2 and LCDR3 sequences of SEQ ID NOs: 16, 17 and 18, respectively, wherein said antibody binds human CD137 extracellular domain at an epitope located in the distal part of the extracellular domain of CD137 within amino acid residues 24-86 of SEQ ID NO: 32, wherein said epitope is characterized by a set of critical residues, determined in accordance with Example 13, comprising the residues Ala33, Asn40, Arg 41, Gln43, Cys45, Cys48, Pro49, Ser52, and Ser80; provided that amino acid residue Asn42 of CD137 is not a critical residue for binding.

2. The antibody of claim 1 , wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 14, and 15; and a light chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 25, 26, and 27.

3. The antibody of claim 2, comprising: (a) a VH sequence of SEQ ID NO: 13 and a VL sequence of SEQ ID NO: 25; (b) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 26; or (c) a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 27.

4. The antibody of claim 1, wherein said antibody does not inhibit the interaction between CD137 and its ligand CD137L as measured by the competition ELISA.

5. The antibody of claim 1, wherein said antibody
   (i) binds to human CD137 with a dissociation constant (KD) of less than 50 nM, as measured by surface plasmon resonance;
   (ii) optionally, binds to Cynomolgus CD137 with a KD of less than 50 nM, as measured by surface plasmon resonance;
   (iii) optionally, does not bind to human CD40 and/or does not bind to human OX40 as measured by SPR;

(iv) when in scFv format, has a melting temperature (Tm), determined by differential scanning fluorimetry, of at least 50°C wherein said antibody is formulated in 50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl;

(v) when in scFv format, has a loss in monomer content, after storage for at least two weeks at 4°C, of less than 7%, when the antibody is at a starting concentration of 10 mg/ml, and wherein the antibody in scFv format is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or

(vi) when in scFv format, has a loss in monomer content, after five consecutive freeze-thaw cycles of less than 5%, when the antibody is at a starting concentration of 10 mg/ml, and wherein the antibody in scFv format is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

6. The antibody of claim 1, wherein the isolated antibody is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, a Fab, an Fv, an scFv, dsFv, an scAb, STAB, and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody Technology), preferably Fv or scFv.

7. The antibody of claim 6, wherein said antibody is an scFv having an amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31.

8. The isolated antibody of claim 1, wherein said epitope characterized by a set of critical residues, determined in accordance with Example 13, further comprises the residues Pro32, Gly34, Thr35, Ser46, Pro47, Pro50, Cys78, and Ser79.

9. The antibody of claim 1, wherein the antibody is a multispecific molecule having at least a second functional molecule.

10. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier.

11. The antibody of claim 1 or the composition of claim 10 for use as a medicament.

12. Use of the antibody of claim 1, or the composition of claim 10 in the manufacture of a medicament for the treatment of a cancer.

13. A nucleic acid encoding the antibody of claim 1.

14. A method of producing the antibody of claim 1, the method comprising the step of culturing a host cell comprising a nucleic acid or a vector encoding the antibody of claim 1.