Agonistic TNF receptor binding agents

Technical Field of the Invention

The present invention relates to binding agents binding to receptors of the TNF superfamily, in particular binding agents binding to at least two different receptors of the TNF superfamily, as well as to their use in medicine. The present invention further relates to nucleic acid molecules encoding such binding agents, to cells comprising such nucleic acid molecules and to pharmaceutical compositions and kits.

Background of the Invention

Receptors for tumor necrosis factor (TNF) superfamily ligands are oligomeric, mostly trimeric, type I or type III transmembrane proteins and contain one to six cysteine-rich domains (CRDs) in their extracellular domain (Naismith J.H. and Sprang S.R. (1998), TRENDS in Biochemical Sciences 23(2):74-79). Moreover, several receptors for TNF superfamily ligands contain death domains (DDs) that recruit caspase-interacting proteins following ligand binding to initiate the extrinsic pathway of caspase activation.

Numerous receptors of the TNF superfamily have been identified so far, including 4-1BB (CD137), BAFFR, BCMA, CD27, CD30, CD40, DCR3, DR3 (TRAMP), DR6, EDAR, Fas (CD95), FN14 (TWEAK-R), GITR, HVEM, LTβR, NGFR (CD271), OPG, OX40 (CD134), RANK (TRANCE-R), RELT, TACI, TNFR1, TNFR2, TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, TROY and XEDAR (Bodmer, J.L. et al. (2002), TRENDS in Biochemical Sciences, 27(1):19-26).

Through timely expression and intricate signaling, receptors of the TNF superfamily and their corresponding ligands act to regulate cell responses, including activation, proliferation, differentiation, and apoptosis. While critical to the regulation of beneficial processes, such as immune defense and hematopoiesis, TNF signaling is also implicated in tumorigenesis, transplant rejection, virus replication, bone resorption, diabetes and autoimmune diseases.

Agonistic agents specifically binding to one or more receptors of the TNF superfamily are, therefore, considered as potential therapeutics in the prevention and/or treatment of various diseases and disorders.

Accordingly, it was an object of the present invention to provide novel agonistic binding agents targeting receptors of the TNF superfamily, in particular agonistic binding agents targeting at least two different receptors of the TNF superfamily.

Summary of the Invention

In one aspect, the present invention relates to a binding agent comprising at least two binding domains, wherein a first binding domain binds to a first receptor of the tumor necrosis factor (TNF) superfamily and a second binding domain binds to a second receptor of the TNF superfamily, wherein the first receptor and the second receptor are different.

In one embodiment, the binding agent is an agonistic binding agent.

In one embodiment, the binding agent is a bispecific molecule, preferably a bispecific antibody.

In one embodiment, the binding agent is in the format of a full-length antibody or an antibody fragment.

In one embodiment, the first receptor and the second receptor are selected from the group consisting of CD40, CD27, OX40 (CD134) and 4-1BB (CD137).

In one embodiment, the first binding domain or the second binding domain binds to CD27, wherein the binding agent comprises

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or
the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3 of an antibody selected from the group consisting of AB27_1 to AB27_186 as shown in Table 7; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof

of an antibody selected from the group consisting of AB27_1 to AB27_186 as shown in Table 7.

In one embodiment, the first binding domain or the second binding domain binds to OX40 (CD134), wherein the binding agent comprises

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or
the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3
of an antibody selected from the group consisting of AB134_1 to AB134_92 as shown in Table 8; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof
of an antibody selected from the group consisting of AB134_1 to AB134_92 as shown in Table 8.

In one embodiment, the first binding domain or the second binding domain binds to 4-1BB (CD137), wherein the binding agent comprises

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or
the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3
of an antibody selected from the group consisting of AB137_1 to AB137_12 as shown in Table 9; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof
of an antibody selected from the group consisting of AB137_1 to AB137_12 as shown in Table 9.

In one embodiment, the first binding domain or the second binding domain binds to CD40, wherein the binding agent comprises

(a) heavy chain complementarity determining region(s) HCDR1 having the amino acid sequence of SEQ ID NO: 2323, HCDR2 having the amino acid sequence of SEQ ID NO: 2324 and/or HCDR3 having the amino acid sequence of SEQ ID NO: 2325, and/or
light chain complementarity determining region(s) LCDR1 having the amino acid sequence of SEQ ID NO: 2326, LCDR2 having the amino acid sequence of SEQ ID NO: 2327 and/or LCDR3 having the amino acid sequence of SEQ ID NO: 2328; and/or

(b) heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2321 or a variant or fragment thereof and/or light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2322 or a variant or fragment thereof.

In one embodiment,

(a) the first binding domain binds to CD40 and the second binding domain binds to CD27;

(b) the first binding domain binds to CD40 and the second binding domain binds to OX40 (CD134);

(c) the first binding domain binds to CD40 and the second binding domain binds to 4-1BB (CD137);

(d) the first binding domain binds to 4-1BB (CD137) and the second binding domain binds to CD27;

(e) the first binding domain binds to 4-1BB (CD137) and the second binding domain binds to OX40 (CD134); or

(f) the first binding domain binds to OX40 (CD134) and the second binding domain binds to CD27.

In one embodiment, the binding agent comprises one or more heavy chain constant domains of an immunoglobulin and/or light chain constant domains of an immunoglobulin.

In another aspect, the present invention relates to a binding agent that binds to CD27, comprising

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or
the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3
of an antibody selected from the group consisting of AB27_1 to AB27_186 as shown in Table 7; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof
of an antibody selected from the group consisting of AB27_1 to AB27_186 as shown in Table 7.

In another aspect, the present invention relates to a binding agent that binds to OX40 (CD134), comprising

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or

the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3 of an antibody selected from the group consisting of AB134_1 to AB134_92 as shown in Table 8; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof of an antibody selected from the group consisting of AB134_1 to AB134_92 as shown in Table 8.

In another aspect, the present invention relates to a binding agent that binds to 4-1BB (CD137), comprising

(a) the heavy chain complementarity determining region(s) HCDR1, HCDR2 and/or HCDR3, and/or
the light chain complementarity determining region(s) LCDR1, LCDR2 and/or LCDR3 of an antibody selected from the group consisting of AB137_1 to AB137_12 as shown in Table 9; and/or

(b) the heavy chain variable domain (VH) or a variant or fragment thereof and/or the light chain variable domain (VL) or a variant or fragment thereof of an antibody selected from the group consisting of AB137_1 to AB137_12 as shown in Table 9.

In one embodiment, the binding agent is an agonistic binding agent.

In one embodiment, the binding agent is in the format of a full-length antibody or an antibody fragment.

In one embodiment, the binding agent comprises one or more heavy chain constant domains of an immunoglobulin and/or light chain constant domains of an immunoglobulin.

In another aspect, the present invention relates to a binding agent binding to the same epitope(s) as a binding agent as defined above.

In another aspect, the present invention relates to a nucleic acid molecule encoding a binding agent as defined above, wherein, preferably, the nucleic acid molecule is contained in a vector.

In another aspect, the present invention relates to a cell comprising a nucleic acid molecule as defined above.

In another aspect, the present invention relates to a pharmaceutical composition comprising, as an active agent, a binding agent as defined above, a nucleic acid molecule as defined above, or a cell as defined above.

In one embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

In another aspect, the present invention relates to a kit comprising a binding agent as defined above, a nucleic acid molecule as defined above, a cell as defined above, or a pharmaceutical composition as defined above.

In another aspect, the present invention relates to a binding agent as defined above, a nucleic acid molecule as defined above, a cell as defined above, or a pharmaceutical composition as defined above for use as a medicament.

In another aspect, the present invention relates to a binding agent as defined above, a nucleic acid molecule as defined above, a cell as defined above, or a pharmaceutical composition as defined above for use in the treatment of a disease selected from the group consisting of cancer, infectious diseases, inflammatory diseases, metabolic diseases, autoimmune disorders, degenerative diseases, apoptosis-associated diseases and transplant rejections.

Brief Description of the Figures

Figure 1 shows binding curves of reference antibodies against CD27, CD134 and CD137 to the respective recombinant protein, as determined by enzyme-linked immunosorbent assay (ELISA).

Figure 2 shows the expression of CD27, CD134 and CD137 on the cell surface of HT1080 cell lines overexpressing CD27, CD134 and CD137, respectively, as determined by fluorescence-activated cell sorting (FACS).

Figure 3 shows binding curves of reference antibodies against CD27, CD134 and CD137 to CD27, CD134 and CD137 expressed on the cell surface of corresponding HT1080 cell lines, as determined by a cellular binding assay.

Figure 4 shows the activation curve of an agonistic anti-CD27 reference antibody with or without cross-linking, as determined by ELISA measuring the IL-8 concentration in the supernatants of CD27-overexpressing HT1080 cells.

Figure 5 shows exemplary activation curves of agonistic anti-CD27, anti-CD134 and anti-CD137 antibodies under cross-linking conditions, as determined by ELISA measuring the IL-8 concentration in the supernatants of target-overexpressing HT1080 cells.

Figure 6 shows the expression of CD27, CD40, CD134 and CD137 on the cell surface of stable transfectants of HEK293_NFK_gfp_luc cells (**A**) and of stable transfectants of K562 cells (**B**) overexpressing CD27, CD40, CD134 and CD137, respectively, as determined by FACS (white curves: control without antibody; grey curves: antibody staining).

Figure 7 shows the analysis of two examples (**A** and **B**) of a bispecific antibody simultaneously targeting CD40 and CD137 (CD137xCD40). Activation of CD137 was measured by luciferase activity (relative light units, RLU) of HEK293_NFK_CD137_gfp_luc upon incubation with indicated bispecific antibodies and K562_CD40 for trans-presentation or K562_wt for control. Activation of CD40 was measured by luciferase activity (RLU) of HEK293_NFK_CD40_gfp_luc upon incubation with indicated bispecific antibodies and K562_CD137 for trans-presentation or K562_wt for control. The two parental monospecific, bivalent antibodies (CD137 IgG1 or CD40 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD137 x control, CD40 x control) served as controls for the bispecific CD137xCD40 antibodies. Only bispecific CD137xCD40 antibodies resulted in activation of CD137 and CD40 upon trans-presentation by CD40 and CD137, respectively. Used sequences of variable domains of anti-CD137 and anti-CD40 antibodies are identified by the abbreviation AB137 and AB40, respectively.

Figure 8 shows the analysis of a bispecific antibody simultaneously targeting CD40 and CD27 (CD27xCD40). Activation of CD27 was measured by luciferase activity (RLU) of HEK293_NFK_CD27_gfp_luc upon incubation with the indicated bispecific antibody and K562_CD40 for trans-presentation or K562_wt for control. Activation of CD40 was measured

by luciferase activity (RLU) of HEK293_NFK_CD40_gfp_luc upon incubation with the indicated bispecific antibody and K562_CD27 for trans-presentation or K562_wt for control. The two parental monospecific, bivalent antibodies (CD27 IgG1 or CD40 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD27 x control, CD40 x control) served as controls for the bispecific CD27xCD40 antibody. The bispecific CD27xCD40 antibody resulted in superior activation of CD27 and CD40 upon trans-presentation by CD40 and CD27, respectively. Used sequences of variable domains of anti-CD27 and anti-CD40 antibodies are identified by the abbreviation AB27 and AB40, respectively.

Figure 9 shows the analysis of two examples (**A** and **B**) of a bispecific antibody simultaneously targeting CD40 and CD134 (CD134xCD40). Activation of CD134 was measured by luciferase activity (RLU) of HEK293_NFK_CD134_gfp_luc upon incubation with the indicated bispecific antibodies and K562_CD40 for trans-presentation or K562_wt for control. Activation of CD40 was measured by luciferase activity (RLU) of HEK293_NFK_CD40_gfp_luc upon incubation with antibodies and K562_CD134 for trans-presentation or K562_wt for control. The two parental monospecific, bivalent antibodies (CD134 IgG1 or CD40 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD134 x control, CD40 x control) served as controls for the bispecific CD134xCD40 antibodies. Bispecific CD134xCD40 antibodies resulted in superior activation of CD134 and CD40 upon trans-presentation by CD40 and CD134, respectively. Used sequences of variable domains of anti-CD134 and anti-CD40 antibodies are identified by the abbreviation AB134 and AB40, respectively.

Figure 10 shows the expression of CD27, CD134 and CD137 on the cell surface of double transfectants of HEK293_NFK_gfp_luc cells overexpressing CD27 + CD134 (HEK293_NFK_CD27_CD134_gfp_luc), CD134 + CD137 (HEK293_NFK_CD134_CD137_gfp_luc) and CD27 + CD137 (HEK293_NFK_CD27_CD137_gfp_luc), respectively, as determined by FACS (white curves: control without antibody; grey curves: antibody staining).

Figure 11 shows the analysis of a bispecific antibody simultaneously targeting CD27 and CD134 (CD134xCD27). Activation of CD27 and CD134 was measured by luciferase activity of HEK293_NFK_CD27_CD134_gfp_luc upon incubation with the indicated bispecific antibody; maximum RLUs are shown. The two parental monospecific, bivalent antibodies (CD134 IgG1 or CD27 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD134 x control, CD27 x control) served as controls for the bispecific CD134xCD27 antibody. The bispecific

CD134xCD27 antibody resulted in superior activation of HEK293_NFK_CD27_CD134_gfp_luc as compared to the four corresponding controls. Used sequences of variable domains of anti-CD134 and anti-CD27 antibodies are identified by the abbreviation AB134 and AB27, respectively.

Figure 12 shows the analysis of two examples (**A** and **B**) of a bispecific antibody simultaneously targeting CD134 and CD137 (CD134xCD137). Activation of CD134 and CD137 was measured by luciferase activity of HEK293_NFK_CD134_CD137_gfp_luc upon incubation with the indicated bispecific antibodies; maximum RLUs are shown. The two parental monospecific, bivalent antibodies (CD134 IgG1 or CD137 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD134 x control, CD137 x control) served as controls for the bispecific CD134xCD137 antibodies. Bispecific CD134xCD137 antibodies resulted in activation of HEK293_NFK_CD134_CD137_gfp_luc, whereas the four corresponding controls did not result in measurable activation of the double transfectant. Used sequences of variable domains of anti-CD134 and anti-CD137 antibodies are identified by the abbreviation AB134 and AB137, respectively.

Figure 13 shows the analysis of two examples (A and B) of a bispecific antibody simultaneously targeting CD27 and CD137 (CD137xCD27). Activation of CD27 and CD137 was measured by luciferase activity of HEK293_NFK_CD27_CD137_gfp_luc upon incubation with the indicated bispecific antibodies; maximum RLUs are shown. The two parental monospecific, bivalent antibodies (CD137 IgG1 or CD27 IgG1) and (ii) the two monovalent antibodies with one irrelevant arm (CD137 x control, CD27 x control) served as controls for the bispecific CD137xCD27 antibodies. Bispecific CD134xCD27 antibodies resulted in superior activation of HEK293_NFK_CD27_CD137_gfp_luc as compared to the two control antibodies with one irrelevant arm. Used sequences of variable domains of anti-CD137 and anti-CD27 antibodies are identified by the abbreviation AB137 and AB27, respectively.

Detailed Description of the Invention

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all

technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., Helvetica Chimica Acta, CH-4010 Basel, Switzerland, (1995).

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, cell biology, immunology, and recombinant DNA techniques which are explained in the literature in the field (cf., e.g., Molecular Cloning: A Laboratory Manual, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step or group of members, integers or steps but not the exclusion of any other member, integer or step or group of members, integers or steps although in some embodiments such other member, integer or step or group of members, integers or steps may be excluded, i.e. the subject-matter consists in the inclusion of a stated member, integer or step or group of members, integers or steps. The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into

the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), provided herein is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether *supra* or *infra*, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The term "receptor of the TNF superfamily" preferably refers to a receptor selected from the group consisting of 4-1BB (CD137), BAFFR, BCMA, CD27, CD30, CD40, DCR3, DR3 (TRAMP), DR6, EDAR, Fas (CD95), FN14 (TWEAK-R), GITR, HVEM, LTβR, NGFR (CD271), OPG, OX40 (CD134), RANK (TRANCE-R), RELT, TACI, TNFR1, TNFR2, TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, TROY and XEDAR.

The term "receptor of the TNF superfamily", as used herein, also includes variants of a given receptor of the TNF superfamily.

The term "variant" according to the invention refers, in particular, to mutants, splice variants, conformation variants, isoforms, allelic variants, species variants and species homologs, in particular those which are naturally present. An allelic variant relates to an alteration in the normal sequence of a gene, the significance of which is often unclear. Complete gene sequencing often identifies numerous allelic variants for a given gene. A species homolog is a nucleic acid or amino acid sequence with a different species of origin from that of a given nucleic acid or amino acid sequence. The term "variant" shall encompass any posttranslationally modified variants and conformation variants.

According to the present invention, the first receptor and the second receptor of the TNF superfamily are preferably selected from the group consisting of CD40, CD27, OX40 (CD134) and 4-1BB (CD137).

CD40, also referred to as tumor necrosis factor receptor superfamily member 5 (TNFRSF5), is the receptor for the ligand TNFSF5/CD40L. CD40 is known to transduce TRAF6- and MAP3K8-mediated signals that activate ERK in macrophages and B-cells, leading to induction of immunoglobulin secretion. In one embodiment, CD40 is human CD40, having UniProt accession number P25942.

CD27, also referred to as TNFRSF7, is the receptor for the ligand CD70/CD27L. CD27 is believed to play a role in survival of activated T cells and a role in apoptosis through association with SIVA1. In one embodiment, CD27 is human CD27, having UniProt accession number P26842.

OX40 (CD134), also referred to as TNFRSF4, is the receptor for the ligand TNFSF4/OX40L/GP34. OX40 (CD134) is a costimulatory molecule implicated in long-term T cell immunity. In one embodiment, OX40 (CD134) is human OX40 (CD134), having UniProt accession number P43489.

4-1BB (CD137), also referred to as TNFRSF9, is the receptor for the ligand TNFSF9/4-1BBL. 4-1BB (CD137) is believed to be involved in T cell activation. In one embodiment, 4-1BB (CD137) is human 4-1BB (CD137), having UniProt accession number Q07011.

The term "binding domain" characterizes in connection with the present invention a structure, e.g., of an antibody, which binds to/interacts with a given target structure/antigen/epitope. Thus, the binding domain according to the invention designates an "antigen-interaction site" or "antigen-binding site".

The term "antigen" relates to an agent such as a protein or peptide comprising an epitope against which an immune response is directed and/or is to be directed.

The term "peptide" according to the invention comprises oligo- and polypeptides and refers to substances comprising two or more, preferably 3 or more, preferably 4 or more, preferably 6 or more, preferably 8 or more, preferably 9 or more, preferably 10 or more, preferably 13 or more, preferably 16 more, preferably 21 or more and up to preferably 8, 10, 20, 30, 40 or 50, in particular 100 amino acids joined covalently by peptide bonds. The term "protein" refers to large peptides, preferably to peptides with more than 100 amino acid residues, but in general the terms "peptides" and "proteins" are synonyms and are used interchangeably herein.

The term "epitope" refers to an antigenic determinant in a molecule, i.e., to the part in a molecule that is recognized by the immune system, for example, that is recognized by an antibody. For example, epitopes are the discrete, three-dimensional sites on an antigen, which are recognized by the immune system. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. An epitope of a protein preferably comprises a continuous or discontinuous portion of said protein and is preferably between 5 and 100, preferably between 5 and 50, more preferably between 8 and 30, most preferably between 10 and 25 amino acids in length, for example, the epitope may be preferably 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length.

Mapping of epitopes recognized by binding agents can be performed as described in detail in "Epitope Mapping Protocols" (Methods in Molecular Biology) by Glenn E. Morris ISBN-089603-375-9 and in "Epitope Mapping: A Practical Approach" Practical Approach Series, 248 by Olwyn M. R. Westwood, Frank C. Hay.

The term "agonistic binding agent", as used herein, refers to a binding agent, which activates the receptor(s) of the TNF superfamily it is binding to. In one embodiment, the binding agent induces IL-8 release from and/or activates the NF-κB pathway in the cell(s) expressing the receptor(s) of the TNF superfamily it is binding to. In one embodiment, the binding agent is a trans-activating binding agent, simultaneously activating receptors of the TNF superfamily, e.g., said first and second receptor, located on different cells. In another embodiment, the binding agent is a cis-activating binding agent, simultaneously activating receptors of the TNF superfamily, e.g., said first and second receptor, located on the same cell.

The term "binding" according to the invention preferably relates to a specific binding. A binding agent, such as an antibody or fragment thereof, is specific for a predetermined target if it is capable of binding to said predetermined target while it is not (substantially) capable of binding to other targets.

According to the present invention, a binding agent, such as an antibody or fragment thereof, is capable of binding to a predetermined target if it has a significant affinity for said predetermined target and binds to said predetermined target in standard assays. "Affinity" or "binding affinity"

is often measured by equilibrium dissociation constant (K_D). Preferably, the term "significant affinity" refers to the binding to a predetermined target with a dissociation constant (K_D) of 10^{-5} M or lower, 10^{-6} M or lower, 10^{-7} M or lower, 10^{-8} M or lower, 10^{-9} M or lower, 10^{-10} M or lower, 10^{-11} M or lower, or 10^{-12} M or lower.

An agent is not (substantially) capable of binding to a target if it has no significant affinity for said target and does not bind significantly, in particular does not bind detectably, to said target in standard assays. Preferably, the agent does not detectably bind to said target if present in a concentration of up to 2, preferably 10, more preferably 20, in particular 50 or 100 µg/ml or higher. Preferably, an agent has no significant affinity for a target if it binds to said target with a K_D that is at least 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold, or 10^6-fold higher than the K_D for binding to the predetermined target to which the agent is capable of binding. For example, if the K_D for binding of an agent to the target to which the agent is capable of binding is 10^{-7} M, the K_D for binding to a target for which the agent has no significant affinity would be at least 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M, or 10^{-1} M.

Binding of an agent to a target can be determined experimentally using any suitable method; see, for example, Berzofsky et al., "Antibody-Antigen Interactions" In Fundamental Immunology, Paul, W. E., Ed., Raven Press New York, N Y (1984), Kuby, Janis Immunology, W. H. Freeman and Company New York, N Y (1992), and methods described herein. Affinities may be readily determined using conventional techniques, such as by equilibrium dialysis; by using the BIAcore 2000 instrument, using general procedures outlined by the manufacturer; by radioimmunoassay using radiolabeled target antigen; or by another method known to the skilled artisan. The affinity data may be analyzed, for example, by the method of Scatchard et al., Ann N.Y. Acad. ScL, 51:660 (1949). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions, e.g., salt concentration, pH. Thus, measurements of affinity and other antigen-binding parameters, e.g., K_D, IC_{50}, are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

The term "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. The term "antibody" includes monoclonal antibodies, recombinant antibodies, human antibodies, humanized antibodies, chimeric antibodies and combinations of any of the foregoing. Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (CH). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The

variable regions and constant regions are also referred to herein as variable domains and constant domains, respectively. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). 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 CDRs of a VH are termed HCDR1, HCDR2 and HCDR3, the CDRs of a VL are termed LCDR1, LCDR2 and LCDR3. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of an antibody comprise the heavy chain constant region (CH) and the light chain constant region (CL), wherein CH can be further subdivided into constant domain CH1, a hinge region, and constant domains CH2 and CH3 (arranged from amino-terminus to carboxy-terminus in the following order: CH1, CH2, CH3). 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.

Antibodies may be derived from different species, including but not limited to mouse, rat, rabbit, guinea pig and human.

Antibodies described herein include IgA such as IgA1 or IgA2, IgG1, IgG2, IgG3, IgG4, IgE, IgM, and IgD antibodies. In various embodiments, the antibody is an IgG1 antibody, more particularly an IgG1, kappa or IgG1, lambda isotype (i.e. IgG1, κ, λ), an IgG2a antibody (e.g. IgG2a, κ, λ), an IgG2b antibody (e.g. IgG2b, κ, λ), an IgG3 antibody (e.g. IgG3, κ, λ) or an IgG4 antibody (e.g. IgG4, κ, λ).

As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes. "Isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

The term "monoclonal antibody", as used herein, refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity. In one embodiment, the monoclonal antibodies are produced by a hybridoma which includes a B-cell obtained from a non-human animal, e.g., a mouse, fused to an immortalized cell.

The term "recombinant antibody", as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal with respect to the immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

The term "recombinant" in the context of the present invention means "made through genetic engineering". Preferably, a "recombinant object", such as a recombinant antibody or nucleic acid" is not naturally occurring.

The term "naturally occurring", as used herein, as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*).

The term "humanized antibody" refers to a molecule having an antigen binding site that is substantially derived from an immunoglobulin from a non-human species, wherein the remaining immunoglobulin structure of the molecule is based upon the structure and/or sequence of a human immunoglobulin. The antigen binding site may either comprise complete variable regions (VH and VL) fused onto constant domains (e.g., the constant domains of IgG1/κ), or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable regions. Antigen binding sites may be wild-type or modified by one or more amino acid substitutions, e.g. modified to resemble human immunoglobulins more closely. Some forms of humanized antibodies preserve all CDR sequences (for example a humanized mouse antibody which contains all six CDRs from the mouse antibody). Other forms have one or more CDRs which are altered with respect to the original antibody.

The term "chimeric antibody" refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chain is homologous to corresponding sequences in another. Typically, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are homologous to sequences of antibodies derived from another. One clear advantage to such chimeric forms is that the variable region can conveniently be derived from presently known sources using readily available B-cells or hybridomas from non-human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation and the specificity is not affected by the source, the constant region being human, is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non human source. However, the definition is not limited to this particular example.

The antibodies described herein are preferably isolated. The term "isolated antibody", as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to an epitope, isoform or variant of a given receptor of the TNF superfamily may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., species homologs of said receptor). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "antibody fragment" refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen and may also be referred to as "antigen-binding fragment" of an antibody. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antibody fragment" include (i) Fab fragments, monovalent fragments consisting of the VL, VH, CL and CH regions; (ii) $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) Fd fragments consisting of the VH and CH regions; (iv) Fv fragments consisting of the VL and VH regions of a single arm of an antibody, (v) dAb fragments (Ward et al., (1989) Nature 341: 544-546), which consist of a VH region; (vi) isolated complementarity determining regions (CDR), and (vii) combinations of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two regions/domains of the Fv fragment, VL and VH,

are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242: 423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85: 5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antibody fragment". A further example is a binding-domain immunoglobulin fusion protein comprising (i) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain CH2 constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain CH3 constant region fused to the CH2 constant region. The binding domain polypeptide can be a heavy chain variable region or a light chain variable region. The binding-domain immunoglobulin fusion proteins are further disclosed in US 2003/0118592 and US 2003/0133939. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The terms "part" or "fragment" are used interchangeably herein and refer to a continuous element. For example, a part of a structure such as an amino acid sequence or protein refers to a continuous element of said structure. A portion, a part or a fragment of a structure preferably comprises one or more functional properties of said structure. For example, a portion, a part or a fragment of an epitope or peptide is preferably immunologically equivalent to the epitope or peptide it is derived from. A part or fragment of a protein sequence preferably comprises a sequence of at least 6, in particular at least 8, at least 12, at least 15, at least 20, at least 30, at least 50, or at least 100 consecutive amino acids of the protein sequence.

When used in connection with VH and VL domains, the term "fragment" preferably refers to one or more of the complementarity-determining regions (CDRs), preferably at least the CDR3 variable region, of the heavy chain variable region (VH) and/or of the light chain variable region (VL). In one embodiment, said one or more of the complementarity-determining regions (CDRs) are selected from a set of complementarity-determining regions CDR1, CDR2 and CDR3. In a particularly preferred embodiment, the term "fragment" refers to the complementarity-determining regions CDR1, CDR2 and CDR3 of the heavy chain variable region (VH), i.e. HCDR1, HCDR2 and HCDR3, and/or of the light chain variable region (VL), i.e. LCDR1, LCDR2 and LCDR3.

The teaching given herein with respect to specific amino acid sequences, e.g., those shown in the sequence listing, is to be construed so as to also relate to variants of said specific sequences resulting in sequences which are functionally equivalent to said specific sequences, e.g., amino acid sequences exhibiting properties identical or similar to those of the specific amino acid sequences. One important property is to retain binding to a target.

For example, the sequences shown in the sequence listing can be modified so as to remove one or more, preferably all free cysteine residues, in particular by replacing the cysteine residues by amino acids other than cysteine, preferably serine, alanine, threonine, glycine, tyrosine, leucine or methionine, most preferably alanine or serine.

It will be appreciated by those skilled in the art that, in particular, the sequences of the CDRs and variable regions can be modified without losing the ability to bind the first and/or the second receptor of the TNF superfamily. For example, CDR regions will be either identical or highly homologous to the regions of antibodies specified herein. By "highly homologous" it is contemplated that from 1 to 5, preferably from 1 to 4, such as 1 to 3 or 1 or 2 substitutions may be made in the CDRs. In addition, the hypervariable and variable regions may be modified so that they show substantial homology with the regions of antibodies specifically disclosed herein.

For the purposes of the present invention, "variants" of an amino acid sequence, in particular variants of a heavy chain variable domain (VH) or of a light chain variable domain (VL), comprise amino acid insertion variants, amino acid addition variants, amino acid deletion variants and/or amino acid substitution variants. Amino acid deletion variants that comprise the deletion at the N-terminal and/or C-terminal end of the protein are also called N-terminal and/or C-terminal truncation variants.

Amino acid insertion variants comprise insertions of single or two or more amino acids in a particular amino acid sequence. In the case of amino acid sequence variants having an insertion, one or more amino acid residues are inserted into a particular site in an amino acid sequence, although random insertion with appropriate screening of the resulting product is also possible.

Amino acid addition variants comprise amino- and/or carboxy-terminal fusions of one or more amino acids, such as 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids.

Amino acid deletion variants are characterized by the removal of one or more amino acids from

the sequence, such as by removal of 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids. The deletions may be in any position of the protein.

Amino acid substitution variants are characterized by at least one residue in the sequence being removed and another residue being inserted in its place. Preference is given to modifications being in positions in the amino acid sequence which are not conserved between homologous proteins or peptides and/or to replacing amino acids with other ones having similar properties. Preferably, amino acid substitutions in protein variants are conservative amino acid substitutions. A conservative amino acid substitution involves substitution of an amino acid with another one of the same family of amino acids, i.e., amino acids which are related in their side chains (e.g., in terms of the electrical charge and/or size). Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids.

Preferably the degree of similarity, preferably identity between a given amino acid sequence and an amino acid sequence which is a variant of said given amino acid sequence will be at least about 60%, 65%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. The degree of similarity or identity is given preferably for an amino acid region which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference amino acid sequence. For example, if the reference amino acid sequence consists of 200 amino acids, the degree of similarity or identity is given preferably for at least about 20, at least about 40, at least about 60, at least about 80, at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 amino acids, preferably continuous amino acids. In preferred embodiments, the degree of similarity or identity is given for the entire length of the reference amino acid sequence. The alignment for determining sequence similarity, preferably sequence identity can be done with art known tools, preferably using the best sequence alignment, for example, using Align, using standard settings, preferably EMBOSS::needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5.

"Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions. "Sequence identity" between two amino acid sequences indicates the percentage of amino acids that are identical between the sequences.

The term "percentage identity" is intended to denote a percentage of amino acid residues which are identical between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly and over their entire length. Sequence comparisons between two amino acid sequences are conventionally carried out by comparing these sequences after having aligned them optimally, said comparison being carried out by segment or by "window of comparison" in order to identify and compare local regions of sequence similarity. The optimal alignment of the sequences for comparison may be produced, besides manually, by means of the local homology algorithm of Smith and Waterman, 1981, Ads App. Math. 2, 482, by means of the local homology algorithm of Neddleman and Wunsch, 1970, J. Mol. Biol. 48, 443, by means of the similarity search method of Pearson and Lipman, 1988, Proc. Natl Acad. Sci. USA 85, 2444, or by means of computer programs which use these algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.).

The percentage identity is calculated by determining the number of identical positions between the two sequences being compared, dividing this number by the number of positions compared and multiplying the result obtained by 100 so as to obtain the percentage identity between these two sequences.

Binding agents in accordance with the present invention may be at least bispecific or multispecific, such as trispecific, tetraspecific and so on.

The term "bispecific molecule" refers to a molecule that has two different binding specificities and thus may bind to two different types of antigen, such as a first and a second receptor of the TNF superfamily. Such bispecific molecule may be in the format of an antibody molecule or of an antibody-like molecule or of a protein scaffold with antibody-like properties or of a cyclic peptide with at least two binding specificities.

The term "bispecific antibody", as used herein, refers to an antibody comprising two antigen-binding sites, a first binding site having affinity for a first antigen or epitope and a second

binding site having binding affinity for a second antigen or epitope distinct from the first. In particular, a bispecific antibody is an artificial protein that is composed of fragments of two different antibodies (said fragments of two different antibodies forming two binding domains) and consequently binds to two different types of antigen.

A bispecific molecule/antibody according to the invention is engineered to simultaneously bind to a first receptor of the TNF superfamily and to a second receptor of the TNF superfamily, wherein the first receptor and the second receptor are different and may be located on the same cell ("cis") or different cells ("trans").

In one embodiment, the first receptor and the second receptor are located on the same cell and are selected from the group consisting of CD27, OX40 (CD134) and 4-1BB (CD137). In one embodiment, said cell is a T cell.

In another embodiment, the first receptor and the second receptor are located on different cells, wherein the first receptor is CD40 and the second receptor is selected from the group consisting of CD27, OX40 (CD134) and 4-1BB (CD137). In one embodiment, said different cells are an antigen-presenting cell (APC), such as a dendritic cell, expressing CD40 and a T cell expressing the second receptor selected from the group consisting of CD27, OX40 (CD134) and 4-1BB (CD137).

Bispecific (full-length) antibodies may be obtained by covalently/chemically linking two (monoclonal) antibodies or by conventional hybrid-hybridoma techniques. Covalent linking of two monoclonal antibodies is described, for example, in Anderson (1992), Blood 80:2826-34. In the context of this invention, one of the antibodies is specific for the first receptor of the TNF superfamily and the other one for the second receptor of the TNF superfamily.

Alternatively, bispecific (full-length) antibodies may be obtained by using the knobs-into-holes technology, which allows the hetero-dimerization of antibody heavy chains by introducing specific mutations in the CH3 dimer interface, e.g., in the CH3 dimer interface of IgG1 or IgG4. This technology is described, for example, in Spiess C. et al. (2013), Journal of Biological Chemistry 288(37):26583-93; Labrijn A.F. et al. (2013), PNAS 110(13):5145-50; and WO 2008/119353 A1.

In one embodiment, the bispecific antibody is a bispecific single-chain antibody.

As used herein, a "bispecific single-chain antibody" denotes a single polypeptide chain comprising two binding domains. Each binding domain comprises one VH region, wherein the VH region of the first binding domain specifically binds to the first receptor of the TNF superfamily (REC1), and the VH region of the second binding domain specifically binds to the second receptor of the TNF superfamily (REC2). The two binding domains are optionally linked to one another by a short peptide linker. Each binding domain may additionally comprise one variable region from an antibody light chain (VL), the VH region and VL region within each of the first and second binding domains being linked to one another via a long peptide linker, which is long enough to allow the VH region and VL region of the first binding domain and the VH region and VL region of the second binding domain to pair with one another.

In one embodiment, the corresponding variable heavy chain regions (VH) and the corresponding variable light chain regions (VL) are arranged, from N-terminus to C-terminus, in the order VH(REC1)-VL(REC1)-VH(REC2)-VL(REC2), VH(REC2)-VL(REC2)-VH(REC1)-VL(REC1) or VH(REC2)-VL(REC2)-VL(REC1)-VH(REC1). It is, however, also envisaged that the bispecific single chain antibodies of the invention comprise other domain arrangements, such as VL(REC1)-VH(REC1)-VH(REC2)-VL(REC2), VL(REC1)-VH(REC1)-VL(REC2)-VH(REC2), VH(REC1)-VL(REC1)-VL(REC2)-VH(REC2), VL(REC2)-VH(REC2)-VH(REC1)-VL(REC1), VL(REC2)-VH(REC2)-VL(REC1)-VH(REC1).

Peptide linkers are generally designed to provide flexibility and protease resistance. In one embodiment, the peptide linker is a glycine-serine-threonine-rich linker or glycine-serine-rich linker, wherein at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90% of the amino acids are a glycine or serine or threonine residue or a glycine or serine residue, respectively. In another embodiment, the amino acids are selected from glycine, serine and threonine, i.e., the peptide linker is exclusively composed of glycine, serine and threonine residues (referred to as a glycine-serine-threonine linker). In yet another embodiment, the peptide linker is exclusively composed of glycine and serine residues (referred to as a glycine-serine linker). Short peptide linkers may consist of 12 or less such as 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 amino acids, and preferably, 5 or 6 amino acids. Short peptide linkers preferably comprise the amino acid sequences SGGGGS or GGGGS. Long peptide linkers may consist of 12 or more, such as 15 to 25 or 15 to 20 or 15 to 18 amino acids, and may, for example, be selected from $(GGGGS)_3$,$(GGGGS)_4$ and $(GGGGS)_5$. In one embodiment, the binding agent of the invention is in the format of a bispecific antibody fragment.

The binding agent according to the invention may be one of various types of bivalent and trivalent single-chain variable fragments (scFvs), fusion proteins mimicking the variable domains of two antibodies. A single-chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a peptide linker of ten to about 25 amino acids. The peptide linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. Divalent (or bivalent) single-chain variable fragments (di-scFvs, bi-scFvs) can be engineered by linking two scFvs. This can be done by producing a single peptide chain with two VH and two VL regions, yielding tandem scFvs. The invention also includes multispecific molecules comprising more than two scFvs binding domains. This makes it possible that the molecule comprises either multiple antigen specificities and is a trispecific, tetraspecific, or multispecific molecule, or the molecule is a bispecific molecule comprising more than one scFv binding domain with specificity for the same antigen. In particular, the molecule of the invention may be a multispecific single chain Fv.

In one embodiment, the binding agent comprises two Fab regions, one being directed against the first receptor of the TNF superfamily and the other being directed against the second receptor of the TNF superfamily. In one embodiment, the binding agent is an antigen-binding fragment (Fab)2 complex. The (Fab)2 complex is composed of two Fab fragments, one Fab fragment comprising a Fv domain, i.e. VH and VL domains, binding to the first receptor of the TNF superfamily, and the other Fab fragment comprising a Fv domain binding to the second receptor of the TNF superfamily. Each of the Fab fragments may be composed of two single chains, a VL-CL module and a VH-CH module. Alternatively, each of the individual Fab fragments may be arranged in a single chain, preferably, VL-CL-CH-VH, and the individual variable and constant domains may be connected with a peptide linker. In general, the individual single chains and Fab fragments may be connected via disulfide bonds, adhesive domains, chemical linkage and/or peptide linker(s). The binding agent may also comprise more than two Fab fragments, in particular, the molecule may be a Fab3, Fab4, or a multimeric Fab complex with specificity for 2, 3, 4, or more different antigens. The invention also includes chemically linked Fabs.

Another possibility is the creation of scFvs with peptide linkers that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Still shorter peptide linkers (one or two amino acids) lead to the formation of trimers, so-called triabodies or tribodies. Tetrabodies have also been produced. They exhibit an even higher affinity to their targets than diabodies.

A diabody (Kipriyanov, Int. J. Cancer 77 (1998), 763-772) is a small bivalent and bispecific antibody fragment. Diabodies comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) on the same polypeptide chain (VH-VL) connected by a peptide linker that is too short to allow pairing between the two domains on the same chain. This forces pairing with the complementary domains of another chain and promotes the assembly of a dimeric molecule with two functional antigen-binding sites. To construct bispecific diabodies of the invention, the V-domains of an antibody binding to the first receptor of the TNF superfamily (REC1) and an antibody binding to the second receptor of the TNF superfamily (REC2) may be fused to create the two chains VH(REC1)-VL(REC2) and VH(REC2)-VL(REC1). Each chain by itself is not able to bind to the respective antigen, but recreates the functional antigen binding sites of an anti-REC1 antibody and an anti-REC2 antibody on pairing with the other chain. To this end, a peptide linker that is too short to allow pairing between the two domains on the same chain is used. The two scFv molecules, with a linker between heavy chain variable domain and light chain variable domain that is too short for intramolecular dimerization, are co-expressed and self assemble to form bi-specific molecules with the two binding sites at opposite ends.

In one embodiment, the bispecific antibody fragment binding to the first receptor of the TNF superfamily (REC1) and the second receptor of the TNF superfamily (REC2) is a minibody, preferably, a minibody comprising two single VH-VL-C chains that are connected with each other via the constant domain (C) of each chain. According to this aspect, the corresponding variable heavy chain regions (VH), corresponding variable light chain regions (VL) and constant domains (C) are arranged, from N-terminus to C-terminus, in the order VH(REC1)-VL(REC1)-(C) and VH(REC2)-VL(REC2)-C, wherein C is preferably a CH3 domain. Pairing of the constant domains results in formation of the minibody.

In one embodiment, the bispecific binding agent is in the format of an antibody-like molecule with a heavy chain containing two consecutive N-terminal variable domains with different specificities and a light chain with two consecutive variable domains with different specificities resulting in four binding domains with two different specificities (Wu et al. (2007), Nat. Biotechnology 25(11))

Binding agents according to the invention may also comprises an amino acid sequence for facilitating secretion of the molecule, such as a N-terminal secretion signal, and/or one or more labels or tags facilitating binding, purification or detection of the molecule.

Preferably, the secretion signal is a signal sequence that allows a sufficient passage through the secretory pathway and/or secretion of the binding agent into the extracellular environment. Preferably, the secretion signal sequence is cleavable and is removed from the mature binding agent. The secretion signal sequence preferably is chosen with respect to the cell or organism wherein the binding agent is produced in.

A "label or tag facilitating binding, purification or detection of the molecule" is meant to include any labels/tags known in the art for these purposes. Particularly preferred are affinity tags, such as chitin binding protein (CBP), maltose binding protein (MBP), glutathione-S-transferase (GST) and poly(His) (e.g., His_6); solubilization tags, such as thioredoxin (TRX) and poly(NANP); chromatography tags, such as a FLAG-tag; epitope tags, such as V5-tag, myc-tag and HA-tag; and fluorescent or luminescent labels or tags, such as fluorescent proteins (e.g., GFP, YFP, RFP etc.), fluorescent dyes and luciferase.

The amino acid sequence of a (poly)peptide label or tag may be introduced at any position within the amino acid sequence of the binding agent, and may, for example, take the shape of a loop within the encoded protein structure or it may be N-terminally or C-terminally fused. The label or tag may further contain a cleavage site that allows a removal of the label or tag from the binding agent. Similarly, non-peptidic labels or tags, e.g., fluorescent dyes, may be conjugated to the binding agent at any suitable site.

Binding agents of the invention may contain, e.g., in addition to said first and second binding domain, a further binding domain which serves e.g. to enhance selectivity for a specific cell type. This can be achieved, e.g., by providing a binding domain that binds to another antigen expressed on said specific cell type.

Binding agents of the invention may further comprise one or more modifications increasing the stability of the binding agent. The term "stability" of the binding agent relates to the "half-life" of the binding agent, e.g., *in vivo*. "Half-life" relates to the period of time which is needed to eliminate half of the activity, amount, or number of molecules.

The binding agent may, for example, be conjugated to a half-life extension module. Such modules are known to a person skilled in the art and include, for example, albumin, an albumin-binding domain, an immunoglobulin-binding domain, an FcRn-binding motif, and, in particular,

a polymer. Particularly preferred polymers include polyethylene glycol (PEG), hydroxyethyl starch (HES), polysialic acid and PEG-mimetic peptide sequences.

The binding agents described herein may be conjugated to a therapeutic moiety or agent, such as a cytotoxin, a drug (e.g., an immunosuppressant) or a radioisotope. A cytotoxin or cytotoxic agent includes any agent that is detrimental to and, in particular, kills cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Suitable therapeutic agents for forming conjugates include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabin, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC), and anti-mitotic agents (e.g., vincristine and vinblastine). In a preferred embodiment, the therapeutic agent is a cytotoxic agent or a radiotoxic agent. In another embodiment, the therapeutic agent is an immunosuppressant. In yet another embodiment, the therapeutic agent is GM-CSF. In a preferred embodiment, the therapeutic agent is doxorubicin, cisplatin, bleomycin, sulfate, carmustine, chlorambucil, cyclophosphamide or ricin A.

Binding agents also can be conjugated to a radioisotope, e.g., iodine-131, yttrium-90 or indium-111, to generate cytotoxic radiopharmaceuticals.

Techniques for conjugating such therapeutic moiety to binding agents, such as antibodies or fragments thereof, are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pincheraet al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in

Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62: 119-58 (1982).

The present invention provides a binding agent comprising at least two binding domains, wherein a first binding domain binds to a first receptor of the tumor necrosis factor (TNF) superfamily and a second binding domain binds to a second receptor of the TNF superfamily, wherein the first receptor and the second receptor are different.

In one embodiment, the first binding domain binds to CD40 and the second binding domain binds to CD27, wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of antibody AB40_1, and
one or more CDRs or one or more variable domains of an antibody AB27_X, wherein X is selected from the group consisting of 1 to 186, as shown in Table 7.

"AB40_1" refers to an antibody binding to CD40, which comprises

(a) heavy chain complementarity determining regions HCDR1 having the amino acid sequence of SEQ ID NO: 2323, HCDR2 having the amino acid sequence of SEQ ID NO: 2324 and HCDR3 having the amino acid sequence of SEQ ID NO: 2325, and
light chain complementarity determining regions LCDR1 having the amino acid sequence of SEQ ID NO: 2326, LCDR2 having the amino acid sequence of SEQ ID NO: 2327 and LCDR3 having the amino acid sequence of SEQ ID NO: 2328; and

(b) heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2321 and light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2322.

In various embodiments, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 1; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 2; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 3; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 4; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 5; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 6; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 7; the binding agent comprises one or

more CDRs or one or more variable domains of antibody AB40_1 and X is 8; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 9; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 10; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 11; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 12; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 13; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 14; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 15; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 16; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 17; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 18; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 19; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 20; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 21; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 22; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 23; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 24; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 25; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 26; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 27; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 28; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 29; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 30; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 31; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 32; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 33; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 34; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 35; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 36;

the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 37; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 38; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 39; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 40; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 41; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 42; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 43; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 44; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 45; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 46; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 47; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 48; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 49; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 50; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 51; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 52; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 53; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 54; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 55; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 56; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 57; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 58; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 59; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 60; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 61; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 62; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 63; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 64; the binding agent comprises one or more CDRs or one or more variable

domains of antibody AB40_1 and X is 65; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 66; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 67; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 68; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 69; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 70; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 71; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 72; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 73; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 74; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 75; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 76; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 77; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 78; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 79; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 80; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 81; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 82; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 83; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 84; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 85; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 86; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 87; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 88; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 89; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 90; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 91; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 92; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 93; the binding agent comprises one

or more CDRs or one or more variable domains of antibody AB40_1 and X is 94; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 95; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 96; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 97; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 98; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 99; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 100; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 101; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 102; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 103; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 104; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 105; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 106; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 107; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 108; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 109; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 110; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 111; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 112; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 113; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 114; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 115; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 116; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 117; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 118; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 119; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 120; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 121; the binding agent comprises one or more CDRs or

one or more variable domains of antibody AB40_1 and X is 122; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 123; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 124; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 125; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 126; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 127; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 128; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 129; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 130; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 131; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 132; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 133; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 134; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 135; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 136; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 137; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 138; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 139; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 140; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 141; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 142; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 143; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 144; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 145; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 146; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 147; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 148; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 149; the binding agent comprises one or more CDRs or

one or more variable domains of antibody AB40_1 and X is 150; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 151; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 152; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 153; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 154; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 155; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 156; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 157; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 158; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 159; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 160; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 161; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 162; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 163; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 164; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 165; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 166; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 167; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 168; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 169; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 170; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 171; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 172; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 173; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 174; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 175; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 176; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 177; the binding agent comprises one or more CDRs or

one or more variable domains of antibody AB40_1 and X is 178; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 179; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 180; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 181; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 182; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 183; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 184; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 185; or the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 186.

In one embodiment, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 5.

In one embodiment, the first binding domain binds to CD40 and the second binding domain binds to OX40 (CD134), wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of antibody AB40_1, as defined above, and
one or more CDRs or one or more variable domains of an antibody AB134_X, wherein X is selected from the group consisting of 1 to 92, as shown in Table 8.

In various embodiments, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 1; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 2; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 3; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 4; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 5; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 6; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 7; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 8; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 9; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 10; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 11; the binding agent comprises one or more CDRs or

one or more variable domains of antibody AB40_1 and X is 12; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 13; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 14; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 15; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 16; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 17; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 18; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 19; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 20; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 21; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 22; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 23; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 24; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 25; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 26; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 27; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 28; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 29; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 30; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 31; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 32; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 33; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 34; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 35; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 36; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 37; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 38; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 39; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 40; the binding

agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 41; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 42; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 43; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 44; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 45; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 46; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 47; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 48; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 49; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 50; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 51; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 52; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 53; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 54; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 55; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 56; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 57; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 58; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 59; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 60; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 61; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 62; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 63; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 64; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 65; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 66; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 67; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 68; the binding agent comprises one or more CDRs or one or more variable domains of

antibody AB40_1 and X is 69; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 70; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 71; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 72; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 73; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 74; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 75; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 76; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 77; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 78; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 79; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 80; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 81; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 82; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 83; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 84; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 85; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 86; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 87; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 88; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 89; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 90; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 91; or the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 92.

In one embodiment, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 3. In another embodiment, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 81.

In one embodiment, the first binding domain binds to CD40 and the second binding domain binds to 4-1BB (CD137), wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of antibody AB40_1, as defined above, and
one or more CDRs or one or more variable domains of an antibody AB137_X, wherein X is selected from the group consisting of 1 to 12, as shown in Table 9.

In various embodiments, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 1; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 2; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 3; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 4; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 5; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 6; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 7; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 8; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 9; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 10; the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 11; or the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 12.

In one embodiment, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 2. In another embodiment, the binding agent comprises one or more CDRs or one or more variable domains of antibody AB40_1 and X is 12.

In one embodiment, the first binding domain binds to 4-1BB (CD137) and the second binding domain binds to CD27, wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of an antibody AB137_X, wherein X is selected from the group consisting of 1 to 12, as shown in Table 9, and
one or more CDRs or one or more variable domains of an antibody AB27_Y, wherein Y is selected from the group consisting of 1 to 186, as shown in Table 7.

In various embodiments, X is 1 and Y is selected from the group consisting of 1 to 186; X is 2 and Y is selected from the group consisting of 1 to 186; X is 3 and Y is selected from the group

consisting of 1 to 186; X is 4 and Y is selected from the group consisting of 1 to 186; X is 5 and Y is selected from the group consisting of 1 to 186; X is 6 and Y is selected from the group consisting of 1 to 186; X is 7 and Y is selected from the group consisting of 1 to 186; X is 8 and Y is selected from the group consisting of 1 to 186; X is 9 and Y is selected from the group consisting of 1 to 186; X is 10 and Y is selected from the group consisting of 1 to 186; X is 11 and Y is selected from the group consisting of 1 to 186; or X is 12 and Y is selected from the group consisting of 1 to 186.

In various embodiments,

X is 1 and Y is 1; X is 1 and Y is 2; X is 1 and Y is 3; X is 1 and Y is 4; X is 1 and Y is 5; X is 1 and Y is 6; X is 1 and Y is 7; X is 1 and Y is 8; X is 1 and Y is 9; X is 1 and Y is 10; X is 1 and Y is 11; X is 1 and Y is 12; X is 1 and Y is 13; X is 1 and Y is 14; X is 1 and Y is 15; X is 1 and Y is 16; X is 1 and Y is 17; X is 1 and Y is 18; X is 1 and Y is 19; X is 1 and Y is 20; X is 1 and Y is 21; X is 1 and Y is 22; X is 1 and Y is 23; X is 1 and Y is 24; X is 1 and Y is 25; X is 1 and Y is 26; X is 1 and Y is 27; X is 1 and Y is 28; X is 1 and Y is 29; X is 1 and Y is 30; X is 1 and Y is 31; X is 1 and Y is 32; X is 1 and Y is 33; X is 1 and Y is 34; X is 1 and Y is 35; X is 1 and Y is 36; X is 1 and Y is 37; X is 1 and Y is 38; X is 1 and Y is 39; X is 1 and Y is 40; X is 1 and Y is 41; X is 1 and Y is 42; X is 1 and Y is 43; X is 1 and Y is 44; X is 1 and Y is 45; X is 1 and Y is 46; X is 1 and Y is 47; X is 1 and Y is 48; X is 1 and Y is 49; X is 1 and Y is 50; X is 1 and Y is 51; X is 1 and Y is 52; X is 1 and Y is 53; X is 1 and Y is 54; X is 1 and Y is 55; X is 1 and Y is 56; X is 1 and Y is 57; X is 1 and Y is 58; X is 1 and Y is 59; X is 1 and Y is 60; X is 1 and Y is 61; X is 1 and Y is 62; X is 1 and Y is 63; X is 1 and Y is 64; X is 1 and Y is 65; X is 1 and Y is 66; X is 1 and Y is 67; X is 1 and Y is 68; X is 1 and Y is 69; X is 1 and Y is 70; X is 1 and Y is 71; X is 1 and Y is 72; X is 1 and Y is 73; X is 1 and Y is 74; X is 1 and Y is 75; X is 1 and Y is 76; X is 1 and Y is 77; X is 1 and Y is 78; X is 1 and Y is 79; X is 1 and Y is 80; X is 1 and Y is 81; X is 1 and Y is 82; X is 1 and Y is 83; X is 1 and Y is 84; X is 1 and Y is 85; X is 1 and Y is 86; X is 1 and Y is 87; X is 1 and Y is 88; X is 1 and Y is 89; X is 1 and Y is 90; X is 1 and Y is 91; X is 1 and Y is 92; X is 1 and Y is 93; X is 1 and Y is 94; X is 1 and Y is 95; X is 1 and Y is 96; X is 1 and Y is 97; X is 1 and Y is 98; X is 1 and Y is 99; X is 1 and Y is 100; X is 1 and Y is 101; X is 1 and Y is 102; X is 1 and Y is 103; X is 1 and Y is 104; X is 1 and Y is 105; X is 1 and Y is 106; X is 1 and Y is 107; X is 1 and Y is 108; X is 1 and Y is 109; X is 1 and Y is 110; X is 1 and Y is 111; X is 1 and Y is 112; X is 1 and Y is 113; X is 1 and Y is 114; X is 1 and Y is 115; X is 1 and Y is 116; X is 1 and Y is 117; X is 1 and Y is 118; X is 1 and Y is 119; X is 1 and Y is 120; X is 1 and Y is 121; X is 1 and Y is 122; X is 1 and Y is 123; X is 1 and Y is 124; X is 1 and Y is 125; X is 1 and Y is 126; X is 1 and Y is 127; X is 1 and Y is 128; X is 1

and Y is 129; X is 1 and Y is 130; X is 1 and Y is 131; X is 1 and Y is 132; X is 1 and Y is 133; X is 1 and Y is 134; X is 1 and Y is 135; X is 1 and Y is 136; X is 1 and Y is 137; X is 1 and Y is 138; X is 1 and Y is 139; X is 1 and Y is 140; X is 1 and Y is 141; X is 1 and Y is 142; X is 1 and Y is 143; X is 1 and Y is 144; X is 1 and Y is 145; X is 1 and Y is 146; X is 1 and Y is 147; X is 1 and Y is 148; X is 1 and Y is 149; X is 1 and Y is 150; X is 1 and Y is 151; X is 1 and Y is 152; X is 1 and Y is 153; X is 1 and Y is 154; X is 1 and Y is 155; X is 1 and Y is 156; X is 1 and Y is 157; X is 1 and Y is 158; X is 1 and Y is 159; X is 1 and Y is 160; X is 1 and Y is 161; X is 1 and Y is 162; X is 1 and Y is 163; X is 1 and Y is 164; X is 1 and Y is 165; X is 1 and Y is 166; X is 1 and Y is 167; X is 1 and Y is 168; X is 1 and Y is 169; X is 1 and Y is 170; X is 1 and Y is 171; X is 1 and Y is 172; X is 1 and Y is 173; X is 1 and Y is 174; X is 1 and Y is 175; X is 1 and Y is 176; X is 1 and Y is 177; X is 1 and Y is 178; X is 1 and Y is 179; X is 1 and Y is 180; X is 1 and Y is 181; X is 1 and Y is 182; X is 1 and Y is 183; X is 1 and Y is 184; X is 1 and Y is 185; X is 1 and Y is 186;

X is 2 and Y is 1; X is 2 and Y is 2; X is 2 and Y is 3; X is 2 and Y is 4; X is 2 and Y is 5; X is 2 and Y is 6; X is 2 and Y is 7; X is 2 and Y is 8; X is 2 and Y is 9; X is 2 and Y is 10; X is 2 and Y is 11; X is 2 and Y is 12; X is 2 and Y is 13; X is 2 and Y is 14; X is 2 and Y is 15; X is 2 and Y is 16; X is 2 and Y is 17; X is 2 and Y is 18; X is 2 and Y is 19; X is 2 and Y is 20; X is 2 and Y is 21; X is 2 and Y is 22; X is 2 and Y is 23; X is 2 and Y is 24; X is 2 and Y is 25; X is 2 and Y is 26; X is 2 and Y is 27; X is 2 and Y is 28; X is 2 and Y is 29; X is 2 and Y is 30; X is 2 and Y is 31; X is 2 and Y is 32; X is 2 and Y is 33; X is 2 and Y is 34; X is 2 and Y is 35; X is 2 and Y is 36; X is 2 and Y is 37; X is 2 and Y is 38; X is 2 and Y is 39; X is 2 and Y is 40; X is 2 and Y is 41; X is 2 and Y is 42; X is 2 and Y is 43; X is 2 and Y is 44; X is 2 and Y is 45; X is 2 and Y is 46; X is 2 and Y is 47; X is 2 and Y is 48; X is 2 and Y is 49; X is 2 and Y is 50; X is 2 and Y is 51; X is 2 and Y is 52; X is 2 and Y is 53; X is 2 and Y is 54; X is 2 and Y is 55; X is 2 and Y is 56; X is 2 and Y is 57; X is 2 and Y is 58; X is 2 and Y is 59; X is 2 and Y is 60; X is 2 and Y is 61; X is 2 and Y is 62; X is 2 and Y is 63; X is 2 and Y is 64; X is 2 and Y is 65; X is 2 and Y is 66; X is 2 and Y is 67; X is 2 and Y is 68; X is 2 and Y is 69; X is 2 and Y is 70; X is 2 and Y is 71; X is 2 and Y is 72; X is 2 and Y is 73; X is 2 and Y is 74; X is 2 and Y is 75; X is 2 and Y is 76; X is 2 and Y is 77; X is 2 and Y is 78; X is 2 and Y is 79; X is 2 and Y is 80; X is 2 and Y is 81; X is 2 and Y is 82; X is 2 and Y is 83; X is 2 and Y is 84; X is 2 and Y is 85; X is 2 and Y is 86; X is 2 and Y is 87; X is 2 and Y is 88; X is 2 and Y is 89; X is 2 and Y is 90; X is 2 and Y is 91; X is 2 and Y is 92; X is 2 and Y is 93; X is 2 and Y is 94; X is 2 and Y is 95; X is 2 and Y is 96; X is 2 and Y is 97; X is 2 and Y is 98; X is 2 and Y is 99; X is 2 and Y is 100; X is 2 and Y is 101; X is 2 and Y is 102; X is 2 and Y is 103; X is 2 and Y is 104; X is 2 and Y is 105; X is 2 and Y is 106; X is 2 and Y is 107; X is 2 and Y is 108; X is 2 and Y is 109; X is 2 and Y is

110; X is 2 and Y is 111; X is 2 and Y is 112; X is 2 and Y is 113; X is 2 and Y is 114; X is 2 and Y is 115; X is 2 and Y is 116; X is 2 and Y is 117; X is 2 and Y is 118; X is 2 and Y is 119; X is 2 and Y is 120; X is 2 and Y is 121; X is 2 and Y is 122; X is 2 and Y is 123; X is 2 and Y is 124; X is 2 and Y is 125; X is 2 and Y is 126; X is 2 and Y is 127; X is 2 and Y is 128; X is 2 and Y is 129; X is 2 and Y is 130; X is 2 and Y is 131; X is 2 and Y is 132; X is 2 and Y is 133; X is 2 and Y is 134; X is 2 and Y is 135; X is 2 and Y is 136; X is 2 and Y is 137; X is 2 and Y is 138; X is 2 and Y is 139; X is 2 and Y is 140; X is 2 and Y is 141; X is 2 and Y is 142; X is 2 and Y is 143; X is 2 and Y is 144; X is 2 and Y is 145; X is 2 and Y is 146; X is 2 and Y is 147; X is 2 and Y is 148; X is 2 and Y is 149; X is 2 and Y is 150; X is 2 and Y is 151; X is 2 and Y is 152; X is 2 and Y is 153; X is 2 and Y is 154; X is 2 and Y is 155; X is 2 and Y is 156; X is 2 and Y is 157; X is 2 and Y is 158; X is 2 and Y is 159; X is 2 and Y is 160; X is 2 and Y is 161; X is 2 and Y is 162; X is 2 and Y is 163; X is 2 and Y is 164; X is 2 and Y is 165; X is 2 and Y is 166; X is 2 and Y is 167; X is 2 and Y is 168; X is 2 and Y is 169; X is 2 and Y is 170; X is 2 and Y is 171; X is 2 and Y is 172; X is 2 and Y is 173; X is 2 and Y is 174; X is 2 and Y is 175; X is 2 and Y is 176; X is 2 and Y is 177; X is 2 and Y is 178; X is 2 and Y is 179; X is 2 and Y is 180; X is 2 and Y is 181; X is 2 and Y is 182; X is 2 and Y is 183; X is 2 and Y is 184; X is 2 and Y is 185; X is 2 and Y is 186;

X is 3 and Y is 1; X is 3 and Y is 2; X is 3 and Y is 3; X is 3 and Y is 4; X is 3 and Y is 5; X is 3 and Y is 6; X is 3 and Y is 7; X is 3 and Y is 8; X is 3 and Y is 9; X is 3 and Y is 10; X is 3 and Y is 11; X is 3 and Y is 12; X is 3 and Y is 13; X is 3 and Y is 14; X is 3 and Y is 15; X is 3 and Y is 16; X is 3 and Y is 17; X is 3 and Y is 18; X is 3 and Y is 19; X is 3 and Y is 20; X is 3 and Y is 21; X is 3 and Y is 22; X is 3 and Y is 23; X is 3 and Y is 24; X is 3 and Y is 25; X is 3 and Y is 26; X is 3 and Y is 27; X is 3 and Y is 28; X is 3 and Y is 29; X is 3 and Y is 30; X is 3 and Y is 31; X is 3 and Y is 32; X is 3 and Y is 33; X is 3 and Y is 34; X is 3 and Y is 35; X is 3 and Y is 36; X is 3 and Y is 37; X is 3 and Y is 38; X is 3 and Y is 39; X is 3 and Y is 40; X is 3 and Y is 41; X is 3 and Y is 42; X is 3 and Y is 43; X is 3 and Y is 44; X is 3 and Y is 45; X is 3 and Y is 46; X is 3 and Y is 47; X is 3 and Y is 48; X is 3 and Y is 49; X is 3 and Y is 50; X is 3 and Y is 51; X is 3 and Y is 52; X is 3 and Y is 53; X is 3 and Y is 54; X is 3 and Y is 55; X is 3 and Y is 56; X is 3 and Y is 57; X is 3 and Y is 58; X is 3 and Y is 59; X is 3 and Y is 60; X is 3 and Y is 61; X is 3 and Y is 62; X is 3 and Y is 63; X is 3 and Y is 64; X is 3 and Y is 65; X is 3 and Y is 66; X is 3 and Y is 67; X is 3 and Y is 68; X is 3 and Y is 69; X is 3 and Y is 70; X is 3 and Y is 71; X is 3 and Y is 72; X is 3 and Y is 73; X is 3 and Y is 74; X is 3 and Y is 75; X is 3 and Y is 76; X is 3 and Y is 77; X is 3 and Y is 78; X is 3 and Y is 79; X is 3 and Y is 80; X is 3 and Y is 81; X is 3 and Y is 82; X is 3 and Y is 83; X is 3 and Y is 84; X is 3 and Y is 85; X is 3 and Y is 86; X is 3 and Y is 87; X is 3 and Y is 88; X is 3 and Y is 89; X is 3 and Y is 90; X is 3 and

Y is 91; X is 3 and Y is 92; X is 3 and Y is 93; X is 3 and Y is 94; X is 3 and Y is 95; X is 3 and Y is 96; X is 3 and Y is 97; X is 3 and Y is 98; X is 3 and Y is 99; X is 3 and Y is 100; X is 3 and Y is 101; X is 3 and Y is 102; X is 3 and Y is 103; X is 3 and Y is 104; X is 3 and Y is 105; X is 3 and Y is 106; X is 3 and Y is 107; X is 3 and Y is 108; X is 3 and Y is 109; X is 3 and Y is 110; X is 3 and Y is 111; X is 3 and Y is 112; X is 3 and Y is 113; X is 3 and Y is 114; X is 3 and Y is 115; X is 3 and Y is 116; X is 3 and Y is 117; X is 3 and Y is 118; X is 3 and Y is 119; X is 3 and Y is 120; X is 3 and Y is 121; X is 3 and Y is 122; X is 3 and Y is 123; X is 3 and Y is 124; X is 3 and Y is 125; X is 3 and Y is 126; X is 3 and Y is 127; X is 3 and Y is 128; X is 3 and Y is 129; X is 3 and Y is 130; X is 3 and Y is 131; X is 3 and Y is 132; X is 3 and Y is 133; X is 3 and Y is 134; X is 3 and Y is 135; X is 3 and Y is 136; X is 3 and Y is 137; X is 3 and Y is 138; X is 3 and Y is 139; X is 3 and Y is 140; X is 3 and Y is 141; X is 3 and Y is 142; X is 3 and Y is 143; X is 3 and Y is 144; X is 3 and Y is 145; X is 3 and Y is 146; X is 3 and Y is 147; X is 3 and Y is 148; X is 3 and Y is 149; X is 3 and Y is 150; X is 3 and Y is 151; X is 3 and Y is 152; X is 3 and Y is 153; X is 3 and Y is 154; X is 3 and Y is 155; X is 3 and Y is 156; X is 3 and Y is 157; X is 3 and Y is 158; X is 3 and Y is 159; X is 3 and Y is 160; X is 3 and Y is 161; X is 3 and Y is 162; X is 3 and Y is 163; X is 3 and Y is 164; X is 3 and Y is 165; X is 3 and Y is 166; X is 3 and Y is 167; X is 3 and Y is 168; X is 3 and Y is 169; X is 3 and Y is 170; X is 3 and Y is 171; X is 3 and Y is 172; X is 3 and Y is 173; X is 3 and Y is 174; X is 3 and Y is 175; X is 3 and Y is 176; X is 3 and Y is 177; X is 3 and Y is 178; X is 3 and Y is 179; X is 3 and Y is 180; X is 3 and Y is 181; X is 3 and Y is 182; X is 3 and Y is 183; X is 3 and Y is 184; X is 3 and Y is 185; X is 3 and Y is 186;

X is 4 and Y is 1; X is 4 and Y is 2; X is 4 and Y is 3; X is 4 and Y is 4; X is 4 and Y is 5; X is 4 and Y is 6; X is 4 and Y is 7; X is 4 and Y is 8; X is 4 and Y is 9; X is 4 and Y is 10; X is 4 and Y is 11; X is 4 and Y is 12; X is 4 and Y is 13; X is 4 and Y is 14; X is 4 and Y is 15; X is 4 and Y is 16; X is 4 and Y is 17; X is 4 and Y is 18; X is 4 and Y is 19; X is 4 and Y is 20; X is 4 and Y is 21; X is 4 and Y is 22; X is 4 and Y is 23; X is 4 and Y is 24; X is 4 and Y is 25; X is 4 and Y is 26; X is 4 and Y is 27; X is 4 and Y is 28; X is 4 and Y is 29; X is 4 and Y is 30; X is 4 and Y is 31; X is 4 and Y is 32; X is 4 and Y is 33; X is 4 and Y is 34; X is 4 and Y is 35; X is 4 and Y is 36; X is 4 and Y is 37; X is 4 and Y is 38; X is 4 and Y is 39; X is 4 and Y is 40; X is 4 and Y is 41; X is 4 and Y is 42; X is 4 and Y is 43; X is 4 and Y is 44; X is 4 and Y is 45; X is 4 and Y is 46; X is 4 and Y is 47; X is 4 and Y is 48; X is 4 and Y is 49; X is 4 and Y is 50; X is 4 and Y is 51; X is 4 and Y is 52; X is 4 and Y is 53; X is 4 and Y is 54; X is 4 and Y is 55; X is 4 and Y is 56; X is 4 and Y is 57; X is 4 and Y is 58; X is 4 and Y is 59; X is 4 and Y is 60; X is 4 and Y is 61; X is 4 and Y is 62; X is 4 and Y is 63; X is 4 and Y is 64; X is 4 and Y is 65; X is 4 and Y is 66; X is 4 and Y is 67; X is 4 and Y is 68; X is 4 and Y is 69; X is 4 and Y is 70; X is 4 and

Y is 71; X is 4 and Y is 72; X is 4 and Y is 73; X is 4 and Y is 74; X is 4 and Y is 75; X is 4 and Y is 76; X is 4 and Y is 77; X is 4 and Y is 78; X is 4 and Y is 79; X is 4 and Y is 80; X is 4 and Y is 81; X is 4 and Y is 82; X is 4 and Y is 83; X is 4 and Y is 84; X is 4 and Y is 85; X is 4 and Y is 86; X is 4 and Y is 87; X is 4 and Y is 88; X is 4 and Y is 89; X is 4 and Y is 90; X is 4 and Y is 91; X is 4 and Y is 92; X is 4 and Y is 93; X is 4 and Y is 94; X is 4 and Y is 95; X is 4 and Y is 96; X is 4 and Y is 97; X is 4 and Y is 98; X is 4 and Y is 99; X is 4 and Y is 100; X is 4 and Y is 101; X is 4 and Y is 102; X is 4 and Y is 103; X is 4 and Y is 104; X is 4 and Y is 105; X is 4 and Y is 106; X is 4 and Y is 107; X is 4 and Y is 108; X is 4 and Y is 109; X is 4 and Y is 110; X is 4 and Y is 111; X is 4 and Y is 112; X is 4 and Y is 113; X is 4 and Y is 114; X is 4 and Y is 115; X is 4 and Y is 116; X is 4 and Y is 117; X is 4 and Y is 118; X is 4 and Y is 119; X is 4 and Y is 120; X is 4 and Y is 121; X is 4 and Y is 122; X is 4 and Y is 123; X is 4 and Y is 124; X is 4 and Y is 125; X is 4 and Y is 126; X is 4 and Y is 127; X is 4 and Y is 128; X is 4 and Y is 129; X is 4 and Y is 130; X is 4 and Y is 131; X is 4 and Y is 132; X is 4 and Y is 133; X is 4 and Y is 134; X is 4 and Y is 135; X is 4 and Y is 136; X is 4 and Y is 137; X is 4 and Y is 138; X is 4 and Y is 139; X is 4 and Y is 140; X is 4 and Y is 141; X is 4 and Y is 142; X is 4 and Y is 143; X is 4 and Y is 144; X is 4 and Y is 145; X is 4 and Y is 146; X is 4 and Y is 147; X is 4 and Y is 148; X is 4 and Y is 149; X is 4 and Y is 150; X is 4 and Y is 151; X is 4 and Y is 152; X is 4 and Y is 153; X is 4 and Y is 154; X is 4 and Y is 155; X is 4 and Y is 156; X is 4 and Y is 157; X is 4 and Y is 158; X is 4 and Y is 159; X is 4 and Y is 160; X is 4 and Y is 161; X is 4 and Y is 162; X is 4 and Y is 163; X is 4 and Y is 164; X is 4 and Y is 165; X is 4 and Y is 166; X is 4 and Y is 167; X is 4 and Y is 168; X is 4 and Y is 169; X is 4 and Y is 170; X is 4 and Y is 171; X is 4 and Y is 172; X is 4 and Y is 173; X is 4 and Y is 174; X is 4 and Y is 175; X is 4 and Y is 176; X is 4 and Y is 177; X is 4 and Y is 178; X is 4 and Y is 179; X is 4 and Y is 180; X is 4 and Y is 181; X is 4 and Y is 182; X is 4 and Y is 183; X is 4 and Y is 184; X is 4 and Y is 185; X is 4 and Y is 186;

X is 5 and Y is 1; X is 5 and Y is 2; X is 5 and Y is 3; X is 5 and Y is 4; X is 5 and Y is 5; X is 5 and Y is 6; X is 5 and Y is 7; X is 5 and Y is 8; X is 5 and Y is 9; X is 5 and Y is 10; X is 5 and Y is 11; X is 5 and Y is 12; X is 5 and Y is 13; X is 5 and Y is 14; X is 5 and Y is 15; X is 5 and Y is 16; X is 5 and Y is 17; X is 5 and Y is 18; X is 5 and Y is 19; X is 5 and Y is 20; X is 5 and Y is 21; X is 5 and Y is 22; X is 5 and Y is 23; X is 5 and Y is 24; X is 5 and Y is 25; X is 5 and Y is 26; X is 5 and Y is 27; X is 5 and Y is 28; X is 5 and Y is 29; X is 5 and Y is 30; X is 5 and Y is 31; X is 5 and Y is 32; X is 5 and Y is 33; X is 5 and Y is 34; X is 5 and Y is 35; X is 5 and Y is 36; X is 5 and Y is 37; X is 5 and Y is 38; X is 5 and Y is 39; X is 5 and Y is 40; X is 5 and Y is 41; X is 5 and Y is 42; X is 5 and Y is 43; X is 5 and Y is 44; X is 5 and Y is 45; X is 5 and Y is 46; X is 5 and Y is 47; X is 5 and Y is 48; X is 5 and Y is 49; X is 5 and Y is 50; X is 5 and

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X is 12 and Y is 1; X is 12 and Y is 2; X is 12 and Y is 3; X is 12 and Y is 4; X is 12 and Y is 5; X is 12 and Y is 6; X is 12 and Y is 7; X is 12 and Y is 8; X is 12 and Y is 9; X is 12 and Y is 10; X is 12 and Y is 11; X is 12 and Y is 12; X is 12 and Y is 13; X is 12 and Y is 14; X is 12 and Y is 15; X is 12 and Y is 16; X is 12 and Y is 17; X is 12 and Y is 18; X is 12 and Y is 19; X is 12 and Y is 20; X is 12 and Y is 21; X is 12 and Y is 22; X is 12 and Y is 23; X is 12 and Y is 24; X is 12 and Y is 25; X is 12 and Y is 26; X is 12 and Y is 27; X is 12 and Y is 28; X is 12 and Y is 29; X is 12 and Y is 30; X is 12 and Y is 31; X is 12 and Y is 32; X is 12 and Y is 33; X is 12 and Y is 34; X is 12 and Y is 35; X is 12 and Y is 36; X is 12 and Y is 37; X is 12 and Y is 38; X is 12 and Y is 39; X is 12 and Y is 40; X is 12 and Y is 41; X is 12 and Y is 42; X is 12 and Y is 43; X is 12 and Y is 44; X is 12 and Y is 45; X is 12 and Y is 46; X is 12 and Y is 47; X is 12 and Y is 48; X is 12 and Y is 49; X is 12 and Y is 50; X is 12 and Y is 51; X is 12 and Y is 52; X is 12 and Y is 53; X is 12 and Y is 54; X is 12 and Y is 55; X is 12 and Y is 56; X is 12 and Y is 57; X is 12 and Y is 58; X is 12 and Y is 59; X is 12 and Y is 60; X is 12 and Y is 61; X

is 12 and Y is 62; X is 12 and Y is 63; X is 12 and Y is 64; X is 12 and Y is 65; X is 12 and Y is 66; X is 12 and Y is 67; X is 12 and Y is 68; X is 12 and Y is 69; X is 12 and Y is 70; X is 12 and Y is 71; X is 12 and Y is 72; X is 12 and Y is 73; X is 12 and Y is 74; X is 12 and Y is 75; X is 12 and Y is 76; X is 12 and Y is 77; X is 12 and Y is 78; X is 12 and Y is 79; X is 12 and Y is 80; X is 12 and Y is 81; X is 12 and Y is 82; X is 12 and Y is 83; X is 12 and Y is 84; X is 12 and Y is 85; X is 12 and Y is 86; X is 12 and Y is 87; X is 12 and Y is 88; X is 12 and Y is 89; X is 12 and Y is 90; X is 12 and Y is 91; X is 12 and Y is 92; X is 12 and Y is 93; X is 12 and Y is 94; X is 12 and Y is 95; X is 12 and Y is 96; X is 12 and Y is 97; X is 12 and Y is 98; X is 12 and Y is 99; X is 12 and Y is 100; X is 12 and Y is 101; X is 12 and Y is 102; X is 12 and Y is 103; X is 12 and Y is 104; X is 12 and Y is 105; X is 12 and Y is 106; X is 12 and Y is 107; X is 12 and Y is 108; X is 12 and Y is 109; X is 12 and Y is 110; X is 12 and Y is 111; X is 12 and Y is 112; X is 12 and Y is 113; X is 12 and Y is 114; X is 12 and Y is 115; X is 12 and Y is 116; X is 12 and Y is 117; X is 12 and Y is 118; X is 12 and Y is 119; X is 12 and Y is 120; X is 12 and Y is 121; X is 12 and Y is 122; X is 12 and Y is 123; X is 12 and Y is 124; X is 12 and Y is 125; X is 12 and Y is 126; X is 12 and Y is 127; X is 12 and Y is 128; X is 12 and Y is 129; X is 12 and Y is 130; X is 12 and Y is 131; X is 12 and Y is 132; X is 12 and Y is 133; X is 12 and Y is 134; X is 12 and Y is 135; X is 12 and Y is 136; X is 12 and Y is 137; X is 12 and Y is 138; X is 12 and Y is 139; X is 12 and Y is 140; X is 12 and Y is 141; X is 12 and Y is 142; X is 12 and Y is 143; X is 12 and Y is 144; X is 12 and Y is 145; X is 12 and Y is 146; X is 12 and Y is 147; X is 12 and Y is 148; X is 12 and Y is 149; X is 12 and Y is 150; X is 12 and Y is 151; X is 12 and Y is 152; X is 12 and Y is 153; X is 12 and Y is 154; X is 12 and Y is 155; X is 12 and Y is 156; X is 12 and Y is 157; X is 12 and Y is 158; X is 12 and Y is 159; X is 12 and Y is 160; X is 12 and Y is 161; X is 12 and Y is 162; X is 12 and Y is 163; X is 12 and Y is 164; X is 12 and Y is 165; X is 12 and Y is 166; X is 12 and Y is 167; X is 12 and Y is 168; X is 12 and Y is 169; X is 12 and Y is 170; X is 12 and Y is 171; X is 12 and Y is 172; X is 12 and Y is 173; X is 12 and Y is 174; X is 12 and Y is 175; X is 12 and Y is 176; X is 12 and Y is 177; X is 12 and Y is 178; X is 12 and Y is 179; X is 12 and Y is 180; X is 12 and Y is 181; X is 12 and Y is 182; X is 12 and Y is 183; X is 12 and Y is 184; X is 12 and Y is 185; or X is 12 and Y is 186.

In one embodiment, X is 2 and Y is 5. In another embodiment, X is 10 and Y is 164.

In one embodiment, the first binding domain binds to 4-1BB (CD137) and the second binding domain binds to OX40 (CD134), wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of an antibody AB137_X, wherein X is selected from the group consisting of 1 to 12, as shown in Table 9, and

one or more CDRs or one or more variable domains of an antibody AB134_Y, wherein Y is selected from the group consisting of 1 to 92, as shown in Table 8.

In various embodiments, X is 1 and Y is selected from the group consisting of 1 to 92; X is 2 and Y is selected from the group consisting of 1 to 92; X is 3 and Y is selected from the group consisting of 1 to 92; X is 4 and Y is selected from the group consisting of 1 to 92; X is 5 and Y is selected from the group consisting of 1 to 92; X is 6 and Y is selected from the group consisting of 1 to 92; X is 7 and Y is selected from the group consisting of 1 to 92; X is 8 and Y is selected from the group consisting of 1 to 92; X is 9 and Y is selected from the group consisting of 1 to 92; X is 10 and Y is selected from the group consisting of 1 to 92; X is 11 and Y is selected from the group consisting of 1 to 92; or X is 12 and Y is selected from the group consisting of 1 to 92.

In various embodiments,

X is 1 and Y is 1; X is 1 and Y is 2; X is 1 and Y is 3; X is 1 and Y is 4; X is 1 and Y is 5; X is 1 and Y is 6; X is 1 and Y is 7; X is 1 and Y is 8; X is 1 and Y is 9; X is 1 and Y is 10; X is 1 and Y is 11; X is 1 and Y is 12; X is 1 and Y is 13; X is 1 and Y is 14; X is 1 and Y is 15; X is 1 and Y is 16; X is 1 and Y is 17; X is 1 and Y is 18; X is 1 and Y is 19; X is 1 and Y is 20; X is 1 and Y is 21; X is 1 and Y is 22; X is 1 and Y is 23; X is 1 and Y is 24; X is 1 and Y is 25; X is 1 and Y is 26; X is 1 and Y is 27; X is 1 and Y is 28; X is 1 and Y is 29; X is 1 and Y is 30; X is 1 and Y is 31; X is 1 and Y is 32; X is 1 and Y is 33; X is 1 and Y is 34; X is 1 and Y is 35; X is 1 and Y is 36; X is 1 and Y is 37; X is 1 and Y is 38; X is 1 and Y is 39; X is 1 and Y is 40; X is 1 and Y is 41; X is 1 and Y is 42; X is 1 and Y is 43; X is 1 and Y is 44; X is 1 and Y is 45; X is 1 and Y is 46; X is 1 and Y is 47; X is 1 and Y is 48; X is 1 and Y is 49; X is 1 and Y is 50; X is 1 and Y is 51; X is 1 and Y is 52; X is 1 and Y is 53; X is 1 and Y is 54; X is 1 and Y is 55; X is 1 and Y is 56; X is 1 and Y is 57; X is 1 and Y is 58; X is 1 and Y is 59; X is 1 and Y is 60; X is 1 and Y is 61; X is 1 and Y is 62; X is 1 and Y is 63; X is 1 and Y is 64; X is 1 and Y is 65; X is 1 and Y is 66; X is 1 and Y is 67; X is 1 and Y is 68; X is 1 and Y is 69; X is 1 and Y is 70; X is 1 and Y is 71; X is 1 and Y is 72; X is 1 and Y is 73; X is 1 and Y is 74; X is 1 and Y is 75; X is 1 and Y is 76; X is 1 and Y is 77; X is 1 and Y is 78; X is 1 and Y is 79; X is 1 and Y is 80; X is 1 and Y is 81; X is 1 and Y is 82; X is 1 and Y is 83; X is 1 and Y is 84; X is 1 and Y is 85; X is 1 and Y is 86; X is 1 and Y is 87; X is 1 and Y is 88; X is 1 and Y is 89; X is 1 and Y is 90; X is 1 and Y is 91; X is 1 and Y is 92;

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Y is 11; X is 2 and Y is 12; X is 2 and Y is 13; X is 2 and Y is 14; X is 2 and Y is 15; X is 2 and Y is 16; X is 2 and Y is 17; X is 2 and Y is 18; X is 2 and Y is 19; X is 2 and Y is 20; X is 2 and Y is 21; X is 2 and Y is 22; X is 2 and Y is 23; X is 2 and Y is 24; X is 2 and Y is 25; X is 2 and Y is 26; X is 2 and Y is 27; X is 2 and Y is 28; X is 2 and Y is 29; X is 2 and Y is 30; X is 2 and Y is 31; X is 2 and Y is 32; X is 2 and Y is 33; X is 2 and Y is 34; X is 2 and Y is 35; X is 2 and Y is 36; X is 2 and Y is 37; X is 2 and Y is 38; X is 2 and Y is 39; X is 2 and Y is 40; X is 2 and Y is 41; X is 2 and Y is 42; X is 2 and Y is 43; X is 2 and Y is 44; X is 2 and Y is 45; X is 2 and Y is 46; X is 2 and Y is 47; X is 2 and Y is 48; X is 2 and Y is 49; X is 2 and Y is 50; X is 2 and Y is 51; X is 2 and Y is 52; X is 2 and Y is 53; X is 2 and Y is 54; X is 2 and Y is 55; X is 2 and Y is 56; X is 2 and Y is 57; X is 2 and Y is 58; X is 2 and Y is 59; X is 2 and Y is 60; X is 2 and Y is 61; X is 2 and Y is 62; X is 2 and Y is 63; X is 2 and Y is 64; X is 2 and Y is 65; X is 2 and Y is 66; X is 2 and Y is 67; X is 2 and Y is 68; X is 2 and Y is 69; X is 2 and Y is 70; X is 2 and Y is 71; X is 2 and Y is 72; X is 2 and Y is 73; X is 2 and Y is 74; X is 2 and Y is 75; X is 2 and Y is 76; X is 2 and Y is 77; X is 2 and Y is 78; X is 2 and Y is 79; X is 2 and Y is 80; X is 2 and Y is 81; X is 2 and Y is 82; X is 2 and Y is 83; X is 2 and Y is 84; X is 2 and Y is 85; X is 2 and Y is 86; X is 2 and Y is 87; X is 2 and Y is 88; X is 2 and Y is 89; X is 2 and Y is 90; X is 2 and Y is 91; X is 2 and Y is 92;

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Y is 86; X is 3 and Y is 87; X is 3 and Y is 88; X is 3 and Y is 89; X is 3 and Y is 90; X is 3 and Y is 91; X is 3 and Y is 92;

X is 4 and Y is 1; X is 4 and Y is 2; X is 4 and Y is 3; X is 4 and Y is 4; X is 4 and Y is 5; X is 4 and Y is 6; X is 4 and Y is 7; X is 4 and Y is 8; X is 4 and Y is 9; X is 4 and Y is 10; X is 4 and Y is 11; X is 4 and Y is 12; X is 4 and Y is 13; X is 4 and Y is 14; X is 4 and Y is 15; X is 4 and Y is 16; X is 4 and Y is 17; X is 4 and Y is 18; X is 4 and Y is 19; X is 4 and Y is 20; X is 4 and Y is 21; X is 4 and Y is 22; X is 4 and Y is 23; X is 4 and Y is 24; X is 4 and Y is 25; X is 4 and Y is 26; X is 4 and Y is 27; X is 4 and Y is 28; X is 4 and Y is 29; X is 4 and Y is 30; X is 4 and Y is 31; X is 4 and Y is 32; X is 4 and Y is 33; X is 4 and Y is 34; X is 4 and Y is 35; X is 4 and Y is 36; X is 4 and Y is 37; X is 4 and Y is 38; X is 4 and Y is 39; X is 4 and Y is 40; X is 4 and Y is 41; X is 4 and Y is 42; X is 4 and Y is 43; X is 4 and Y is 44; X is 4 and Y is 45; X is 4 and Y is 46; X is 4 and Y is 47; X is 4 and Y is 48; X is 4 and Y is 49; X is 4 and Y is 50; X is 4 and Y is 51; X is 4 and Y is 52; X is 4 and Y is 53; X is 4 and Y is 54; X is 4 and Y is 55; X is 4 and Y is 56; X is 4 and Y is 57; X is 4 and Y is 58; X is 4 and Y is 59; X is 4 and Y is 60; X is 4 and Y is 61; X is 4 and Y is 62; X is 4 and Y is 63; X is 4 and Y is 64; X is 4 and Y is 65; X is 4 and Y is 66; X is 4 and Y is 67; X is 4 and Y is 68; X is 4 and Y is 69; X is 4 and Y is 70; X is 4 and Y is 71; X is 4 and Y is 72; X is 4 and Y is 73; X is 4 and Y is 74; X is 4 and Y is 75; X is 4 and Y is 76; X is 4 and Y is 77; X is 4 and Y is 78; X is 4 and Y is 79; X is 4 and Y is 80; X is 4 and Y is 81; X is 4 and Y is 82; X is 4 and Y is 83; X is 4 and Y is 84; X is 4 and Y is 85; X is 4 and Y is 86; X is 4 and Y is 87; X is 4 and Y is 88; X is 4 and Y is 89; X is 4 and Y is 90; X is 4 and Y is 91; X is 4 and Y is 92;

X is 5 and Y is 1; X is 5 and Y is 2; X is 5 and Y is 3; X is 5 and Y is 4; X is 5 and Y is 5; X is 5 and Y is 6; X is 5 and Y is 7; X is 5 and Y is 8; X is 5 and Y is 9; X is 5 and Y is 10; X is 5 and Y is 11; X is 5 and Y is 12; X is 5 and Y is 13; X is 5 and Y is 14; X is 5 and Y is 15; X is 5 and Y is 16; X is 5 and Y is 17; X is 5 and Y is 18; X is 5 and Y is 19; X is 5 and Y is 20; X is 5 and Y is 21; X is 5 and Y is 22; X is 5 and Y is 23; X is 5 and Y is 24; X is 5 and Y is 25; X is 5 and Y is 26; X is 5 and Y is 27; X is 5 and Y is 28; X is 5 and Y is 29; X is 5 and Y is 30; X is 5 and Y is 31; X is 5 and Y is 32; X is 5 and Y is 33; X is 5 and Y is 34; X is 5 and Y is 35; X is 5 and Y is 36; X is 5 and Y is 37; X is 5 and Y is 38; X is 5 and Y is 39; X is 5 and Y is 40; X is 5 and Y is 41; X is 5 and Y is 42; X is 5 and Y is 43; X is 5 and Y is 44; X is 5 and Y is 45; X is 5 and Y is 46; X is 5 and Y is 47; X is 5 and Y is 48; X is 5 and Y is 49; X is 5 and Y is 50; X is 5 and Y is 51; X is 5 and Y is 52; X is 5 and Y is 53; X is 5 and Y is 54; X is 5 and Y is 55; X is 5 and Y is 56; X is 5 and Y is 57; X is 5 and Y is 58; X is 5 and Y is 59; X is 5 and Y is 60; X is 5 and Y is 61; X is 5 and Y is 62; X is 5 and Y is 63; X is 5 and Y is 64; X is 5 and Y is 65; X is 5 and Y is 66; X is 5 and Y is 67; X is 5 and Y is 68; X is 5 and Y is 69; X is 5 and Y is 70; X is 5 and

Y is 71; X is 5 and Y is 72; X is 5 and Y is 73; X is 5 and Y is 74; X is 5 and Y is 75; X is 5 and Y is 76; X is 5 and Y is 77; X is 5 and Y is 78; X is 5 and Y is 79; X is 5 and Y is 80; X is 5 and Y is 81; X is 5 and Y is 82; X is 5 and Y is 83; X is 5 and Y is 84; X is 5 and Y is 85; X is 5 and Y is 86; X is 5 and Y is 87; X is 5 and Y is 88; X is 5 and Y is 89; X is 5 and Y is 90; X is 5 and Y is 91; X is 5 and Y is 92;

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X is 7 and Y is 1; X is 7 and Y is 2; X is 7 and Y is 3; X is 7 and Y is 4; X is 7 and Y is 5; X is 7 and Y is 6; X is 7 and Y is 7; X is 7 and Y is 8; X is 7 and Y is 9; X is 7 and Y is 10; X is 7 and Y is 11; X is 7 and Y is 12; X is 7 and Y is 13; X is 7 and Y is 14; X is 7 and Y is 15; X is 7 and Y is 16; X is 7 and Y is 17; X is 7 and Y is 18; X is 7 and Y is 19; X is 7 and Y is 20; X is 7 and Y is 21; X is 7 and Y is 22; X is 7 and Y is 23; X is 7 and Y is 24; X is 7 and Y is 25; X is 7 and Y is 26; X is 7 and Y is 27; X is 7 and Y is 28; X is 7 and Y is 29; X is 7 and Y is 30; X is 7 and Y is 31; X is 7 and Y is 32; X is 7 and Y is 33; X is 7 and Y is 34; X is 7 and Y is 35; X is 7 and Y is 36; X is 7 and Y is 37; X is 7 and Y is 38; X is 7 and Y is 39; X is 7 and Y is 40; X is 7 and Y is 41; X is 7 and Y is 42; X is 7 and Y is 43; X is 7 and Y is 44; X is 7 and Y is 45; X is 7 and Y is 46; X is 7 and Y is 47; X is 7 and Y is 48; X is 7 and Y is 49; X is 7 and Y is 50; X is 7 and Y is 51; X is 7 and Y is 52; X is 7 and Y is 53; X is 7 and Y is 54; X is 7 and Y is 55; X is 7 and

Y is 56; X is 7 and Y is 57; X is 7 and Y is 58; X is 7 and Y is 59; X is 7 and Y is 60; X is 7 and Y is 61; X is 7 and Y is 62; X is 7 and Y is 63; X is 7 and Y is 64; X is 7 and Y is 65; X is 7 and Y is 66; X is 7 and Y is 67; X is 7 and Y is 68; X is 7 and Y is 69; X is 7 and Y is 70; X is 7 and Y is 71; X is 7 and Y is 72; X is 7 and Y is 73; X is 7 and Y is 74; X is 7 and Y is 75; X is 7 and Y is 76; X is 7 and Y is 77; X is 7 and Y is 78; X is 7 and Y is 79; X is 7 and Y is 80; X is 7 and Y is 81; X is 7 and Y is 82; X is 7 and Y is 83; X is 7 and Y is 84; X is 7 and Y is 85; X is 7 and Y is 86; X is 7 and Y is 87; X is 7 and Y is 88; X is 7 and Y is 89; X is 7 and Y is 90; X is 7 and Y is 91; X is 7 and Y is 92;

X is 8 and Y is 1; X is 8 and Y is 2; X is 8 and Y is 3; X is 8 and Y is 4; X is 8 and Y is 5; X is 8 and Y is 6; X is 8 and Y is 7; X is 8 and Y is 8; X is 8 and Y is 9; X is 8 and Y is 10; X is 8 and Y is 11; X is 8 and Y is 12; X is 8 and Y is 13; X is 8 and Y is 14; X is 8 and Y is 15; X is 8 and Y is 16; X is 8 and Y is 17; X is 8 and Y is 18; X is 8 and Y is 19; X is 8 and Y is 20; X is 8 and Y is 21; X is 8 and Y is 22; X is 8 and Y is 23; X is 8 and Y is 24; X is 8 and Y is 25; X is 8 and Y is 26; X is 8 and Y is 27; X is 8 and Y is 28; X is 8 and Y is 29; X is 8 and Y is 30; X is 8 and Y is 31; X is 8 and Y is 32; X is 8 and Y is 33; X is 8 and Y is 34; X is 8 and Y is 35; X is 8 and Y is 36; X is 8 and Y is 37; X is 8 and Y is 38; X is 8 and Y is 39; X is 8 and Y is 40; X is 8 and Y is 41; X is 8 and Y is 42; X is 8 and Y is 43; X is 8 and Y is 44; X is 8 and Y is 45; X is 8 and Y is 46; X is 8 and Y is 47; X is 8 and Y is 48; X is 8 and Y is 49; X is 8 and Y is 50; X is 8 and Y is 51; X is 8 and Y is 52; X is 8 and Y is 53; X is 8 and Y is 54; X is 8 and Y is 55; X is 8 and Y is 56; X is 8 and Y is 57; X is 8 and Y is 58; X is 8 and Y is 59; X is 8 and Y is 60; X is 8 and Y is 61; X is 8 and Y is 62; X is 8 and Y is 63; X is 8 and Y is 64; X is 8 and Y is 65; X is 8 and Y is 66; X is 8 and Y is 67; X is 8 and Y is 68; X is 8 and Y is 69; X is 8 and Y is 70; X is 8 and Y is 71; X is 8 and Y is 72; X is 8 and Y is 73; X is 8 and Y is 74; X is 8 and Y is 75; X is 8 and Y is 76; X is 8 and Y is 77; X is 8 and Y is 78; X is 8 and Y is 79; X is 8 and Y is 80; X is 8 and Y is 81; X is 8 and Y is 82; X is 8 and Y is 83; X is 8 and Y is 84; X is 8 and Y is 85; X is 8 and Y is 86; X is 8 and Y is 87; X is 8 and Y is 88; X is 8 and Y is 89; X is 8 and Y is 90; X is 8 and Y is 91; X is 8 and Y is 92;

X is 9 and Y is 1; X is 9 and Y is 2; X is 9 and Y is 3; X is 9 and Y is 4; X is 9 and Y is 5; X is 9 and Y is 6; X is 9 and Y is 7; X is 9 and Y is 8; X is 9 and Y is 9; X is 9 and Y is 10; X is 9 and Y is 11; X is 9 and Y is 12; X is 9 and Y is 13; X is 9 and Y is 14; X is 9 and Y is 15; X is 9 and Y is 16; X is 9 and Y is 17; X is 9 and Y is 18; X is 9 and Y is 19; X is 9 and Y is 20; X is 9 and Y is 21; X is 9 and Y is 22; X is 9 and Y is 23; X is 9 and Y is 24; X is 9 and Y is 25; X is 9 and Y is 26; X is 9 and Y is 27; X is 9 and Y is 28; X is 9 and Y is 29; X is 9 and Y is 30; X is 9 and Y is 31; X is 9 and Y is 32; X is 9 and Y is 33; X is 9 and Y is 34; X is 9 and Y is 35; X is 9 and Y is 36; X is 9 and Y is 37; X is 9 and Y is 38; X is 9 and Y is 39; X is 9 and Y is 40; X is 9 and

Y is 41; X is 9 and Y is 42; X is 9 and Y is 43; X is 9 and Y is 44; X is 9 and Y is 45; X is 9 and Y is 46; X is 9 and Y is 47; X is 9 and Y is 48; X is 9 and Y is 49; X is 9 and Y is 50; X is 9 and Y is 51; X is 9 and Y is 52; X is 9 and Y is 53; X is 9 and Y is 54; X is 9 and Y is 55; X is 9 and Y is 56; X is 9 and Y is 57; X is 9 and Y is 58; X is 9 and Y is 59; X is 9 and Y is 60; X is 9 and Y is 61; X is 9 and Y is 62; X is 9 and Y is 63; X is 9 and Y is 64; X is 9 and Y is 65; X is 9 and Y is 66; X is 9 and Y is 67; X is 9 and Y is 68; X is 9 and Y is 69; X is 9 and Y is 70; X is 9 and Y is 71; X is 9 and Y is 72; X is 9 and Y is 73; X is 9 and Y is 74; X is 9 and Y is 75; X is 9 and Y is 76; X is 9 and Y is 77; X is 9 and Y is 78; X is 9 and Y is 79; X is 9 and Y is 80; X is 9 and Y is 81; X is 9 and Y is 82; X is 9 and Y is 83; X is 9 and Y is 84; X is 9 and Y is 85; X is 9 and Y is 86; X is 9 and Y is 87; X is 9 and Y is 88; X is 9 and Y is 89; X is 9 and Y is 90; X is 9 and Y is 91; X is 9 and Y is 92;

X is 10 and Y is 1; X is 10 and Y is 2; X is 10 and Y is 3; X is 10 and Y is 4; X is 10 and Y is 5; X is 10 and Y is 6; X is 10 and Y is 7; X is 10 and Y is 8; X is 10 and Y is 9; X is 10 and Y is 10; X is 10 and Y is 11; X is 10 and Y is 12; X is 10 and Y is 13; X is 10 and Y is 14; X is 10 and Y is 15; X is 10 and Y is 16; X is 10 and Y is 17; X is 10 and Y is 18; X is 10 and Y is 19; X is 10 and Y is 20; X is 10 and Y is 21; X is 10 and Y is 22; X is 10 and Y is 23; X is 10 and Y is 24; X is 10 and Y is 25; X is 10 and Y is 26; X is 10 and Y is 27; X is 10 and Y is 28; X is 10 and Y is 29; X is 10 and Y is 30; X is 10 and Y is 31; X is 10 and Y is 32; X is 10 and Y is 33; X is 10 and Y is 34; X is 10 and Y is 35; X is 10 and Y is 36; X is 10 and Y is 37; X is 10 and Y is 38; X is 10 and Y is 39; X is 10 and Y is 40; X is 10 and Y is 41; X is 10 and Y is 42; X is 10 and Y is 43; X is 10 and Y is 44; X is 10 and Y is 45; X is 10 and Y is 46; X is 10 and Y is 47; X is 10 and Y is 48; X is 10 and Y is 49; X is 10 and Y is 50; X is 10 and Y is 51; X is 10 and Y is 52; X is 10 and Y is 53; X is 10 and Y is 54; X is 10 and Y is 55; X is 10 and Y is 56; X is 10 and Y is 57; X is 10 and Y is 58; X is 10 and Y is 59; X is 10 and Y is 60; X is 10 and Y is 61; X is 10 and Y is 62; X is 10 and Y is 63; X is 10 and Y is 64; X is 10 and Y is 65; X is 10 and Y is 66; X is 10 and Y is 67; X is 10 and Y is 68; X is 10 and Y is 69; X is 10 and Y is 70; X is 10 and Y is 71; X is 10 and Y is 72; X is 10 and Y is 73; X is 10 and Y is 74; X is 10 and Y is 75; X is 10 and Y is 76; X is 10 and Y is 77; X is 10 and Y is 78; X is 10 and Y is 79; X is 10 and Y is 80; X is 10 and Y is 81; X is 10 and Y is 82; X is 10 and Y is 83; X is 10 and Y is 84; X is 10 and Y is 85; X is 10 and Y is 86; X is 10 and Y is 87; X is 10 and Y is 88; X is 10 and Y is 89; X is 10 and Y is 90; X is 10 and Y is 91; X is 10 and Y is 92;

X is 11 and Y is 1; X is 11 and Y is 2; X is 11 and Y is 3; X is 11 and Y is 4; X is 11 and Y is 5; X is 11 and Y is 6; X is 11 and Y is 7; X is 11 and Y is 8; X is 11 and Y is 9; X is 11 and Y is 10; X is 11 and Y is 11; X is 11 and Y is 12; X is 11 and Y is 13; X is 11 and Y is 14; X is 11 and Y is 15; X is 11 and Y is 16; X is 11 and Y is 17; X is 11 and Y is 18; X is 11 and Y is 19; X

is 11 and Y is 20; X is 11 and Y is 21; X is 11 and Y is 22; X is 11 and Y is 23; X is 11 and Y is 24; X is 11 and Y is 25; X is 11 and Y is 26; X is 11 and Y is 27; X is 11 and Y is 28; X is 11 and Y is 29; X is 11 and Y is 30; X is 11 and Y is 31; X is 11 and Y is 32; X is 11 and Y is 33; X is 11 and Y is 34; X is 11 and Y is 35; X is 11 and Y is 36; X is 11 and Y is 37; X is 11 and Y is 38; X is 11 and Y is 39; X is 11 and Y is 40; X is 11 and Y is 41; X is 11 and Y is 42; X is 11 and Y is 43; X is 11 and Y is 44; X is 11 and Y is 45; X is 11 and Y is 46; X is 11 and Y is 47; X is 11 and Y is 48; X is 11 and Y is 49; X is 11 and Y is 50; X is 11 and Y is 51; X is 11 and Y is 52; X is 11 and Y is 53; X is 11 and Y is 54; X is 11 and Y is 55; X is 11 and Y is 56; X is 11 and Y is 57; X is 11 and Y is 58; X is 11 and Y is 59; X is 11 and Y is 60; X is 11 and Y is 61; X is 11 and Y is 62; X is 11 and Y is 63; X is 11 and Y is 64; X is 11 and Y is 65; X is 11 and Y is 66; X is 11 and Y is 67; X is 11 and Y is 68; X is 11 and Y is 69; X is 11 and Y is 70; X is 11 and Y is 71; X is 11 and Y is 72; X is 11 and Y is 73; X is 11 and Y is 74; X is 11 and Y is 75; X is 11 and Y is 76; X is 11 and Y is 77; X is 11 and Y is 78; X is 11 and Y is 79; X is 11 and Y is 80; X is 11 and Y is 81; X is 11 and Y is 82; X is 11 and Y is 83; X is 11 and Y is 84; X is 11 and Y is 85; X is 11 and Y is 86; X is 11 and Y is 87; X is 11 and Y is 88; X is 11 and Y is 89; X is 11 and Y is 90; X is 11 and Y is 91; X is 11 and Y is 92;

X is 12 and Y is 1; X is 12 and Y is 2; X is 12 and Y is 3; X is 12 and Y is 4; X is 12 and Y is 5; X is 12 and Y is 6; X is 12 and Y is 7; X is 12 and Y is 8; X is 12 and Y is 9; X is 12 and Y is 10; X is 12 and Y is 11; X is 12 and Y is 12; X is 12 and Y is 13; X is 12 and Y is 14; X is 12 and Y is 15; X is 12 and Y is 16; X is 12 and Y is 17; X is 12 and Y is 18; X is 12 and Y is 19; X is 12 and Y is 20; X is 12 and Y is 21; X is 12 and Y is 22; X is 12 and Y is 23; X is 12 and Y is 24; X is 12 and Y is 25; X is 12 and Y is 26; X is 12 and Y is 27; X is 12 and Y is 28; X is 12 and Y is 29; X is 12 and Y is 30; X is 12 and Y is 31; X is 12 and Y is 32; X is 12 and Y is 33; X is 12 and Y is 34; X is 12 and Y is 35; X is 12 and Y is 36; X is 12 and Y is 37; X is 12 and Y is 38; X is 12 and Y is 39; X is 12 and Y is 40; X is 12 and Y is 41; X is 12 and Y is 42; X is 12 and Y is 43; X is 12 and Y is 44; X is 12 and Y is 45; X is 12 and Y is 46; X is 12 and Y is 47; X is 12 and Y is 48; X is 12 and Y is 49; X is 12 and Y is 50; X is 12 and Y is 51; X is 12 and Y is 52; X is 12 and Y is 53; X is 12 and Y is 54; X is 12 and Y is 55; X is 12 and Y is 56; X is 12 and Y is 57; X is 12 and Y is 58; X is 12 and Y is 59; X is 12 and Y is 60; X is 12 and Y is 61; X is 12 and Y is 62; X is 12 and Y is 63; X is 12 and Y is 64; X is 12 and Y is 65; X is 12 and Y is 66; X is 12 and Y is 67; X is 12 and Y is 68; X is 12 and Y is 69; X is 12 and Y is 70; X is 12 and Y is 71; X is 12 and Y is 72; X is 12 and Y is 73; X is 12 and Y is 74; X is 12 and Y is 75; X is 12 and Y is 76; X is 12 and Y is 77; X is 12 and Y is 78; X is 12 and Y is 79; X is 12 and Y is 80; X is 12 and Y is 81; X is 12 and Y is 82; X is 12 and Y is 83; X is 12 and Y is 84; X is 12

and Y is 85; X is 12 and Y is 86; X is 12 and Y is 87; X is 12 and Y is 88; X is 12 and Y is 89; X is 12 and Y is 90; X is 12 and Y is 91; or X is 12 and Y is 92.

In one embodiment, X is 2 and Y is 3. In another embodiment, X is 12 and Y is 9.

In one embodiment, the first binding domain binds to OX40 (CD134) and the second binding domain binds to CD27, wherein, preferably, the binding agent comprises
one or more CDRs or one or more variable domains of an antibody AB134_X, wherein X is selected from the group consisting of 1 to 92, as shown in Table 8, and
one or more CDRs or one or more variable domains of an antibody AB27_Y, wherein Y is selected from the group consisting of 1 to 186, as shown in Table 7.

In various embodiments, X is 1 and Y is selected from the group consisting of 1 to 186; X is 2 and Y is selected from the group consisting of 1 to 186; X is 3 and Y is selected from the group consisting of 1 to 186; X is 4 and Y is selected from the group consisting of 1 to 186; X is 5 and Y is selected from the group consisting of 1 to 186; X is 6 and Y is selected from the group consisting of 1 to 186; X is 7 and Y is selected from the group consisting of 1 to 186; X is 8 and Y is selected from the group consisting of 1 to 186; X is 9 and Y is selected from the group consisting of 1 to 186; X is 10 and Y is selected from the group consisting of 1 to 186; X is 11 and Y is selected from the group consisting of 1 to 186; X is 12 and Y is selected from the group consisting of 1 to 186; X is 13 and Y is selected from the group consisting of 1 to 186; X is 14 and Y is selected from the group consisting of 1 to 186; X is 15 and Y is selected from the group consisting of 1 to 186; X is 16 and Y is selected from the group consisting of 1 to 186; X is 17 and Y is selected from the group consisting of 1 to 186; X is 18 and Y is selected from the group consisting of 1 to 186; X is 19 and Y is selected from the group consisting of 1 to 186; X is 20 and Y is selected from the group consisting of 1 to 186; X is 21 and Y is selected from the group consisting of 1 to 186; X is 22 and Y is selected from the group consisting of 1 to 186; X is 23 and Y is selected from the group consisting of 1 to 186; X is 24 and Y is selected from the group consisting of 1 to 186; X is 25 and Y is selected from the group consisting of 1 to 186; X is 26 and Y is selected from the group consisting of 1 to 186; X is 27 and Y is selected from the group consisting of 1 to 186; X is 28 and Y is selected from the group consisting of 1 to 186; X is 29 and Y is selected from the group consisting of 1 to 186; X is 30 and Y is selected from the group consisting of 1 to 186; X is 31 and Y is selected from the group consisting of 1 to 186; X is 32 and Y is selected from the group consisting of 1 to 186; X is 33 and Y is selected from the group consisting of 1 to 186; X is 34 and Y is selected from the group consisting of 1 to 186; X is 35

and Y is selected from the group consisting of 1 to 186; X is 36 and Y is selected from the group consisting of 1 to 186; X is 37 and Y is selected from the group consisting of 1 to 186; X is 38 and Y is selected from the group consisting of 1 to 186; X is 39 and Y is selected from the group consisting of 1 to 186; X is 40 and Y is selected from the group consisting of 1 to 186; X is 41 and Y is selected from the group consisting of 1 to 186; X is 42 and Y is selected from the group consisting of 1 to 186; X is 43 and Y is selected from the group consisting of 1 to 186; X is 44 and Y is selected from the group consisting of 1 to 186; X is 45 and Y is selected from the group consisting of 1 to 186; X is 46 and Y is selected from the group consisting of 1 to 186; X is 47 and Y is selected from the group consisting of 1 to 186; X is 48 and Y is selected from the group consisting of 1 to 186; X is 49 and Y is selected from the group consisting of 1 to 186; X is 50 and Y is selected from the group consisting of 1 to 186; X is 51 and Y is selected from the group consisting of 1 to 186; X is 52 and Y is selected from the group consisting of 1 to 186; X is 53 and Y is selected from the group consisting of 1 to 186; X is 54 and Y is selected from the group consisting of 1 to 186; X is 55 and Y is selected from the group consisting of 1 to 186; X is 56 and Y is selected from the group consisting of 1 to 186; X is 57 and Y is selected from the group consisting of 1 to 186; X is 58 and Y is selected from the group consisting of 1 to 186; X is 59 and Y is selected from the group consisting of 1 to 186; X is 60 and Y is selected from the group consisting of 1 to 186; X is 61 and Y is selected from the group consisting of 1 to 186; X is 62 and Y is selected from the group consisting of 1 to 186; X is 63 and Y is selected from the group consisting of 1 to 186; X is 64 and Y is selected from the group consisting of 1 to 186; X is 65 and Y is selected from the group consisting of 1 to 186; X is 66 and Y is selected from the group consisting of 1 to 186; X is 67 and Y is selected from the group consisting of 1 to 186; X is 68 and Y is selected from the group consisting of 1 to 186; X is 69 and Y is selected from the group consisting of 1 to 186; X is 70 and Y is selected from the group consisting of 1 to 186; X is 71 and Y is selected from the group consisting of 1 to 186; X is 72 and Y is selected from the group consisting of 1 to 186; X is 73 and Y is selected from the group consisting of 1 to 186; X is 74 and Y is selected from the group consisting of 1 to 186; X is 75 and Y is selected from the group consisting of 1 to 186; X is 76 and Y is selected from the group consisting of 1 to 186; X is 77 and Y is selected from the group consisting of 1 to 186; X is 78 and Y is selected from the group consisting of 1 to 186; X is 79 and Y is selected from the group consisting of 1 to 186; X is 80 and Y is selected from the group consisting of 1 to 186; X is 81 and Y is selected from the group consisting of 1 to 186; X is 82 and Y is selected from the group consisting of 1 to 186; X is 83 and Y is selected from the group consisting of 1 to 186; X is 84 and Y is selected from the group consisting of 1 to 186; X is 85 and Y is selected from the group consisting of 1 to 186; X is 86 and Y is selected from the group consisting of 1 to 186 X is 87 and Y is selected from the group

consisting of 1 to 186; X is 88 and Y is selected from the group consisting of 1 to 186; X is 89 and Y is selected from the group consisting of 1 to 186; X is 90 and Y is selected from the group consisting of 1 to 186; X is 91 and Y is selected from the group consisting of 1 to 186; or X is 92 and Y is selected from the group consisting of 1 to 186.

In various embodiments,

X is 1 and Y is 1; X is 1 and Y is 2; X is 1 and Y is 3; X is 1 and Y is 4; X is 1 and Y is 5; X is 1 and Y is 6; X is 1 and Y is 7; X is 1 and Y is 8; X is 1 and Y is 9; X is 1 and Y is 10; X is 1 and Y is 11; X is 1 and Y is 12; X is 1 and Y is 13; X is 1 and Y is 14; X is 1 and Y is 15; X is 1 and Y is 16; X is 1 and Y is 17; X is 1 and Y is 18; X is 1 and Y is 19; X is 1 and Y is 20; X is 1 and Y is 21; X is 1 and Y is 22; X is 1 and Y is 23; X is 1 and Y is 24; X is 1 and Y is 25; X is 1 and Y is 26; X is 1 and Y is 27; X is 1 and Y is 28; X is 1 and Y is 29; X is 1 and Y is 30; X is 1 and Y is 31; X is 1 and Y is 32; X is 1 and Y is 33; X is 1 and Y is 34; X is 1 and Y is 35; X is 1 and Y is 36; X is 1 and Y is 37; X is 1 and Y is 38; X is 1 and Y is 39; X is 1 and Y is 40; X is 1 and Y is 41; X is 1 and Y is 42; X is 1 and Y is 43; X is 1 and Y is 44; X is 1 and Y is 45; X is 1 and Y is 46; X is 1 and Y is 47; X is 1 and Y is 48; X is 1 and Y is 49; X is 1 and Y is 50; X is 1 and Y is 51; X is 1 and Y is 52; X is 1 and Y is 53; X is 1 and Y is 54; X is 1 and Y is 55; X is 1 and Y is 56; X is 1 and Y is 57; X is 1 and Y is 58; X is 1 and Y is 59; X is 1 and Y is 60; X is 1 and Y is 61; X is 1 and Y is 62; X is 1 and Y is 63; X is 1 and Y is 64; X is 1 and Y is 65; X is 1 and Y is 66; X is 1 and Y is 67; X is 1 and Y is 68; X is 1 and Y is 69; X is 1 and Y is 70; X is 1 and Y is 71; X is 1 and Y is 72; X is 1 and Y is 73; X is 1 and Y is 74; X is 1 and Y is 75; X is 1 and Y is 76; X is 1 and Y is 77; X is 1 and Y is 78; X is 1 and Y is 79; X is 1 and Y is 80; X is 1 and Y is 81; X is 1 and Y is 82; X is 1 and Y is 83; X is 1 and Y is 84; X is 1 and Y is 85; X is 1 and Y is 86; X is 1 and Y is 87; X is 1 and Y is 88; X is 1 and Y is 89; X is 1 and Y is 90; X is 1 and Y is 91; X is 1 and Y is 92; X is 1 and Y is 93; X is 1 and Y is 94; X is 1 and Y is 95; X is 1 and Y is 96; X is 1 and Y is 97; X is 1 and Y is 98; X is 1 and Y is 99; X is 1 and Y is 100; X is 1 and Y is 101; X is 1 and Y is 102; X is 1 and Y is 103; X is 1 and Y is 104; X is 1 and Y is 105; X is 1 and Y is 106; X is 1 and Y is 107; X is 1 and Y is 108; X is 1 and Y is 109; X is 1 and Y is 110; X is 1 and Y is 111; X is 1 and Y is 112; X is 1 and Y is 113; X is 1 and Y is 114; X is 1 and Y is 115; X is 1 and Y is 116; X is 1 and Y is 117; X is 1 and Y is 118; X is 1 and Y is 119; X is 1 and Y is 120; X is 1 and Y is 121; X is 1 and Y is 122; X is 1 and Y is 123; X is 1 and Y is 124; X is 1 and Y is 125; X is 1 and Y is 126; X is 1 and Y is 127; X is 1 and Y is 128; X is 1 and Y is 129; X is 1 and Y is 130; X is 1 and Y is 131; X is 1 and Y is 132; X is 1 and Y is 133; X is 1 and Y is 134; X is 1 and Y is 135; X is 1 and Y is 136; X is 1 and Y is 137; X is 1 and Y is 138; X is 1 and Y is 139; X is 1 and Y is 140; X is 1 and Y is 141; X is 1 and Y is 142; X is 1

and Y is 143; X is 1 and Y is 144; X is 1 and Y is 145; X is 1 and Y is 146; X is 1 and Y is 147; X is 1 and Y is 148; X is 1 and Y is 149; X is 1 and Y is 150; X is 1 and Y is 151; X is 1 and Y is 152; X is 1 and Y is 153; X is 1 and Y is 154; X is 1 and Y is 155; X is 1 and Y is 156; X is 1 and Y is 157; X is 1 and Y is 158; X is 1 and Y is 159; X is 1 and Y is 160; X is 1 and Y is 161; X is 1 and Y is 162; X is 1 and Y is 163; X is 1 and Y is 164; X is 1 and Y is 165; X is 1 and Y is 166; X is 1 and Y is 167; X is 1 and Y is 168; X is 1 and Y is 169; X is 1 and Y is 170; X is 1 and Y is 171; X is 1 and Y is 172; X is 1 and Y is 173; X is 1 and Y is 174; X is 1 and Y is 175; X is 1 and Y is 176; X is 1 and Y is 177; X is 1 and Y is 178; X is 1 and Y is 179; X is 1 and Y is 180; X is 1 and Y is 181; X is 1 and Y is 182; X is 1 and Y is 183; X is 1 and Y is 184; X is 1 and Y is 185; X is 1 and Y is 186;

X is 2 and Y is 1; X is 2 and Y is 2; X is 2 and Y is 3; X is 2 and Y is 4; X is 2 and Y is 5; X is 2 and Y is 6; X is 2 and Y is 7; X is 2 and Y is 8; X is 2 and Y is 9; X is 2 and Y is 10; X is 2 and Y is 11; X is 2 and Y is 12; X is 2 and Y is 13; X is 2 and Y is 14; X is 2 and Y is 15; X is 2 and Y is 16; X is 2 and Y is 17; X is 2 and Y is 18; X is 2 and Y is 19; X is 2 and Y is 20; X is 2 and Y is 21; X is 2 and Y is 22; X is 2 and Y is 23; X is 2 and Y is 24; X is 2 and Y is 25; X is 2 and Y is 26; X is 2 and Y is 27; X is 2 and Y is 28; X is 2 and Y is 29; X is 2 and Y is 30; X is 2 and Y is 31; X is 2 and Y is 32; X is 2 and Y is 33; X is 2 and Y is 34; X is 2 and Y is 35; X is 2 and Y is 36; X is 2 and Y is 37; X is 2 and Y is 38; X is 2 and Y is 39; X is 2 and Y is 40; X is 2 and Y is 41; X is 2 and Y is 42; X is 2 and Y is 43; X is 2 and Y is 44; X is 2 and Y is 45; X is 2 and Y is 46; X is 2 and Y is 47; X is 2 and Y is 48; X is 2 and Y is 49; X is 2 and Y is 50; X is 2 and Y is 51; X is 2 and Y is 52; X is 2 and Y is 53; X is 2 and Y is 54; X is 2 and Y is 55; X is 2 and Y is 56; X is 2 and Y is 57; X is 2 and Y is 58; X is 2 and Y is 59; X is 2 and Y is 60; X is 2 and Y is 61; X is 2 and Y is 62; X is 2 and Y is 63; X is 2 and Y is 64; X is 2 and Y is 65; X is 2 and Y is 66; X is 2 and Y is 67; X is 2 and Y is 68; X is 2 and Y is 69; X is 2 and Y is 70; X is 2 and Y is 71; X is 2 and Y is 72; X is 2 and Y is 73; X is 2 and Y is 74; X is 2 and Y is 75; X is 2 and Y is 76; X is 2 and Y is 77; X is 2 and Y is 78; X is 2 and Y is 79; X is 2 and Y is 80; X is 2 and Y is 81; X is 2 and Y is 82; X is 2 and Y is 83; X is 2 and Y is 84; X is 2 and Y is 85; X is 2 and Y is 86; X is 2 and Y is 87; X is 2 and Y is 88; X is 2 and Y is 89; X is 2 and Y is 90; X is 2 and Y is 91; X is 2 and Y is 92; X is 2 and Y is 93; X is 2 and Y is 94; X is 2 and Y is 95; X is 2 and Y is 96; X is 2 and Y is 97; X is 2 and Y is 98; X is 2 and Y is 99; X is 2 and Y is 100; X is 2 and Y is 101; X is 2 and Y is 102; X is 2 and Y is 103; X is 2 and Y is 104; X is 2 and Y is 105; X is 2 and Y is 106; X is 2 and Y is 107; X is 2 and Y is 108; X is 2 and Y is 109; X is 2 and Y is 110; X is 2 and Y is 111; X is 2 and Y is 112; X is 2 and Y is 113; X is 2 and Y is 114; X is 2 and Y is 115; X is 2 and Y is 116; X is 2 and Y is 117; X is 2 and Y is 118; X is 2 and Y is 119; X is 2 and Y is 120; X is 2 and Y is 121; X is 2 and Y is 122; X is 2 and Y is 123; X is 2 and Y is

124; X is 2 and Y is 125; X is 2 and Y is 126; X is 2 and Y is 127; X is 2 and Y is 128; X is 2 and Y is 129; X is 2 and Y is 130; X is 2 and Y is 131; X is 2 and Y is 132; X is 2 and Y is 133; X is 2 and Y is 134; X is 2 and Y is 135; X is 2 and Y is 136; X is 2 and Y is 137; X is 2 and Y is 138; X is 2 and Y is 139; X is 2 and Y is 140; X is 2 and Y is 141; X is 2 and Y is 142; X is 2 and Y is 143; X is 2 and Y is 144; X is 2 and Y is 145; X is 2 and Y is 146; X is 2 and Y is 147; X is 2 and Y is 148; X is 2 and Y is 149; X is 2 and Y is 150; X is 2 and Y is 151; X is 2 and Y is 152; X is 2 and Y is 153; X is 2 and Y is 154; X is 2 and Y is 155; X is 2 and Y is 156; X is 2 and Y is 157; X is 2 and Y is 158; X is 2 and Y is 159; X is 2 and Y is 160; X is 2 and Y is 161; X is 2 and Y is 162; X is 2 and Y is 163; X is 2 and Y is 164; X is 2 and Y is 165; X is 2 and Y is 166; X is 2 and Y is 167; X is 2 and Y is 168; X is 2 and Y is 169; X is 2 and Y is 170; X is 2 and Y is 171; X is 2 and Y is 172; X is 2 and Y is 173; X is 2 and Y is 174; X is 2 and Y is 175; X is 2 and Y is 176; X is 2 and Y is 177; X is 2 and Y is 178; X is 2 and Y is 179; X is 2 and Y is 180; X is 2 and Y is 181; X is 2 and Y is 182; X is 2 and Y is 183; X is 2 and Y is 184; X is 2 and Y is 185; X is 2 and Y is 186;

X is 3 and Y is 1; X is 3 and Y is 2; X is 3 and Y is 3; X is 3 and Y is 4; X is 3 and Y is 5; X is 3 and Y is 6; X is 3 and Y is 7; X is 3 and Y is 8; X is 3 and Y is 9; X is 3 and Y is 10; X is 3 and Y is 11; X is 3 and Y is 12; X is 3 and Y is 13; X is 3 and Y is 14; X is 3 and Y is 15; X is 3 and Y is 16; X is 3 and Y is 17; X is 3 and Y is 18; X is 3 and Y is 19; X is 3 and Y is 20; X is 3 and Y is 21; X is 3 and Y is 22; X is 3 and Y is 23; X is 3 and Y is 24; X is 3 and Y is 25; X is 3 and Y is 26; X is 3 and Y is 27; X is 3 and Y is 28; X is 3 and Y is 29; X is 3 and Y is 30; X is 3 and Y is 31; X is 3 and Y is 32; X is 3 and Y is 33; X is 3 and Y is 34; X is 3 and Y is 35; X is 3 and Y is 36; X is 3 and Y is 37; X is 3 and Y is 38; X is 3 and Y is 39; X is 3 and Y is 40; X is 3 and Y is 41; X is 3 and Y is 42; X is 3 and Y is 43; X is 3 and Y is 44; X is 3 and Y is 45; X is 3 and Y is 46; X is 3 and Y is 47; X is 3 and Y is 48; X is 3 and Y is 49; X is 3 and Y is 50; X is 3 and Y is 51; X is 3 and Y is 52; X is 3 and Y is 53; X is 3 and Y is 54; X is 3 and Y is 55; X is 3 and Y is 56; X is 3 and Y is 57; X is 3 and Y is 58; X is 3 and Y is 59; X is 3 and Y is 60; X is 3 and Y is 61; X is 3 and Y is 62; X is 3 and Y is 63; X is 3 and Y is 64; X is 3 and Y is 65; X is 3 and Y is 66; X is 3 and Y is 67; X is 3 and Y is 68; X is 3 and Y is 69; X is 3 and Y is 70; X is 3 and Y is 71; X is 3 and Y is 72; X is 3 and Y is 73; X is 3 and Y is 74; X is 3 and Y is 75; X is 3 and Y is 76; X is 3 and Y is 77; X is 3 and Y is 78; X is 3 and Y is 79; X is 3 and Y is 80; X is 3 and Y is 81; X is 3 and Y is 82; X is 3 and Y is 83; X is 3 and Y is 84; X is 3 and Y is 85; X is 3 and Y is 86; X is 3 and Y is 87; X is 3 and Y is 88; X is 3 and Y is 89; X is 3 and Y is 90; X is 3 and Y is 91; X is 3 and Y is 92; X is 3 and Y is 93; X is 3 and Y is 94; X is 3 and Y is 95; X is 3 and Y is 96; X is 3 and Y is 97; X is 3 and Y is 98; X is 3 and Y is 99; X is 3 and Y is 100; X is 3 and Y is 101; X is 3 and Y is 102; X is 3 and Y is 103; X is 3 and Y is 104; X is 3 and Y is 105;

X is 3 and Y is 106; X is 3 and Y is 107; X is 3 and Y is 108; X is 3 and Y is 109; X is 3 and Y is 110; X is 3 and Y is 111; X is 3 and Y is 112; X is 3 and Y is 113; X is 3 and Y is 114; X is 3 and Y is 115; X is 3 and Y is 116; X is 3 and Y is 117; X is 3 and Y is 118; X is 3 and Y is 119; X is 3 and Y is 120; X is 3 and Y is 121; X is 3 and Y is 122; X is 3 and Y is 123; X is 3 and Y is 124; X is 3 and Y is 125; X is 3 and Y is 126; X is 3 and Y is 127; X is 3 and Y is 128; X is 3 and Y is 129; X is 3 and Y is 130; X is 3 and Y is 131; X is 3 and Y is 132; X is 3 and Y is 133; X is 3 and Y is 134; X is 3 and Y is 135; X is 3 and Y is 136; X is 3 and Y is 137; X is 3 and Y is 138; X is 3 and Y is 139; X is 3 and Y is 140; X is 3 and Y is 141; X is 3 and Y is 142; X is 3 and Y is 143; X is 3 and Y is 144; X is 3 and Y is 145; X is 3 and Y is 146; X is 3 and Y is 147; X is 3 and Y is 148; X is 3 and Y is 149; X is 3 and Y is 150; X is 3 and Y is 151; X is 3 and Y is 152; X is 3 and Y is 153; X is 3 and Y is 154; X is 3 and Y is 155; X is 3 and Y is 156; X is 3 and Y is 157; X is 3 and Y is 158; X is 3 and Y is 159; X is 3 and Y is 160; X is 3 and Y is 161; X is 3 and Y is 162; X is 3 and Y is 163; X is 3 and Y is 164; X is 3 and Y is 165; X is 3 and Y is 166; X is 3 and Y is 167; X is 3 and Y is 168; X is 3 and Y is 169; X is 3 and Y is 170; X is 3 and Y is 171; X is 3 and Y is 172; X is 3 and Y is 173; X is 3 and Y is 174; X is 3 and Y is 175; X is 3 and Y is 176; X is 3 and Y is 177; X is 3 and Y is 178; X is 3 and Y is 179; X is 3 and Y is 180; X is 3 and Y is 181; X is 3 and Y is 182; X is 3 and Y is 183; X is 3 and Y is 184; X is 3 and Y is 185; X is 3 and Y is 186;

X is 4 and Y is 1; X is 4 and Y is 2; X is 4 and Y is 3; X is 4 and Y is 4; X is 4 and Y is 5; X is 4 and Y is 6; X is 4 and Y is 7; X is 4 and Y is 8; X is 4 and Y is 9; X is 4 and Y is 10; X is 4 and Y is 11; X is 4 and Y is 12; X is 4 and Y is 13; X is 4 and Y is 14; X is 4 and Y is 15; X is 4 and Y is 16; X is 4 and Y is 17; X is 4 and Y is 18; X is 4 and Y is 19; X is 4 and Y is 20; X is 4 and Y is 21; X is 4 and Y is 22; X is 4 and Y is 23; X is 4 and Y is 24; X is 4 and Y is 25; X is 4 and Y is 26; X is 4 and Y is 27; X is 4 and Y is 28; X is 4 and Y is 29; X is 4 and Y is 30; X is 4 and Y is 31; X is 4 and Y is 32; X is 4 and Y is 33; X is 4 and Y is 34; X is 4 and Y is 35; X is 4 and Y is 36; X is 4 and Y is 37; X is 4 and Y is 38; X is 4 and Y is 39; X is 4 and Y is 40; X is 4 and Y is 41; X is 4 and Y is 42; X is 4 and Y is 43; X is 4 and Y is 44; X is 4 and Y is 45; X is 4 and Y is 46; X is 4 and Y is 47; X is 4 and Y is 48; X is 4 and Y is 49; X is 4 and Y is 50; X is 4 and Y is 51; X is 4 and Y is 52; X is 4 and Y is 53; X is 4 and Y is 54; X is 4 and Y is 55; X is 4 and Y is 56; X is 4 and Y is 57; X is 4 and Y is 58; X is 4 and Y is 59; X is 4 and Y is 60; X is 4 and Y is 61; X is 4 and Y is 62; X is 4 and Y is 63; X is 4 and Y is 64; X is 4 and Y is 65; X is 4 and Y is 66; X is 4 and Y is 67; X is 4 and Y is 68; X is 4 and Y is 69; X is 4 and Y is 70; X is 4 and Y is 71; X is 4 and Y is 72; X is 4 and Y is 73; X is 4 and Y is 74; X is 4 and Y is 75; X is 4 and Y is 76; X is 4 and Y is 77; X is 4 and Y is 78; X is 4 and Y is 79; X is 4 and Y is 80; X is 4 and Y is 81; X is 4 and Y is 82; X is 4 and Y is 83; X is 4 and Y is 84; X is 4 and Y is 85; X is 4 and

Y is 86; X is 4 and Y is 87; X is 4 and Y is 88; X is 4 and Y is 89; X is 4 and Y is 90; X is 4 and Y is 91; X is 4 and Y is 92; X is 4 and Y is 93; X is 4 and Y is 94; X is 4 and Y is 95; X is 4 and Y is 96; X is 4 and Y is 97; X is 4 and Y is 98; X is 4 and Y is 99; X is 4 and Y is 100; X is 4 and Y is 101; X is 4 and Y is 102; X is 4 and Y is 103; X is 4 and Y is 104; X is 4 and Y is 105; X is 4 and Y is 106; X is 4 and Y is 107; X is 4 and Y is 108; X is 4 and Y is 109; X is 4 and Y is 110; X is 4 and Y is 111; X is 4 and Y is 112; X is 4 and Y is 113; X is 4 and Y is 114; X is 4 and Y is 115; X is 4 and Y is 116; X is 4 and Y is 117; X is 4 and Y is 118; X is 4 and Y is 119; X is 4 and Y is 120; X is 4 and Y is 121; X is 4 and Y is 122; X is 4 and Y is 123; X is 4 and Y is 124; X is 4 and Y is 125; X is 4 and Y is 126; X is 4 and Y is 127; X is 4 and Y is 128; X is 4 and Y is 129; X is 4 and Y is 130; X is 4 and Y is 131; X is 4 and Y is 132; X is 4 and Y is 133; X is 4 and Y is 134; X is 4 and Y is 135; X is 4 and Y is 136; X is 4 and Y is 137; X is 4 and Y is 138; X is 4 and Y is 139; X is 4 and Y is 140; X is 4 and Y is 141; X is 4 and Y is 142; X is 4 and Y is 143; X is 4 and Y is 144; X is 4 and Y is 145; X is 4 and Y is 146; X is 4 and Y is 147; X is 4 and Y is 148; X is 4 and Y is 149; X is 4 and Y is 150; X is 4 and Y is 151; X is 4 and Y is 152; X is 4 and Y is 153; X is 4 and Y is 154; X is 4 and Y is 155; X is 4 and Y is 156; X is 4 and Y is 157; X is 4 and Y is 158; X is 4 and Y is 159; X is 4 and Y is 160; X is 4 and Y is 161; X is 4 and Y is 162; X is 4 and Y is 163; X is 4 and Y is 164; X is 4 and Y is 165; X is 4 and Y is 166; X is 4 and Y is 167; X is 4 and Y is 168; X is 4 and Y is 169; X is 4 and Y is 170; X is 4 and Y is 171; X is 4 and Y is 172; X is 4 and Y is 173; X is 4 and Y is 174; X is 4 and Y is 175; X is 4 and Y is 176; X is 4 and Y is 177; X is 4 and Y is 178; X is 4 and Y is 179; X is 4 and Y is 180; X is 4 and Y is 181; X is 4 and Y is 182; X is 4 and Y is 183; X is 4 and Y is 184; X is 4 and Y is 185; X is 4 and Y is 186;

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X is 92 and Y is 1; X is 92 and Y is 2; X is 92 and Y is 3; X is 92 and Y is 4; X is 92 and Y is 5; X is 92 and Y is 6; X is 92 and Y is 7; X is 92 and Y is 8; X is 92 and Y is 9; X is 92 and Y is 10; X is 92 and Y is 11; X is 92 and Y is 12; X is 92 and Y is 13; X is 92 and Y is 14; X is 92 and Y is 15; X is 92 and Y is 16; X is 92 and Y is 17; X is 92 and Y is 18; X is 92 and Y is 19; X is 92 and Y is 20; X is 92 and Y is 21; X is 92 and Y is 22; X is 92 and Y is 23; X is 92 and Y is 24; X is 92 and Y is 25; X is 92 and Y is 26; X is 92 and Y is 27; X is 92 and Y is 28; X is 92 and Y is 29; X is 92 and Y is 30; X is 92 and Y is 31; X is 92 and Y is 32; X is 92 and Y is 33; X is 92 and Y is 34; X is 92 and Y is 35; X is 92 and Y is 36; X is 92 and Y is 37; X is 92 and Y is 38; X is 92 and Y is 39; X is 92 and Y is 40; X is 92 and Y is 41; X is 92 and Y is 42; X is 92 and Y is 43; X is 92 and Y is 44; X is 92 and Y is 45; X is 92 and Y is 46; X is 92 and Y is 47; X is 92 and Y is 48; X is 92 and Y is 49; X is 92 and Y is 50; X is 92 and Y is 51; X is 92 and Y is 52; X is 92 and Y is 53; X is 92 and Y is 54; X is 92 and Y is 55; X is 92 and Y is 56; X is 92 and Y is 57; X is 92 and Y is 58; X is 92 and Y is 59; X is 92 and Y is 60; X is 92 and Y is 61; X is 92 and Y is 62; X is 92 and Y is 63; X is 92 and Y is 64; X is 92 and Y is 65; X is 92 and Y is 66; X is 92 and Y is 67; X is 92 and Y is 68; X is 92 and Y is 69; X is 92 and Y is 70; X is 92 and Y is 71; X is 92 and Y is 72; X is 92 and Y is 73; X is 92 and Y is 74; X is 92 and Y is 75; X is 92 and Y is 76; X is 92 and Y is 77; X is 92 and Y is 78; X is 92 and Y is 79; X is 92 and Y is

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In one embodiment, X is 3 and Y is 5.

The term "one or more CDRs", as used in connection with the above embodiments, refers to one of the CDRs HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 (e.g., HCDR3), or several (i.e., 2, 3, 4, 5 or 6) of the CDRs, such as a set of HCDR1, HCDR2 and HCDR3 or a set of LCDR1, LCDR2 and LCDR3. In a preferred embodiment, the term "one or more CDRs" refers to all six CDRs, i.e., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3.

The term "one or more variable domains", as used in connection with the above embodiments, preferably refers to one of the VH domain and the VL domain or, more preferably, to both the VH domain and the VL domain.

The term "nucleic acid molecule", as used herein, is intended to include DNA and RNA, such as genomic DNA, cDNA, mRNA, recombinantly produced and chemically synthesized molecules. A nucleic acid molecule may be single-stranded or double-stranded. RNA includes *in vitro* transcribed RNA (IVT RNA) or synthetic RNA.

In the context of the present invention, the term "DNA" relates to a molecule which comprises deoxyribonucleotide residues and preferably is entirely or substantially composed of deoxyribonucleotide residues. "Deoxyribonucleotide" relates to a nucleotide which lacks a hydroxyl group at the 2'-position of a β-D-ribofuranosyl group. The term "DNA" comprises isolated DNA such as partially or completely purified DNA, essentially pure DNA, synthetic DNA, and recombinantly generated DNA and includes modified DNA which differs from naturally occurring DNA by addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of a DNA or internally, for example at one or more nucleotides of the DNA. Nucleotides in DNA molecules can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides. These altered DNAs can be referred to as analogs or analogs of naturally-occurring DNA.

In the context of the present invention, the term "RNA" relates to a molecule which comprises ribonucleotide residues and preferably being entirely or substantially composed of ribonucleotide residues. "Ribonucleotide" relates to a nucleotide with a hydroxyl group at the 2'-position of a β-D-ribofuranosyl group. The term includes double stranded RNA, single stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as modified RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of a RNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in RNA molecules can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA. According to the present invention, the term "RNA" includes and preferably relates to "mRNA" which means "messenger RNA" and relates to a

transcript which may be produced using DNA as template and encodes a peptide or protein. mRNA typically comprises a 5' non translated region (5'-UTR), a protein or peptide coding region and a 3' non translated region (3'-UTR). mRNA has a limited halftime in cells and *in vitro*. Preferably, mRNA is produced by *in vitro* transcription using a DNA template. In one embodiment of the invention, the RNA is obtained by *in vitro* transcription or chemical synthesis. The *in vitro* transcription methodology is known to the skilled person. For example, there is a variety of *in vitro* transcription kits commercially available.

The nucleic acid molecule according to the present invention may be contained/comprised in a vector. The term "vector", as used herein, includes any vectors known to the skilled person, including plasmid vectors, cosmid vectors, phage vectors, such as lambda phage, viral vectors, such as adenoviral or baculoviral vectors, or artificial chromosome vectors such as bacterial artificial chromosomes (BAC), yeast artificial chromosomes (YAC), or P1 artificial chromosomes (PAC). Said vectors include expression as well as cloning vectors. Expression vectors comprise plasmids as well as viral vectors and generally contain a desired coding sequence and appropriate DNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism (e.g., bacteria, yeast, plant, insect, or mammal) or in *in vitro* expression systems. Cloning vectors are generally used to engineer and amplify a certain desired DNA fragment and may lack functional sequences needed for expression of the desired DNA fragments.

The term "cell" or "host cell" preferably relates to an intact cell, i.e. a cell with an intact membrane that has not released its normal intracellular components such as enzymes, organelles, or genetic material. An intact cell preferably is a viable cell, i.e. a living cell capable of carrying out its normal metabolic functions. Preferably said term relates according to the invention to any cell which can be transfected with an exogenous nucleic acid. Preferably, the cell when transfected with an exogenous nucleic acid and transferred to a recipient can express the nucleic acid in the recipient. The term "cell" includes bacterial cells; other useful cells are yeast cells, fungal cells or mammalian cells. Suitable bacterial cells include cells from gram-negative bacterial strains such as strains of *Escherichia coli*, *Proteus*, and *Pseudomonas*, and gram-positive bacterial strains such as strains of *Bacillus*, *Streptomyces*, *Staphylococcus*, and *Lactococcus*. Suitable fungal cell include cells from species of *Trichoderma*, *Neuro*spora, and *Aspergillus*. Suitable yeast cells include cells from species of *Saccharomyces* (Tor example Saccharomyces cerevisiae), *Schizosaccharomyces* (for example *Schizosaccharomyces pombe*), *Pichia* (for example *Pichia pastoris* and *Pichia methanolica*), and *Hansenula*. Suitable

mammalian cells include for example CHO cells, BHK cells, HeLa cells, COS cells, 293 HEK and the like. However, amphibian cells, insect cells, plant cells, and any other cells used in the art for the expression of heterologous proteins can be used as well. Mammalian cells are particularly preferred for adoptive transfer, such as cells from humans, mice, hamsters, pigs, goats, and primates. The cells may be derived from a large number of tissue types and include primary cells and cell lines such as cells of the immune system, in particular antigen-presenting cells (APCs), such as dendritic cells, and T cells, stem cells, such as hematopoietic stem cells and mesenchymal stem cells, and other cell types. An antigen-presenting cell is a cell that displays antigen in the context of major histocompatibility complex on its surface. T cells may recognize this complex using their T cell receptor (TCR).

The binding agents of the invention can be produced either intracellularly (e.g. in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or they can be produced extracellularly (e.g. in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. Methods and reagents used for the recombinant production of polypeptides, such as specific suitable expression vectors, transformation or transfection methods, selection markers, methods of induction of protein expression, culture conditions, and the like, are known in the art. Similarly, protein isolation and purification techniques are well known to the skilled person.

The binding agents, nucleic acid molecules or cells described herein may be administered in the form of any suitable pharmaceutical composition.

The pharmaceutical compositions of the invention are preferably sterile and contain an effective amount of the binding agents, nucleic acid molecules or cells described herein to generate the desired reaction or the desired effect.

Pharmaceutical compositions are usually provided in a uniform dosage form and may be prepared in a manner known per se. A pharmaceutical composition may, e.g., be in the form of a solution or suspension.

A pharmaceutical composition may further comprise one or more carriers and/or excipients, all of which are preferably pharmaceutically acceptable. The term "pharmaceutically acceptable", as used herein, refers to the non-toxicity of a material which, preferably, does not interact with the action of the active agent of the pharmaceutical composition.

The term "carrier" refers to an organic or inorganic component, of a natural or synthetic nature, in which the active component is combined in order to facilitate, enhance or enable application. According to the invention, the term "carrier" also includes one or more compatible solid or liquid fillers, diluents or encapsulating substances, which are suitable for administration to a subject.

Possible carrier substances for parenteral administration are, e.g., sterile water, Ringer, Ringer lactate, sterile sodium chloride solution, polyalkylene glycols, hydrogenated naphthalenes and, in particular, biocompatible lactide polymers, lactide/glycolide copolymers or polyoxyethylene/polyoxy-propylene copolymers.

The term "excipient", as used herein, is intended to include all substances which may be present in a pharmaceutical composition and which are not active ingredients, such as salts, binders, lubricants, thickeners, surface active agents, preservatives, emulsifiers, buffer substances, flavouring agents, or colorants.

Salts, which are not pharmaceutically acceptable, may be used for preparing pharmaceutically acceptable salts and are included in the invention. Pharmaceutically acceptable salts of this kind comprise in a non limiting way those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic acids, and the like. Pharmaceutically acceptable salts may also be prepared as alkali metal salts or alkaline earth metal salts, such as sodium salts, potassium salts or calcium salts.

Suitable preservatives for use in a pharmaceutical composition include benzalkonium chloride, chlorobutanol, paraben and thimerosal.

Suitable buffer substances for use in a pharmaceutical composition include acetic acid in a salt, citric acid in a salt, boric acid in a salt and phosphoric acid in a salt.

The agents and compositions described herein may be administered via any conventional route, such as by parenteral administration including by injection or infusion. Administration is preferably parenterally, e.g. intravenously, intraarterially, subcutaneously, intradermally or intramuscularly.

Pharmaceutical compositions suitable for parenteral administration usually comprise a sterile aqueous or non-aqueous preparation of the active compound, which is preferably isotonic to the blood of the recipient. Examples of compatible carriers/solvents/diluents are Ringer solution and isotonic sodium chloride solution. In addition, usually sterile, fixed oils are used as solution or suspension medium.

The agents and compositions described herein are administered in effective amounts. An "effective amount" refers to the amount, which achieves a desired reaction or a desired effect alone or together with further doses. In the case of treatment of a particular disease or of a particular condition, the desired reaction preferably relates to inhibition of the course of the disease. This comprises slowing down the progress of the disease and, in particular, interrupting or reversing the progress of the disease. The desired reaction in a treatment of a disease or of a condition may also be delay of the onset or a prevention of the onset of said disease or said condition. An effective amount of an agent or composition described herein will depend on the condition to be treated, the severeness of the disease, the individual parameters of the patient, including age, physiological condition, size and weight, the duration of treatment, the type of an accompanying therapy (if present), the specific route of administration and similar factors. Accordingly, the doses administered of the agents described herein may depend on various of such parameters. In the case that a reaction in a patient is insufficient with an initial dose, higher doses (or effectively higher doses achieved by a different, more localized route of administration) may be used.

As used herein, the term "kit of parts (in short: kit)" refers to an article of manufacture comprising one or more containers and, optionally, a data carrier. Said one or more containers may be filled with one or more of the above mentioned means or reagents. Additional containers may be included in the kit that contain, e.g., diluents, buffers and further reagents. Said data carrier may be a non-electronical data carrier, e.g., a graphical data carrier such as an information leaflet, an information sheet, a bar code or an access code, or an electronical data carrier such as a floppy disk, a compact disk (CD), a digital versatile disk (DVD), a microchip or another semiconductor-based electronical data carrier. The access code may allow the access to a database, e.g., an internet database, a centralized, or a decentralized database. Said data carrier may comprise instructions for the use of the binding agent, nucleic acid molecule, cell and/or pharmaceutical composition of the present invention.

The agents and compositions described herein can be administered to patients, e.g., *in vivo*, to treat or prevent a variety of disorders such as those described herein.

The term "medicament", as used herein, refers to a substance/composition used in therapy, i.e., in the treatment of a disease.

According to the invention, the term "disease" refers to any pathological state, in particular cancer, infectious diseases, inflammatory diseases, metabolic diseases, autoimmune disorders, degenerative diseases, apoptosis-associated diseases and transplant rejections.

As used herein, the term "cancer" includes a disease characterized by aberrantly regulated cellular growth, proliferation, differentiation, adhesion, and/or migration. By "cancer cell" is meant an abnormal cell that grows by a rapid, uncontrolled cellular proliferation and continues to grow after the stimuli that initiated the new growth cease. The term "cancer" according to the invention comprises leukemias, seminomas, melanomas, teratomas, lymphomas, neuroblastomas, gliomas, rectal cancer, endometrial cancer, kidney cancer, adrenal cancer, thyroid cancer, blood cancer, skin cancer, cancer of the brain, cervical cancer, intestinal cancer, liver cancer, colon cancer, stomach cancer, intestine cancer, head and neck cancer, gastrointestinal cancer, lymph node cancer, esophagus cancer, colorectal cancer, pancreas cancer, ear, nose and throat (ENT) cancer, breast cancer, prostate cancer, cancer of the uterus, ovarian cancer and lung cancer and the metastases thereof. Examples thereof are lung carcinomas, mamma carcinomas, prostate carcinomas, colon carcinomas, renal cell carcinomas, cervical carcinomas, or metastases of the cancer types or tumors described above.

The term "cancer" according to the invention also comprises cancer metastases. By "metastasis" is meant the spread of cancer cells from its original site to another part of the body. The formation of metastasis is a very complex process and depends on detachment of malignant cells from the primary tumor, invasion of the extracellular matrix, penetration of the endothelial basement membranes to enter the body cavity and vessels, and then, after being transported by the blood, infiltration of target organs. Finally, the growth of a new tumor, i.e. a secondary tumor or metastatic tumor, at the target site depends on angiogenesis. Tumor metastasis often occurs even after the removal of the primary tumor because tumor cells or components may remain and develop metastatic potential. In one embodiment, the term "metastasis" according to the invention relates to "distant metastasis" which relates to a metastasis which is remote from the primary tumor and the regional lymph node system.

The term "infectious disease" refers to any disease which can be transmitted from individual to individual or from organism to organism, and is caused by a microbial agent (e.g. common cold). Examples of infectious diseases include viral infectious diseases, such as AIDS (HIV), hepatitis A, B or C, herpes, herpes zoster (chicken-pox), German measles (rubella virus), yellow fever, dengue etc. flaviviruses, influenza viruses, hemorrhagic infectious diseases (Marburg or Ebola viruses), and severe acute respiratory syndrome (SARS), bacterial infectious diseases, such as Legionnaire's disease (*Legionella*), sexually transmitted diseases (e.g. chlamydia or gonorrhea), gastric ulcer (*Helicobacter*), cholera (*Vibrio*), tuberculosis, diphtheria, infections by *E. coli*, *Staphylococci*, *Salmonella* or *Streptococci* (tetanus); infections by protozoan pathogens such as malaria, sleeping sickness, leishmaniasis; toxoplasmosis, i.e. infections by Plasmodium, Trypanosoma, Leishmania and Toxoplasma; or fungal infections, which are caused, e.g., by *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis* or *Candida albicans*.

The term "inflammatory disease" refers to any disease, which is characterized by or associated with high levels of inflammation in tissues, in particular connective tissues, or degeneration of these tissues. A chronic inflammatory disease is a medical condition which is characterized by persistent inflammation. Examples of (chronic) inflammatory diseases include celiac disease, vasculitis, lupus, chronic obstructive pulmonary disease (COPD), irritable bowel disease, atherosclerosis, arthritis, ankylosing spondylitis, Crohn's disease, colitis, chronic active hepatitis, dermatitis and psoriasis.

The term "metabolic disease" refers to any disease or disorder that disrupts normal metabolism. Examples include cystinosis, diabetes, dyslipidemia, hyperthyroidism, hypothyroidism, hyperlipidemia, hypolipidemia, galactosemia, Gaucher's disease, obesity and phenylketonuria.

The term "autoimmune disorder" refers to any disease/disorder in which the body produces an immunogenic (i.e. immune system) response to some constituent of its own tissue. In other words, the immune system loses its ability to recognize some tissue or system within the body as self and targets and attacks it as if it were foreign. Autoimmune diseases can be classified into those in which predominantly one organ is affected (e.g. hemolytic anemia and anti-immune thyroiditis), and those in which the autoimmune disease process is diffused through many tissues (e.g. systemic lupus erythematosus). For example, multiple sclerosis is thought to be caused by T cells attacking the sheaths that surround the nerve fibers of the brain and spinal cord. This results in loss of coordination, weakness, and blurred vision. Autoimmune diseases are known in the art

and include, for instance, Hashimoto's thyroiditis, Grave's disease, lupus, multiple sclerosis, rheumatic arthritis, hemolytic anemia, anti-immune thyroiditis, systemic lupus erythematosus, celiac disease, Crohn's disease, colitis, diabetes, scleroderma, psoriasis, and the like.

The term "degenerative disease" refers to any disease in which the function or structure of the affected tissues or organs will increasingly deteriorate over time. Examples include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, macular degeneration, multiple sclerosis, muscular dystrophy, Niemann Pick disease, osteoporosis and rheumatoid arthritis.

The term "apoptosis-associated diseases" refers to any disease in which alterations of apoptosis are involved. Examples include cancer, neurological disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) and stroke, heart diseases, such as ischemia reperfusion and chronic heart failure, infectious diseases and autoimmune diseases.

The term "transplant rejection" refers to the rejection of a transplanted tissue or organ by the recipient's immune system, which may, ultimately, destroy the transplanted tissue or organ.

By "treat" is meant to administer a compound or composition or a combination of compounds or compositions to a subject in order to prevent or eliminate a disease, including reducing the size of a tumor or the number of tumors in a subject; arrest or slow a disease in a subject; inhibit or slow the development of a new disease in a subject; decrease the frequency or severity of symptoms and/or recurrences in a subject who currently has or who previously has had a disease; and/or prolong, i.e. increase the lifespan of the subject.

In particular, the term "treatment of a disease" includes curing, shortening the duration, ameliorating, preventing, slowing down or inhibiting progression or worsening, or preventing or delaying the onset of a disease or the symptoms thereof.

The term "subject" means according to the invention a subject for treatment, in particular a diseased subject (also referred to as "patient"), including human beings, non-human primates or other animals, in particular mammals, such as cows, horses, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, hamsters or rodents, such as mice and rats. In a particularly preferred embodiment, the subject/patient is a human being.

The agents and compositions provided herein may be used alone or in combination with therapeutic regimens.

Examples

Example 1: Generation of agonistic anti-CD27, anti-CD134 and anti-CD137 antibodies

1.1 Immunization and collection of B-cell clones

9 rabbits were immunized with a mixture of human CD27 (Enzo Life Sciences, ALX-522-031-0000), human CD134 (OX40; AdipoGen, AG-40B-0014) and human CD137 (4-1BB; Enzo Life Sciences, ALX-522-029-0000), wherein each protein was fused to the Fc portion of human IgG1. Blood was taken at 4 different time points and enriched for B-lymphocytes (also referred to as B-cells). Single B-cells were sorted by fluorescence-activated cell sorting (FACS) into wells of a 96-well microtiter plate and cultivated for 8 days, releasing antibodies into the cultivation medium. The B-cell supernatants were removed and prepared for testing.

1.2 Screening for target-binding antibodies by ELISA

In order to identify antibodies that bind to human CD27, CD134 and CD137, respectively, an enzyme-linked immunosorbent assay (ELISA) was established. For this purpose, CD27 protein (His-tagged, Sino Biological Inc. 10039-H08B1), CD134 protein (His-tagged, Sino Biological Inc. 10481-H08H) and CD137 protein (His-tagged, Sino Biological Inc. 10041-H08H), respectively, were coated onto microtiter plates. PBS containing 2% BSA and 0.05% Tween was used for blocking. After incubation with B-cell supernatants, bound antibodies were detected by using a peroxidase-labeled anti-rabbit-Fc antibody.

Commercially available antibodies were used as reference antibodies for assay development. **Figure 1A** shows binding curves of reference anti-CD27 antibodies (Armenian hamster monoclonal anti-human CD27 clone LG7F9 and mouse monoclonal anti-human CD27 clone 0323, both purchased from eBiosciences) to recombinant protein. **Figure 1B** shows binding curves of a reference anti-CD134 antibody (mouse monoclonal anti-human CD134 clone Ber-ACT35, purchased from eBiosciences) to recombinant protein. **Figure 1C** shows binding curves of reference anti-CD137 antibodies (mouse anti-human CD137 clone 4B4-1 from Biolegend and rat monoclonal anti-human CD137 from Biozol BZL06614) to recombinant protein. For all three

ELISAs, OD 450 nm/620 nm values > 0,5 indicated binding, and the maximum value was at about 3,6.

In total, 47.471 B-cells were sorted, cultivated and screened for binding to CD27, CD134 and CD137 by ELISA. 5.096 antibodies were identified to bind to at least to one of the three targets.

1.3 Identification of cell-binding antibodies

The 5.096 ELISA hits were then tested in a cellular binding assay for binding to cell surface expressed CD27, CD134 and CD137, respectively. For this purpose, following stable transfectants of the HT1080 cell line were used: HT1080_CD27, HT1080_OX40 and HT1080_4-1BB. Cells were cultivated in RPMI 1640 GlutaMAX supplemented with 5% FCS, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cell surface expression of TNF receptors on HT1080-transfectants was analyzed by FACS, wherein cells were stained using antibodies against CD27-PE (BD), OX40-PE (BD) and 4-1BB-PE (BD) (**Figure 2**).

In order to test cellular binding of the antibodies, HT1080 cells overexpressing the respective target (1.000 cells per well of a 384-well microtiter plate) were incubated together with samples for 18 hours. After gentle washing, an Alexa-labeled detection antibody and Hoechst dye were added, and fluorescence intensity was read in the cell imager CellInsight (Thermo). Data analysis was based on the calculation of the mean object spot average intensity.

Commercially available antibodies were used as reference antibodies for assay development. **Figure 3A** shows binding curves of reference anti-CD27 antibodies (Armenian hamster monoclonal anti-human CD27 clone LG7F9) to HT1080_CD27 cells. **Figure 3B** shows binding curves of reference anti-CD134 antibodies (mouse monoclonal anti-human CD134 clone Ber-ACT35) to HT1080_OX40 cells. **Figure 3C** shows binding curves of reference anti-CD137 antibodies (mouse anti-human CD137 clone 4B4-1) to HT1080_4-1BB cells. For all three cell lines, a mean spot intensity > 25 indicated binding, and the maximum values were at about 300.

3.176 antibodies out of the 5.096 ELISA hits were identified to bind at least to one of the three cell surface expressed targets by the cellular binding assay.

1.4 Recombinant antibody production

644 antibody hits, identified by ELISA and the cellular binding assay as described above, were selected. These hits were picked, their RNA was extracted, and sequencing was performed. 582 unique sequences were identified, and the corresponding variable regions of heavy and light chains were gene synthesized. The variable regions were cloned in front of human immunoglobulin constant parts (IgG1/κ). HEK293 cell transient transfections protocols were executed by a Tecan Freedom Evo device on a Dionex Ultimate 3000 with a plate auto-sampler. After normalization, 531 antibodies were available for confirmation of the primary screening data set (ELISA, cellular binding) and for functional assays.

1.5 Analysis of recombinantly expressed antibodies

In order to confirm the primary screening data set, all 531 recombinantly produced antibodies were retested (i) for their binding to the corresponding recombinant protein in the ELISA and (ii) for their binding to the corresponding target expressed on the cell surface of HT1080 cells in the cellular binding assay (both assays as described above).

Furthermore, for the analysis of agonistic antibody functions, target-overexpressing HT1080 cells were used, wherein stimulation of the targets (CD27, CD134 and CD137, respectively) induced IL-8 release from these cell lines. The recombinant CD27-, CD134- and CD137-overexpressing HT1080 cells (5.000 cells per well of a 384-well microtiter plate) were incubated for 6 hours at 37°C with the antibody samples. IL-8 concentrations in the supernatants were then determined by using an IL-8 ELISA kit (BD Bioscience). In a second approach, antibodies were cross-linked. Therefor, the target-overexpressing HT1080 cells were incubated with a mixture of the antibody sample and a F(ab')2 fragment goat anti-human IgG (Dianova). An agonistic reference anti-CD27 antibody [described in US 2013/0183316 A1 (sequences 3 and 4, hCD27.15)] was used for assay development. **Figure 4** shows results of the IL-8 ELISA upon antibody titration with and without cross-linking. For all three IL-8 ELISAs, OD 450 nm/620 nm values > 0,5 indicated agonistic antibody activities, the maximum values being at about 3.

Altogether, 186 agonistic anti-CD27 antibodies, 92 agonistic anti-CD134 antibodies and 12 agonistic anti-CD137 antibodies were identified by the functional assay under cross-linking conditions. To some extent, antibodies already showed agonistic activity without cross-linking. The data of the ELISAs, the cellular binding assays and the functional assays are summarized in **Table 1** for the agonistic anti-CD27 antibodies, in **Table 2** for the agonistic anti-CD134 antibodies and in **Table 3** for the agonistic anti-CD137 antibodies.

EC50 values of the agonistic function of anti-CD27, anti-CD134 and anti-CD137, respectively, upon cross-linking were determined by measuring IL-8 release values of 12 different antibody concentrations applied in the above-described functional assay. The EC50 value was calculated using Xlfit-analysis tools (Fit Models 205). Resulting typical sigmoidal curve progression is exemplarily shown in **Figure 5** for one anti-CD27 antibody (P018.S09.A.A06; EC50: 387,2 ng/mL), for one anti-CD134 antibody (P018.S02.A.H03; EC50: 214,9 ng/mL) and for one anti-CD137 antibody (P018.S08.A.E03; EC50: 4963,8 ng/mL). EC50 values of the antibodies are listed in **Table 4** (anti-CD27), **Table 5** (anti-CD134) and **Table 6** (anti-CD137).

Sequence information of the tested antibodies are listed in **Table 7** (anti-CD27), **Table 8** (anti-CD134) as well as **Table 9** (anti-CD137), wherein the sequences of the CDR regions are given according to the IMGT unique numbering scheme for V- and V-like-domains (Lefranc, M.-P., The Immunologist, 7, 132-136 (1999)).

Anti-CD27 Antibody ID	ELISA	Cellular binding assay	Functional assay	
	[abs. corr.]	[RFU]	IL-8 [abs. corr.]	Cross-linked by F(ab')2 fragment IL-8 [abs. corr.]
P018.S.01.A.G04	**3,765**	**388**	0,273	**1,145**
P018.S.04.A.D04	**3,696**	**365**	0,486	**1,322**
P018.S.01.A.G02	**3,673**	**363**	**0,848**	**1,878**
P018.S.07.A.B04	**3,635**	**382**	0,304	**1,667**
P018.S.06.A.B03	**3,594**	**424**	**0,566**	**1,220**
P018.S.03.A.H04	**3,589**	**334**	0,211	**1,226**
P018.S.08.A.H02	**3,576**	**352**	0,328	**1,909**
P018.S.09.A.H03	**3,536**	**268**	**0,578**	**2,130**
P018.S.09.A.E06	**3,535**	**307**	**1,228**	**1,733**
P018.S.09.A.C03	**3,533**	**319**	0,464	**2,253**
P018.S.07.A.F03	**3,528**	**380**	0,444	**1,413**
P018.S.03.A.C04	**3,524**	**337**	0,394	**1,483**
P018.S.09.A.E05	**3,523**	**353**	**0,872**	**2,169**
P018.S.04.A.E02	**3,516**	**463**	**0,676**	**1,911**
P018.S.01.A.B05	**3,503**	**376**	0,453	**1,882**
P018.S.04.A.B05	**3,502**	**414**	0,308	**1,084**
P018.S.01.A.G03	**3,495**	**358**	-0,105	**1,214**
P018.S.10.A.F05	**3,491**	**335**	0,267	**1,341**
P018.S.02.A.B05	**3,485**	**374**	0,201	**1,761**
P018.S.04.A.G04	**3,479**	**335**	0,451	**1,571**
P018.S.07.A.H02	**3,471**	**283**	0,107	**1,222**
P018.S.05.A.H02	**3,469**	**336**	0,369	**1,899**
P018.S.03.A.F02	**3,464**	**276**	**0,736**	**1,221**
P018.S.04.A.H04	**3,463**	**291**	**0,579**	**1,982**
P018.S.07.A.C04	**3,453**	**300**	0,302	**1,645**
P018.S.09.A.C06	**3,452**	**219**	**1,943**	**2,304**
P018.S.01.A.E06	**3,446**	**291**	0,448	**1,401**
P018.S.01.A.H06	**3,445**	**422**	0,270	**2,181**
P018.S.07.A.A05	**3,441**	**379**	0,408	**1,033**
P018.S.02.A.F05	**3,440**	**307**	0,395	**1,165**
P018.S.03.A.A02	**3,438**	**373**	**0,651**	**2,203**
P018.S.03.A.G02	**3,435**	**275**	0,412	**1,280**
P018.S.10.A.G05	**3,424**	**408**	0,137	**1,249**
P018.S.08.A.D01	**3,421**	**394**	-0,001	**1,120**
P018.S.01.A.F02	**3,420**	**326**	0,495	**2,052**
P018.S.07.A.B05	**3,418**	**295**	0,203	**1,407**
P018.S.10.A.H01	**3,418**	**443**	0,412	**2,288**
P018.S.08.A.A01	**3,417**	**448**	**0,876**	**1,570**
P018.S.04.A.D02	**3,416**	**335**	0,294	**1,346**
P018.S.03.A.B02	**3,411**	**308**	0,366	**1,489**
P018.S.06.A.A03	**3,411**	**387**	**0,576**	**1,861**

P018.S.02.A.G01	**3,408**	**332**	0,127	**1,518**
P018.S.01.A.D04	**3,405**	**341**	0,175	**2,199**
P018.S.07.A.H04	**3,403**	**386**	0,158	**1,864**
P018.S.03.A.G06	**3,402**	**302**	**0,560**	**1,364**
P018.S.06.A.F01	**3,402**	**310**	0,405	**1,082**
P018.S.10.A.C06	**3,390**	**395**	0,280	**1,175**
P018.S.04.A.B01	**3,383**	**330**	**0,508**	**1,670**
P018.S.04.A.E04	**3,381**	**283**	**0,641**	**1,344**
P018.S.02.A.H05	**3,375**	**252**	0,067	**1,403**
P018.S.04.A.C02	**3,373**	**415**	0,235	**1,534**
P018.S.09.A.D02	**3,373**	**337**	**1,013**	**1,746**
P018.S.08.A.D02	**3,372**	**407**	**0,534**	**1,668**
P018.S.05.A.D04	**3,372**	**317**	0,246	**1,527**
P018.S.03.A.D02	**3,370**	**397**	0,463	**1,469**
P018.S.07.A.B03	**3,369**	**346**	**0,612**	**1,340**
P018.S.02.A.C06	**3,366**	**348**	0,418	**1,590**
P018.S.01.A.B04	**3,362**	**382**	**0,582**	**2,121**
P018.S.03.A.C02	**3,362**	**317**	0,373	**1,582**
P018.S.03.A.H05	**3,359**	**294**	0,401	**1,391**
P018.S.09.A.H04	**3,356**	**314**	0,274	**1,399**
P018.S.04.A.F04	**3,355**	**320**	**1,600**	**2,494**
P018.S.05.A.E04	**3,354**	**332**	**0,813**	**1,329**
P018.S.07.A.B06	**3,353**	**372**	0,218	**1,074**
P018.S.05.A.C06	**3,351**	**351**	**0,539**	**1,111**
P018.S.07.A.F04	**3,351**	**296**	0,424	**1,582**
P018.S.01.A.F05	**3,350**	**369**	**0,559**	**1,180**
P018.S.05.A.G04	**3,349**	**375**	0,393	**1,191**
P018.S.09.A.D04	**3,348**	**376**	**0,688**	**1,660**
P018.S.07.A.H06	**3,345**	**327**	0,422	**1,228**
P018.S.06.A.E01	**3,345**	**354**	**0,525**	**1,551**
P018.S.07.A.D03	**3,340**	**409**	**0,569**	**1,676**
P018.S.07.A.D04	**3,335**	**393**	0,449	**1,810**
P018.S.08.A.E02	**3,333**	**387**	0,315	**1,555**
P018.S.04.A.D05	**3,331**	**292**	0,241	**1,081**
P018.S.07.A.G02	**3,330**	**453**	**0,725**	**2,408**
P018.S.02.A.E06	**3,324**	**331**	0,308	**1,292**
P018.S.03.A.C05	**3,321**	**303**	**0,544**	**1,630**
P018.S.04.A.A01	**3,319**	**297**	0,176	**1,471**
P018.S.05.A.B02	**3,318**	**259**	**0,633**	**1,954**
P018.S.08.A.G02	**3,316**	**315**	**0,598**	**2,483**
P018.S.03.A.F04	**3,307**	**299**	0,253	**1,593**
P018.S.10.A.F01	**3,306**	**329**	-0,011	**1,052**
P018.S.10.A.G03	**3,306**	**386**	-0,180	**1,300**
P018.S.01.A.B03	**3,304**	**387**	0,060	**1,699**
P018.S.05.A.A02	**3,301**	**334**	0,212	**1,534**
P018.S.05.A.A04	**3,301**	**369**	0,226	**1,371**

P018.S.06.A.E02	**3,299**	**357**	**0,582**	**1,223**
P018.S.10.A.E01	**3,298**	**227**	0,257	**2,544**
P018.S.01.A.E03	**3,297**	**335**	0,436	**1,220**
P018.S.01.A.H02	**3,296**	**317**	0,300	**2,536**
P018.S.01.A.C06	**3,296**	**369**	0,325	**1,788**
P018.S.05.A.F02	**3,294**	**472**	**0,526**	**1,913**
P018.S.03.A.E04	**3,294**	**356**	0,200	**1,193**
P018.S.04.A.A05	**3,294**	**206**	**1,666**	**2,238**
P018.S.07.A.C06	**3,293**	**336**	0,408	**1,044**
P018.S.09.A.D05	**3,282**	**400**	0,187	**2,109**
P018.S.10.A.E06	**3,281**	**215**	0,262	**2,061**
P018.S.06.A.D04	**3,280**	**394**	0,444	**1,064**
P018.S.01.A.E04	**3,280**	**295**	0,264	**1,149**
P018.S.07.A.E03	**3,276**	**227**	0,123	**1,513**
P018.S.04.A.B03	**3,276**	**338**	0,394	**1,971**
P018.S.09.A.E03	**3,272**	**334**	0,099	**1,545**
P018.S.02.A.D04	**3,269**	**390**	**0,569**	**1,369**
P018.S.01.A.D02	**3,258**	**323**	**0,675**	**1,667**
P018.S.10.A.B06	**3,254**	**308**	0,455	**1,461**
P018.S.10.A.H02	**3,252**	**144**	**1,573**	**2,104**
P018.S.02.A.B03	**3,251**	**332**	0,120	**1,161**
P018.S.01.A.C02	**3,250**	**324**	0,395	**1,478**
P018.S.07.A.H05	**3,248**	**256**	-0,160	**1,186**
P018.S.01.A.A04	**3,244**	**315**	0,236	**1,030**
P018.S.01.A.C04	**3,243**	**368**	0,383	**1,081**
P018.S.02.A.F06	**3,240**	**294**	0,094	**1,728**
P018.S.06.A.H04	**3,234**	**331**	0,173	**1,692**
P018.S.06.A.C02	**3,232**	**283**	**0,519**	**1,610**
P018.S.06.A.A02	**3,232**	**366**	**0,737**	**1,811**
P018.S.02.A.A01	**3,225**	**350**	0,400	**1,616**
P018.S.01.A.E05	**3,222**	**420**	0,209	**1,332**
P018.S.03.A.D03	**3,216**	**320**	0,281	**1,051**
P018.S.08.A.A03	**3,214**	**342**	0,276	**1,639**
P018.S.10.A.C03	**3,214**	**360**	0,079	**1,561**
P018.S.03.A.G03	**3,207**	**292**	**0,546**	**1,585**
P018.S.07.A.E05	**3,198**	**317**	0,055	**1,151**
P018.S.03.A.D06	**3,194**	**360**	0,245	**1,053**
P018.S.01.A.C05	**3,189**	**349**	0,327	**1,400**
P018.S.02.A.G04	**3,188**	**196**	0,205	**1,674**
P018.S.10.A.B01	**3,187**	**333**	0,080	**1,037**
P018.S.10.A.A06	**3,181**	**414**	-0,130	**1,121**
P018.S.06.A.F04	**3,176**	**338**	**0,678**	**1,160**
P018.S.09.A.A05	**3,173**	**214**	**0,504**	**1,953**
P018.S.04.A.C05	**3,173**	**292**	0,382	**1,429**
P018.S.01.A.G06	**3,171**	**313**	0,491	**1,088**
P018.S.06.A.F06	**3,170**	**326**	**0,700**	**1,030**

P018.S.09.A.A03	**3,160**	**360**	0,263	**1,542**
P018.S.06.A.E04	**3,160**	**281**	0,327	**1,223**
P018.S.06.A.F03	**3,157**	**304**	0,063	**1,082**
P018.S.07.A.G04	**3,156**	**177**	0,274	**1,741**
P018.S.10.A.E04	**3,154**	**280**	0,339	**2,287**
P018.S.03.A.H02	**3,152**	**395**	0,130	**1,071**
P018.S.03.A.H03	**3,152**	**289**	**0,588**	**1,093**
P018.S.03.A.F03	**3,151**	**223**	0,106	**1,355**
P018.S.01.A.A05	**3,148**	**300**	0,328	**1,086**
P018.S.06.A.C03	**3,143**	**269**	0,272	**1,131**
P018.S.10.A.C05	**3,143**	11	0,206	**2,098**
P018.S.01.A.D05	**3,140**	**273**	0,089	**1,101**
P018.S.03.A.B05	**3,137**	**206**	0,325	**1,213**
P018.S.10.A.A04	**3,132**	**175**	**1,778**	**2,159**
P018.S.06.A.C06	**3,132**	**379**	0,128	**1,078**
P018.S.02.A.G03	**3,129**	**357**	-0,061	**1,484**
P018.S.07.A.C02	**3,128**	**380**	**0,735**	**2,020**
P018.S.02.A.A06	**3,126**	**293**	0,299	**1,476**
P018.S.01.A.E02	**3,126**	**360**	0,276	**1,172**
P018.S.09.A.F05	**3,125**	**400**	0,158	**1,683**
P018.S.09.A.G06	**3,120**	**338**	0,467	**1,338**
P018.S.03.A.D05	**3,118**	**324**	**0,500**	**1,504**
P018.S.10.A.B02	**3,114**	**249**	0,470	**1,907**
P018.S.04.A.E05	**3,111**	**319**	**0,747**	**1,766**
P018.S.05.A.A06	**3,107**	**374**	0,324	**1,582**
P018.S.05.A.D03	**3,105**	**321**	0,100	**1,278**
P018.S.08.A.F01	**3,102**	**395**	0,211	**1,113**
P018.S.05.A.B06	**3,098**	**309**	0,220	**1,700**
P018.S.03.A.D04	**3,098**	**270**	**0,513**	**1,230**
P018.S.01.A.A06	**3,091**	**287**	**0,566**	**1,919**
P018.S.09.A.A06	**3,088**	**113**	**2,192**	**2,251**
P018.S.07.A.A02	**3,081**	**314**	0,059	**1,852**
P018.S.07.A.D05	**3,080**	**329**	-0,032	**1,639**
P018.S.05.A.E03	**3,069**	**322**	0,208	**1,915**
P018.S.10.A.E02	**3,016**	**395**	0,230	**1,389**
P018.S.03.A.G05	**2,981**	**269**	0,031	**1,272**
P018.S.09.A.E02	**2,981**	**305**	0,172	**1,610**
P018.S.09.A.F06	**2,980**	**218**	**0,580**	**2,032**
P018.S.06.A.H05	**2,977**	**308**	0,278	**1,557**
P018.S.10.A.H05	**2,961**	**313**	-0,155	**1,108**
P018.S.06.A.A05	**2,954**	**140**	0,433	**1,086**
P018.S.09.A.A02	**2,949**	**155**	**0,839**	**2,458**
P018.S.09.A.C02	**2,943**	**323**	0,070	**1,068**
P018.S.06.A.B01	**2,943**	**279**	0,106	**1,176**
P018.S.10.A.C01	**2,934**	**266**	-0,046	**1,104**
P018.S.05.A.D06	**2,931**	**323**	0,338	**1,228**

P018.S.02.A.B01	**2,930**	**332**	**0,538**	**1,423**
P018.S.10.A.C02	**2,905**	**238**	**0,631**	**2,284**
P018.S.10.A.F04	**2,889**	**133**	0,273	**2,324**
P018.S.10.A.A03	**2,806**	**198**	**1,021**	**2,063**
P018.S.05.A.F05	**2,310**	11	0,189	**2,212**
P018.S.10.A.B03	**2,243**	19	-0,032	**2,074**

Table 1. Binding and functional activity properties of agonistic anti-CD27 antibodies

Anti-CD134 Antibody ID	ELISA	Cellular binding assay	Functional assay	
	[abs. corr.]	[RFU]	IL-8 [abs. corr.]	Cross-linked by F(ab´)2 fragment IL-8 [abs. corr.]
P018.S.03.A.E03	**2,718**	**419**	0,314	**2,544**
P018.S.04.A.G05	**2,865**	**451**	-0,022	**2,414**
P018.S.04.A.F05	**2,439**	**368**	0,181	**2,397**
P018.S.05.A.G03	0,056	**40**	0,032	**2,391**
P018.S.05.A.G05	**2,619**	**463**	**2,616**	**2,387**
P018.S.04.A.H05	**2,972**	**451**	0,356	**2,386**
P018.S.04.A.E06	0,206	**140**	0,135	**2,381**
P018.S.02.A.F01	**2,690**	**498**	0,115	**2,341**
P018.S.04.A.D06	**2,493**	**510**	**1,009**	**2,302**
P018.S.04.A.B06	**2,638**	**482**	**1,824**	**2,271**
P018.S.12.A.E04	**2,208**	**659**	0,455	**2,234**
P018.S.04.A.A06	**2,853**	**486**	**0,879**	**2,211**
P018.S.04.A.F06	**2,358**	**402**	**2,071**	**2,210**
P018.S.11.A.C04	**1,963**	**406**	0,253	**2,195**
P018.S.12.A.H05	**2,373**	**527**	0,348	**2,190**
P018.S.12.A.G05	**2,045**	**570**	0,130	**2,190**
P018.S.11.A.C03	**1,535**	**507**	**0,586**	**2,189**
P018.S.12.A.D06	**0,865**	**180**	**0,832**	**2,171**
P018.S.12.A.F05	**2,124**	**511**	0,341	**2,165**
P018.S.11.A.A06	**1,845**	**537**	**0,732**	**2,164**
P018.S.11.A.F05	**1,669**	**396**	0,330	**2,155**
P018.S.12.A.F03	**2,450**	**485**	**0,527**	**2,153**
P018.S.12.A.D05	**2,130**	**451**	-0,007	**2,152**
P018.S.11.A.A05	**1,898**	**550**	0,260	**2,149**
P018.S.12.A.B05	**2,103**	**536**	**0,960**	**2,147**
P018.S.12.A.C02	**2,052**	**571**	**0,936**	**2,143**
P018.S.12.A.A01	**2,414**	**600**	0,071	**2,137**
P018.S.11.A.F04	**2,251**	**486**	**0,568**	**2,113**
P018.S.04.A.B04	**3,208**	**331**	**1,990**	**2,112**
P018.S.12.A.C03	**1,956**	**483**	0,238	**2,108**
P018.S.12.A.B01	**2,341**	**607**	0,396	**2,107**

P018.S.12.A.E05	**1,933**	**523**	0,132	**2,107**
P018.S.12.A.G03	**2,521**	**452**	0,209	**2,105**
P018.S.11.A.B06	**2,133**	**441**	0,403	**2,105**
P018.S.11.A.B03	**2,660**	**527**	-0,075	**2,104**
P018.S.12.A.C06	**2,346**	**525**	**0,881**	**2,101**
P018.S.11.A.G03	**2,112**	**450**	**1,573**	**2,101**
P018.S.12.A.B06	**1,404**	**451**	**1,845**	**2,071**
P018.S.11.A.G02	**1,070**	**357**	0,270	**2,061**
P018.S.12.A.C01	**2,493**	**496**	-0,026	**2,058**
P018.S.12.A.B03	**1,182**	**446**	**1,074**	**2,054**
P018.S.11.A.G05	**2,363**	**531**	0,031	**2,053**
P018.S.12.A.F06	**1,645**	**500**	0,374	**2,052**
P018.S.12.A.A06	**1,613**	**515**	0,062	**2,043**
P018.S.11.A.D04	**2,070**	**530**	0,380	**2,043**
P018.S.11.A.E02	**2,652**	**525**	0,214	**2,043**
P018.S.12.A.E01	**2,547**	**463**	0,439	**2,038**
P018.S.11.A.A03	**1,930**	**459**	**1,381**	**2,011**
P018.S.11.A.H05	**1,691**	**478**	**1,207**	**2,010**
P018.S.04.A.C06	**2,501**	**458**	**0,538**	**2,005**
P018.S.12.A.E03	**2,043**	**482**	0,461	**2,005**
P018.S.12.A.G04	**2,082**	**534**	0,407	**1,994**
P018.S.11.A.A04	**2,105**	**582**	**0,533**	**1,993**
P018.S.12.A.F02	**2,074**	**549**	0,185	**1,991**
P018.S.11.A.D06	**0,744**	**130**	0,019	**1,966**
P018.S.12.A.B02	**2,208**	**560**	-0,167	**1,966**
P018.S.02.A.H03	**0,468**	**198**	**2,557**	**1,954**
P018.S.11.A.H06	**2,516**	**498**	**0,567**	**1,949**
P018.S.12.A.F04	**2,109**	**551**	0,289	**1,946**
P018.S.12.A.A03	**1,440**	**493**	0,393	**1,945**
P018.S.11.A.F03	**1,852**	**522**	0,380	**1,936**
P018.S.08.A.B03	**2,661**	**477**	**0,709**	**1,935**
P018.S.12.A.H04	**1,984**	**295**	-0,121	**1,889**
P018.S.12.A.E06	**2,302**	**493**	0,054	**1,866**
P018.S.12.A.D04	**2,227**	**488**	0,139	**1,865**
P018.S.12.A.G01	**1,560**	**526**	**1,326**	**1,862**
P018.S.11.A.D03	**1,993**	**482**	**2,243**	**1,861**
P018.S.11.A.E04	**1,314**	**416**	-0,194	**1,834**
P018.S.12.A.H01	**1,888**	**523**	0,430	**1,834**
P018.S.12.A.D03	**1,345**	**559**	**0,707**	**1,830**
P018.S.12.A.C04	**2,044**	**509**	0,215	**1,829**
P018.S.11.A.B05	**1,651**	**456**	**1,360**	**1,826**
P018.S.12.A.G02	**2,331**	**530**	0,212	**1,806**
P018.S.11.A.H02	**2,802**	**515**	**0,783**	**1,803**
P018.S.11.A.E03	0,276	**318**	0,481	**1,791**
P018.S.12.A.D02	**2,642**	**472**	0,333	**1,773**
P018.S.11.A.G04	**2,128**	**482**	0,014	**1,752**

P018.S.02.A.F03	**2,851**	**140**	0,090	**1,732**
P018.S.11.A.E06	**2,047**	**554**	0,278	**1,722**
P018.S.05.A.F05	**2,411**	**392**	0,187	**1,711**
P018.S.11.A.B04	**1,426**	**444**	-0,087	**1,657**
P018.S.12.A.C05	**2,941**	**494**	0,228	**1,653**
P018.S.12.A.E02	**2,589**	**497**	0,355	**1,629**
P018.S.11.A.H04	**2,648**	**382**	**0,610**	**1,612**
P018.S.13.A.G06	0,002	0	-0,114	**1,585**
P018.S.12.A.A02	**1,577**	**502**	0,145	**1,569**
P018.S.12.A.A05	**2,862**	**503**	0,267	**1,526**
P018.S.11.A.E05	**1,660**	**407**	-0,265	**1,422**
P018.S.04.A.E01	**2,876**	22	0,084	**1,405**
P018.S.12.A.A04	**1,985**	**514**	-0,007	**1,401**
P018.S.11.A.F02	**2,426**	**503**	-0,011	**1,382**
P018.S.12.A.H03	**2,477**	**496**	-0,074	**1,275**
P018.S.04.A.F04	0,000	11	-0,129	**1,132**

Table 2. Binding and functional activity properties of agonistic anti-CD134 antibodies

Anti-CD137 Antibody ID	**ELISA**	**Cellular binding assay**	**Functional assay**	
	[abs. corr.]	**[RFU]**	**IL-8 [abs. corr.]**	**Cross-linked by F(ab')2 fragment IL-8 [abs. corr.]**
P018.S.13.A.A05	**0,687**	0	**0,559**	**2,570**
P018.S.14.A.H01	**2,968**	6	**2,015**	**2,193**
P018.S.08.A.E03	**3,047**	9	-0,304	**2,019**
P018.S.13.A.G04	**0,577**	1	-0,179	**1,801**
P018.S.13.A.A06	**3,388**	**38**	-0,153	**1,712**
P018.S.08.A.A04	**3,418**	**46**	0,368	**1,546**
P018.S.14.A.D06	**2,896**	1	-0,011	**1,523**
P018.S.13.A.C02	**2,551**	8	0,113	**1,399**
P018.S.08.A.F03	**2,330**	7	-0,346	**1,356**
P018.S.14.A.G03	**2,072**	**19**	0,222	**1,140**
P018.S.14.A.A04	**3,440**	**81**	**0,785**	**0,972**
P018.S.13.A.G03	**1,725**	**13**	-0,164	**0,789**

Table 3. Binding and functional activity properties of agonistic anti-CD137 antibodies

5

Anti-CD27 Antibody ID	**EC 50 [ng/mL]**
P018.S.10.A.H02	122,2
P018.S.09.A.A02	144,1
P018.S.09.A.C06	220,9

P018.S.10.A.E04	233,6
P018.S.09.A.H06	329,1
P018.S.10.A.A04	334,7
P018.S.10.A.C05	340,1
P018.S.05.A.F05	376,4
P018.S.09.A.H03	387,0
P018.S.09.A.A06	387,2
P018.S.04.A.A05	407,7
P018.S.10.A.C02	414,3
P018.S.07.A.G02	430,4
P018.S.10.A.H01	431,0
P018.S.09.A.E05	502,0
P018.S.10.A.A03	512,8
P018.S.02.A.G03	526,1
P018.S.09.A.D05	561,2
P018.S.06.A.A02	575,3
P018.S.10.A.E01	721,5
P018.S.07.A.C04	801,5
P018.S.04.A.A04	819,8
P018.S.03.A.A02	834,9
P018.S.01.A.B04	855,7
P018.S.09.A.C03	1022,1
P018.S.01.A.H02	1109,2
P018.S.10.A.F04	1266,5
P018.S.01.A.D04	1302,2
P018.S.01.A.F05	1322,3
P018.S.04.A.F04	1444,2
P018.S.08.A.G02	1935,4
P018.S.10.A.B03	1936,1
P018.S.01.A.H06	2346,5

Table 4. EC50 values of agonistic anti-CD27 antibodies

Anti-CD134 Antibody ID	**EC 50 [ng/mL]**
P018.S.12.A.B05	102,1
P018.S.11.A.D03	175,8
P018.S.02.A.H03	214,9
P018.S.11.A.C03	217,2
P018.S.04.A.F05	224,6
P018.S.04.A.D06	354,6
P018.S.12.A.C01	431,5
P018.S.04.A.F06	440,1
P018.S.04.A.B06	460,8
P018.S.03.A.E03	528,9
P018.S.04.A.A06	629,9
P018.S.04.A.G05	669,5
P018.S.12.A.F05	732,5

P018.S.12.A.G05	745,0
P018.S.11.A.C04	785,3
P018.S.11.A.A05	865,3
P018.S.11.A.G03	893,9
P018.S.05.A.G03	966,4
P018.S.05.A.G05	974,5
P018.S.12.A.E04	1005,0
P018.S.04.A.H05	1167,0
P018.S.04.A.E06	1390,5
P018.S.12.A.F03	1630,3
P018.S.11.A.A06	2094,9
P018.S.12.A.D05	2401,8
P018.S.12.A.D06	2773,7
P018.S.05.A.F05	7119,8

Table 5. EC50 values of agonistic anti-CD134 antibodies

Anti-CD137 Antibody ID	EC 50 [ng/mL]
P018.S.08.A.F03	2710,1
P018.S.13.A.C02	3498,0
P018.S.14.A.G03	3499,7
P018.S.13.A.A05	3722,8
P018.S.13.A.G03	4776,3
P018.S.08.A.E03	4963,8
P018.S.08.A.A04	5152,8
P018.S.14.A.H01	5818,0
P018.S.14.A.A04	6789,0
P018.S.13.A.A06	7785,1
P018.S.14.A.D06	10208,0
P018.S.13.A.G04	255179,8

Table 6. EC50 values of agonistic anti-CD137 antibodies

5

Short ID	Anti-CD27 Antibody ID	SEQ ID NOs of Variable Domains VH/VL	SEQ ID NOs of CDRs HCDR1/HCDR2/HCDR3 LCDR1/LCDR2/LCDR3
AB27_1	P018.S.01.A.A04	1 / 2	373 / 374 / 375 376 / 377 / 378
AB27_2	P018.S.01.A.A05	3 / 4	379 / 380 / 381 382 / 383 / 384
AB27_3	P018.S.01.A.A06	5 / 6	385 / 386 / 387 388 / 389 / 390
AB27_4	P018.S.01.A.B03	7 / 8	391 / 392 / 393 394 / 395 / 396
AB27_5	P018.S.01.A.B04	9 / 10	397 / 398 / 399 400 / 401 / 402
AB27_6	P018.S.01.A.B05	11 / 12	403 / 404 / 405 406 / 407 / 408
AB27_7	P018.S.01.A.C02	13 / 14	409 / 410 / 411 412 / 413 / 414
AB27_8	P018.S.01.A.C04	15 / 16	415 / 416 / 417 418 / 419 / 420
AB27_9	P018.S.01.A.C05	17 / 18	421 / 422 / 423 424 / 425 / 426
AB27_10	P018.S.01.A.C06	19 / 20	427 / 428 / 429 430 / 431 / 432
AB27_11	P018.S.01.A.D02	21 / 22	433 / 434 / 435 436 / 437 / 438
AB27_12	P018.S.01.A.D04	23 / 24	439 / 440 / 441 442 / 443 / 444
AB27_13	P018.S.01.A.D05	25 / 26	445 / 446 / 447 448 / 449 / 450
AB27_14	P018.S.01.A.E02	27 / 28	451 / 452 / 453 454 / 455 / 456
AB27_15	P018.S.01.A.E03	29 / 30	457 / 458 / 459 460 / 461 / 462
AB27_16	P018.S.01.A.E04	31 / 32	463 / 464 / 465 466 / 467 / 468
AB27_17	P018.S.01.A.E05	33 / 34	469 / 470 / 471 472 / 473 / 474
AB27_18	P018.S.01.A.E06	35 / 36	475 / 476 / 477 478 / 479 / 480
AB27_19	P018.S.01.A.F02	37 / 38	481 / 482 / 483 484 / 485 / 486
AB27_20	P018.S.01.A.F05	39 / 40	487 / 488 / 489 490 / 491 / 492
AB27_21	P018.S.01.A.G02	41 / 42	493 / 494 / 495 496 / 497 / 498
AB27_22	P018.S.01.A.G03	43 / 44	499 / 500 / 501 502 / 503 / 504
AB27_23	P018.S.01.A.G04	45 / 46	505 / 506 / 507 508 / 509 / 510
AB27_24	P018.S.01.A.G06	47 / 48	511 / 512 / 513 514 / 515 / 516
AB27_25	P018.S.01.A.H02	49 / 50	517 / 518 / 519 520 / 521 / 522
AB27_26	P018.S.01.A.H06	51 / 52	523 / 524 / 525 526 / 527 / 528
AB27_27	P018.S.02.A.A01	53 / 54	529 / 530 / 531 532 / 533 / 534
AB27_28	P018.S.02.A.A06	55 / 56	535 / 536 / 537

			538 / 539 / 540
AB27_29	P018.S.02.A.B01	57 / 58	541 / 542 / 543 544 / 545 / 546
AB27_30	P018.S.02.A.B03	59 / 60	547 / 548 / 549 550 / 551 / 552
AB27_31	P018.S.02.A.B05	61 / 62	553 / 554 / 555 556 / 557 / 558
AB27_32	P018.S.02.A.C06	63 / 64	559 / 560 / 561 562 / 563 / 564
AB27_33	P018.S.02.A.D04	65 / 66	565 / 566 / 567 568 / 569 / 570
AB27_34	P018.S.02.A.E06	67 / 68	571 / 572 / 573 574 / 575 / 576
AB27_35	P018.S.02.A.F05	69 / 70	577 / 578 / 579 580 / 581 / 582
AB27_36	P018.S.02.A.F06	71 / 72	583 / 584 / 585 586 / 587 / 588
AB27_37	P018.S.02.A.G01	73 / 74	589 / 590 / 591 592 / 593 / 594
AB27_38	P018.S.02.A.G03	75 / 76	595 / 596 / 597 598 / 599 / 600
AB27_39	P018.S.02.A.G04	77 / 78	601 / 602 / 603 604 / 605 / 606
AB27_40	P018.S.02.A.H05	79 / 80	607 / 608 / 609 610 / 611 / 612
AB27_41	P018.S.03.A.A02	81 / 82	613 / 614 / 615 616 / 617 / 618
AB27_42	P018.S.03.A.B02	83 / 84	619 / 620 / 621 622 / 623 / 624
AB27_43	P018.S.03.A.B05	85 / 86	625 / 626 / 627 628 / 629 / 630
AB27_44	P018.S.03.A.C02	87 / 88	631 / 632 / 633 634 / 635 / 636
AB27_45	P018.S.03.A.C04	89 / 90	637 / 638 / 639 640 / 641 / 642
AB27_46	P018.S.03.A.C05	91 / 92	643 / 644 / 645 646 / 647 / 648
AB27_47	P018.S.03.A.D02	93 / 94	649 / 650 / 651 652 / 653 / 654
AB27_48	P018.S.03.A.D03	95 / 96	655 / 656 / 657 658 / 659 / 660
AB27_49	P018.S.03.A.D04	97 / 98	661 / 662 / 663 664 / 665 / 666
AB27_50	P018.S.03.A.D05	99 / 100	667 / 668 / 669 670 / 671 / 672
AB27_51	P018.S.03.A.D06	101 / 102	673 / 674 / 675 676 / 677 / 678
AB27_52	P018.S.03.A.E04	103 / 104	679 / 680 / 681 682 / 683 / 684
AB27_53	P018.S.03.A.F02	105 / 106	685 / 686 / 687 688 / 689 / 690
AB27_54	P018.S.03.A.F03	107 / 108	691 / 692 / 693 694 / 695 / 696
AB27_55	P018.S.03.A.F04	109 / 110	697 / 698 / 699 700 / 701 / 702
AB27_56	P018.S.03.A.G02	111 / 112	703 / 704 / 705 706 / 707 / 708
AB27_57	P018.S.03.A.G03	113 / 114	709 / 710 / 711

			712 / 713 / 714
AB27_58	P018.S.03.A.G05	115 / 116	715 / 716 / 717 718 / 719 / 720
AB27_59	P018.S.03.A.G06	117 / 118	721 / 722 / 723 724 / 725 / 726
AB27_60	P018.S.03.A.H02	119 / 120	727 / 728 / 729 730 / 731 / 732
AB27_61	P018.S.03.A.H03	121 / 122	733 / 734 / 735 736 / 737 / 738
AB27_62	P018.S.03.A.H04	123 / 124	739 / 740 / 741 742 / 743 / 744
AB27_63	P018.S.03.A.H05	125 / 126	745 / 746 / 747 748 / 749 / 750
AB27_64	P018.S.04.A.A01	127 / 128	751 / 752 / 753 754 / 755 / 756
AB27_65	P018.S.04.A.A05	129 / 130	757 / 758 / 759 760 / 761 / 762
AB27_66	P018.S.04.A.B01	131 / 132	763 / 764 / 765 766 / 767 / 768
AB27_67	P018.S.04.A.B03	133 / 134	769 / 770 / 771 772 / 773 / 774
AB27_68	P018.S.04.A.B05	135 / 136	775 / 776 / 777 778 / 779 / 780
AB27_69	P018.S.04.A.C02	137 / 138	781 / 782 / 783 784 / 785 / 786
AB27_70	P018.S.04.A.C05	139 / 140	787 / 788 / 789 790 / 791 / 792
AB27_71	P018.S.04.A.D02	141 / 142	793 / 794 / 795 796 / 797 / 798
AB27_72	P018.S.04.A.D04	143 / 144	799 / 800 / 801 802 / 803 / 804
AB27_73	P018.S.04.A.D05	145 / 146	805 / 806 / 807 808 / 809 / 810
AB27_74	P018.S.04.A.E02	147 / 148	811 / 812 / 813 814 / 815 / 816
AB27_75	P018.S.04.A.E04	149 / 150	817 / 818 / 819 820 / 821 / 822
AB27_76	P018.S.04.A.E05	151 / 152	823 / 824 / 825 826 / 827 / 828
AB27_77	P018.S.04.A.F04	153 / 154	829 / 830 / 831 832 / 833 / 834
AB27_78	P018.S.04.A.G04	155 / 156	835 / 836 / 837 838 / 839 / 840
AB27_79	P018.S.04.A.H04	157 / 158	841 / 842 / 843 844 / 845 / 846
AB27_80	P018.S.05.A.A02	159 / 160	847 / 848 / 849 850 / 851 / 852
AB27_81	P018.S.05.A.A04	161 / 162	853 / 854 / 855 856 / 857 / 858
AB27_82	P018.S.05.A.A06	163 / 164	859 / 860 / 861 862 / 863 / 864
AB27_83	P018.S.05.A.B02	165 / 166	865 / 866 / 867 868 / 869 / 870
AB27_84	P018.S.05.A.B06	167 / 168	871 / 872 / 873 874 / 875 / 876
AB27_85	P018.S.05.A.C06	169 / 170	877 / 878 / 879 880 / 881 / 882
AB27_86	P018.S.05.A.D03	171 / 172	883 / 884 / 885

			886 / 887 / 888
AB27_87	P018.S.05.A.D04	173 / 174	889 / 890 / 891 892 / 893 / 894
AB27_88	P018.S.05.A.D06	175 / 176	895 / 896 / 897 898 / 899 / 900
AB27_89	P018.S.05.A.E03	177 / 178	901 / 902 / 903 904 / 905 / 906
AB27_90	P018.S.05.A.E04	179 / 180	907 / 908 / 909 910 / 911 / 912
AB27_91	P018.S.05.A.F02	181 / 182	913 / 914 / 915 916 / 917 / 918
AB27_92	P018.S.05.A.F05	183 / 184	919 / 920 / 921 922 / 923 / 924
AB27_93	P018.S.05.A.G04	185 / 186	925 / 926 / 927 928 / 929 / 930
AB27_94	P018.S.05.A.H02	187 / 188	931 / 932 / 933 934 / 935 / 936
AB27_95	P018.S.06.A.A02	189 / 190	937 / 938 / 939 940 / 941 / 942
AB27_96	P018.S.06.A.A03	191 / 192	943 / 944 / 945 946 / 947 / 948
AB27_97	P018.S.06.A.A05	193 / 194	949 / 950 / 951 952 / 953 / 954
AB27_98	P018.S.06.A.B01	195 / 196	955 / 956 / 957 958 / 959 / 960
AB27_99	P018.S.06.A.B03	197 / 198	961 / 962 / 963 964 / 965 / 966
AB27_100	P018.S.06.A.C02	199 / 200	967 / 968 / 969 970 / 971 / 972
AB27_101	P018.S.06.A.C03	201 / 202	973 / 974 / 975 976 / 977 / 978
AB27_102	P018.S.06.A.C06	203 / 204	979 / 980 / 981 982 / 983 / 984
AB27_103	P018.S.06.A.D04	205 / 206	985 / 986 / 987 988 / 989 / 990
AB27_104	P018.S.06.A.E01	207 / 208	991 / 992 / 993 994 / 995 / 996
AB27_105	P018.S.06.A.E02	209 / 210	997 / 998 / 999 1000 / 1001 / 1002
AB27_106	P018.S.06.A.E04	211 / 212	1003 / 1004 / 1005 1006 / 1007 / 1008
AB27_107	P018.S.06.A.F01	213 / 214	1009 / 1010 / 1011 1012 / 1013 / 1014
AB27_108	P018.S.06.A.F03	215 / 216	1015 / 1016 / 1017 1018 / 1019 / 1020
AB27_109	P018.S.06.A.F04	217 / 218	1021 / 1022 / 1023 1024 / 1025 / 1026
AB27_110	P018.S.06.A.F06	219 / 220	1027 / 1028 / 1029 1030 / 1031 / 1032
AB27_111	P018.S.06.A.H04	221 / 222	1033 / 1034 / 1035 1036 / 1037 / 1038
AB27_112	P018.S.06.A.H05	223 / 224	1039 / 1040 / 1041 1042 / 1043 / 1044
AB27_113	P018.S.07.A.A02	225 / 226	1045 / 1046 / 1047 1048 / 1049 / 1050
AB27_114	P018.S.07.A.A05	227 / 228	1051 / 1052 / 1053 1054 / 1055 / 1056
AB27_115	P018.S.07.A.B03	229 / 230	1057 / 1058 / 1059

			1060 / 1061 / 1062
AB27_116	P018.S.07.A.B04	231 / 232	1063 / 1064 / 1065 1066 / 1067 / 1068
AB27_117	P018.S.07.A.B05	233 / 234	1069 / 1070 / 1071 1072 / 1073 / 1074
AB27_118	P018.S.07.A.B06	235 / 236	1075 / 1076 / 1077 1078 / 1079 / 1080
AB27_119	P018.S.07.A.C02	237 / 238	1081 / 1082 / 1083 1084 / 1085 / 1086
AB27_120	P018.S.07.A.C04	239 / 240	1087 / 1088 / 1089 1090 / 1091 / 1092
AB27_121	P018.S.07.A.C06	241 / 242	1093 / 1094 / 1095 1096 / 1097 / 1098
AB27_122	P018.S.07.A.D03	243 / 244	1099 / 1100 / 1101 1102 / 1103 / 1104
AB27_123	P018.S.07.A.D04	245 / 246	1105 / 1106 / 1107 1108 / 1109 / 1110
AB27_124	P018.S.07.A.D05	247 / 248	1111 / 1112 / 1113 1114 / 1115 / 1116
AB27_125	P018.S.07.A.E03	249 / 250	1117 / 1118 / 1119 1120 / 1121 / 1122
AB27_126	P018.S.07.A.E05	251 / 252	1123 / 1124 / 1125 1126 / 1127 / 1128
AB27_127	P018.S.07.A.F03	253 / 254	1129 / 1130 / 1131 1132 / 1133 / 1134
AB27_128	P018.S.07.A.F04	255 / 256	1135 / 1136 / 1137 1138 / 1139 / 1140
AB27_129	P018.S.07.A.G02	257 / 258	1141 / 1142 / 1143 1144 / 1145 / 1146
AB27_130	P018.S.07.A.G04	259 / 260	1147 / 1148 / 1149 1150 / 1151 / 1152
AB27_131	P018.S.07.A.H02	261 / 262	1153 / 1154 / 1155 1156 / 1157 / 1158
AB27_132	P018.S.07.A.H04	263 / 264	1159 / 1160 / 1161 1162 / 1163 / 1164
AB27_133	P018.S.07.A.H05	265 / 266	1165 / 1166 / 1167 1168 / 1169 / 1170
AB27_134	P018.S.07.A.H06	267 / 268	1171 / 1172 / 1173 1174 / 1175 / 1176
AB27_135	P018.S.08.A.A01	269 / 270	1177 / 1178 / 1179 1180 / 1181 / 1182
AB27_136	P018.S.08.A.A03	271 / 272	1183 / 1184 / 1185 1186 / 1187 / 1188
AB27_137	P018.S.08.A.D01	273 / 274	1189 / 1190 / 1191 1192 / 1193 / 1194
AB27_138	P018.S.08.A.D02	275 / 276	1195 / 1196 / 1197 1198 / 1199 / 1200
AB27_139	P018.S.08.A.E02	277 / 278	1201 / 1202 / 1203 1204 / 1205 / 1206
AB27_140	P018.S.08.A.F01	279 / 280	1207 / 1208 / 1209 1210 / 1211 / 1212
AB27_141	P018.S.08.A.G02	281 / 282	1213 / 1214 / 1215 1216 / 1217 / 1218
AB27_142	P018.S.08.A.H02	283 / 284	1219 / 1220 / 1221 1222 / 1223 / 1224
AB27_143	P018.S.09.A.A02	285 / 286	1225 / 1226 / 1227 1228 / 1229 / 1230
AB27_144	P018.S.09.A.A03	287 / 288	1231 / 1232 / 1233

			1234 / 1235 / 1236
AB27_145	P018.S.09.A.A05	289 / 290	1237 / 1238 / 1239 1240 / 1241 / 1242
AB27_146	P018.S.09.A.A06	291 / 292	1243 / 1244 / 1245 1246 / 1247 / 1248
AB27_147	P018.S.09.A.C02	293 / 294	1249 / 1250 / 1251 1252 / 1253 / 1254
AB27_148	P018.S.09.A.C03	295 / 296	1255 / 1256 / 1257 1258 / 1259 / 1260
AB27_149	P018.S.09.A.C06	297 / 298	1261 / 1262 / 1263 1264 / 1265 / 1266
AB27_150	P018.S.09.A.D02	299 / 300	1267 / 1268 / 1269 1270 / 1271 / 1272
AB27_151	P018.S.09.A.D04	301 / 302	1273 / 1274 / 1275 1276 / 1277 / 1278
AB27_152	P018.S.09.A.D05	303 / 304	1279 / 1280 / 1281 1282 / 1283 / 1284
AB27_153	P018.S.09.A.E02	305 / 306	1285 / 1286 / 1287 1288 / 1289 / 1290
AB27_154	P018.S.09.A.E03	307 / 308	1291 / 1292 / 1293 1294 / 1295 / 1296
AB27_155	P018.S.09.A.E05	309 / 310	1297 / 1298 / 1299 1300 / 1301 / 1302
AB27_156	P018.S.09.A.E06	311 / 312	1303 / 1304 / 1305 1306 / 1307 / 1308
AB27_157	P018.S.09.A.F05	313 / 314	1309 / 1310 / 1311 1312 / 1313 / 1314
AB27_158	P018.S.09.A.F06	315 / 316	1315 / 1316 / 1317 1318 / 1319 / 1320
AB27_159	P018.S.09.A.G06	317 / 318	1321 / 1322 / 1323 1324 / 1325 / 1326
AB27_160	P018.S.09.A.H03	319 / 320	1327 / 1328 / 1329 1330 / 1331 / 1332
AB27_161	P018.S.09.A.H04	321 / 322	1333 / 1334 / 1335 1336 / 1337 / 1338
AB27_162	P018.S.09.A.H06	323 / 324	1339 / 1340 / 1341 1342 / 1343 / 1344
AB27_163	P018.S.10.A.A03	325 / 326	1345 / 1346 / 1347 1348 / 1349 / 1350
AB27_164	P018.S.10.A.A04	327 / 328	1351 / 1352 / 1353 1354 / 1355 / 1356
AB27_165	P018.S.10.A.A06	329 / 330	1357 / 1358 / 1359 1360 / 1361 / 1362
AB27_166	P018.S.10.A.B01	331 / 332	1363 / 1364 / 1365 1366 / 1367 / 1368
AB27_167	P018.S.10.A.B02	333 / 334	1369 / 1370 / 1371 1372 / 1373 / 1374
AB27_168	P018.S.10.A.B03	335 / 336	1375 / 1376 / 1377 1378 / 1379 / 1380
AB27_169	P018.S.10.A.B06	337 / 338	1381 / 1382 / 1383 1384 / 1385 / 1386
AB27_170	P018.S.10.A.C01	339 / 340	1387 / 1388 / 1389 1390 / 1391 / 1392
AB27_171	P018.S.10.A.C02	341 / 342	1393 / 1394 / 1395 1396 / 1397 / 1398
AB27_172	P018.S.10.A.C03	343 / 344	1399 / 1400 / 1401 1402 / 1403 / 1404
AB27_173	P018.S.10.A.C05	345 / 346	1405 / 1406 / 1407

			1408 / 1409 / 1410
AB27_174	P018.S.10.A.C06	347 / 348	1411 / 1412 / 1413 1414 / 1415 / 1416
AB27_175	P018.S.10.A.E01	349 / 350	1417 / 1418 / 1419 1420 / 1421 / 1422
AB27_176	P018.S.10.A.E02	351 / 352	1423 / 1424 / 1425 1426 / 1427 / 1428
AB27_177	P018.S.10.A.E04	353 / 354	1429 / 1430 / 1431 1432 / 1433 / 1434
AB27_178	P018.S.10.A.E06	355 / 356	1435 / 1436 / 1437 1438 / 1439 / 1440
AB27_179	P018.S.10.A.F01	357 / 358	1441 / 1442 / 1443 1444 / 1445 / 1446
AB27_180	P018.S.10.A.F04	359 / 360	1447 / 1448 / 1449 1450 / 1451 / 1452
AB27_181	P018.S.10.A.F05	361 / 362	1453 / 1454 / 1455 1456 / 1457 / 1458
AB27_182	P018.S.10.A.G03	363 / 364	1459 / 1460 / 1461 1462 / 1463 / 1464
AB27_183	P018.S.10.A.G05	365 / 366	1465 / 1466 / 1467 1468 / 1469 / 1470
AB27_184	P018.S.10.A.H01	367 / 368	1471 / 1472 / 1473 1474 / 1475 / 1476
AB27_185	P018.S.10.A.H02	369 / 370	1477 / 1478 / 1479 1480 / 1481 / 1482
AB27_186	P018.S.10.A.H05	371 / 372	1483 / 1484 / 1485 1486 / 1487 / 1488

Table 7. Sequence information of agonistic anti-CD27 antibodies (variable domains and CDRs)

Short ID	Anti-CD134 Antibody ID	SEQ ID NOs of Variable Domains VH/VL	SEQ ID NOs of CDRs HCDR1/HCDR2/HCDR3 LCDR1/LCDR2/LCDR3
AB134_1	P018.S.02.A.F01	1489 / 1490	1673 / 1674 / 1675 1676 / 1677 / 1678
AB134_2	P018.S.02.A.F03	1491 / 1492	1679 / 1680 / 1681 1682 / 1683 / 1684
AB134_3	P018.S.02.A.H03	1493 / 1494	1685 / 1686 / 1687 1688 / 1689 /1690
AB134_4	P018.S.03.A.E03	1495 / 1496	1691 / 1692 / 1693 1694 / 1695 / 1696
AB134_5	P018.S.04.A.A06	1497 / 1498	1697 / 1698 / 1699 1700 / 1701 / 1702
AB134_6	P018.S.04.A.B04	1499 / 1500	1703 / 1704 / 1705 1706 / 1707 / 1708
AB134_7	P018.S.04.A.B06	1501 / 1502	1709 / 1710 / 1711 1712 / 1713 / 1714
AB134_8	P018.S.04.A.C06	1503 / 1504	1715 / 1716 / 1717 1718 / 1719 / 1720
AB134_9	P018.S.04.A.D06	1505 / 1506	1721 / 1722 / 1723 1724 / 1725 / 1726
AB134_10	P018.S.04.A.E01	1507 / 1508	1727 / 1728 / 1729 1730 / 1731 / 1732
AB134_11	P018.S.04.A.E06	1509 / 1510	1733 / 1734 / 1735 1736 / 1737 / 1738
AB134_12	P018.S.04.A.F05	1511 / 1512	1739 / 1740 / 1741 1742 / 1743 / 1744

AB134_13	P018.S.04.A.F06	1513 / 1514	1745 / 1746 / 1747 1748 / 1749 / 1750
AB134_14	P018.S.04.A.G05	1515 / 1516	1751 / 1752 / 1753 1754 / 1755 / 1756
AB134_15	P018.S.04.A.H05	1517 / 1518	1757 / 1758 / 1759 1760 / 1761 / 1762
AB134_16	P018.S.05.A.F05	1519 / 1520	1763 / 1764 / 1765 1766 / 1767 / 1768
AB134_17	P018.S.05.A.G03	1521 / 1522	1769 / 1770 / 1771 1772 / 1773 / 1774
AB134_18	P018.S.05.A.G05	1523 / 1524	1775 / 1776 / 1777 1778 / 1779 / 1780
AB134_19	P018.S.08.A.B03	1525 / 1526	1781 / 1782 / 1783 1784 / 1785 / 1786
AB134_20	P018.S.11.A.A03	1527 / 1528	1787 / 1788 / 1789 1790 / 1791 / 1792
AB134_21	P018.S.11.A.A04	1529 / 1530	1793 / 1794 / 1795 1796 / 1797 / 1798
AB134_22	P018.S.11.A.A05	1531 / 1532	1799 / 1800 / 1801 1802 / 1803 / 1804
AB134_23	P018.S.11.A.A06	1533 / 1534	1805 / 1806 / 1807 1808 / 1809 / 1810
AB134_24	P018.S.11.A.B03	1535 / 1536	1811 / 1812 / 1813 1814 / 1815 / 1816
AB134_25	P018.S.11.A.B04	1537 / 1538	1817 / 1818 / 1819 1820 / 1821 / 1822
AB134_26	P018.S.11.A.B05	1539 / 1540	1823 / 1824 / 1825 1826 / 1827 / 1828
AB134_27	P018.S.11.A.B06	1541 / 1542	1829 / 1830 / 1831 1832 / 1833 / 1834
AB134_28	P018.S.11.A.C03	1543 / 1544	1835 / 1836 / 1837 1838 / 1839 / 1840
AB134_29	P018.S.11.A.C04	1545 / 1546	1841 / 1842 / 1843 1844 / 1845 / 1846
AB134_30	P018.S.11.A.D03	1547 / 1548	1847 / 1848 / 1849 1850 / 1851 / 1852
AB134_31	P018.S.11.A.D04	1549 / 1550	1853 / 1854 / 1855 1856 / 1857 / 1858
AB134_32	P018.S.11.A.D06	1551 / 1552	1859 / 1860 / 1861 1862 / 1863 / 1864
AB134_33	P018.S.11.A.E02	1553 / 1554	1865 / 1866 / 1867 1868 / 1869 / 1870
AB134_34	P018.S.11.A.E03	1555 / 1556	1871 / 1872 / 1873 1874 / 1875 / 1876
AB134_35	P018.S.11.A.E04	1557 / 1558	1877 / 1878 / 1879 1880 / 1881 / 1882
AB134_36	P018.S.11.A.E05	1559 / 1560	1883 / 1884 / 1885 1886 / 1887 / 1888
AB134_37	P018.S.11.A.E06	1561 / 1562	1889 / 1890 / 1891 1892 / 1893 / 1894
AB134_38	P018.S.11.A.F02	1563 / 1564	1895 / 1896 / 1897 1898 / 1899 / 1900
AB134_39	P018.S.11.A.F03	1565 / 1566	1901 / 1902 / 1903 1904 / 1905 / 1906
AB134_40	P018.S.11.A.F04	1567 / 1568	1907 / 1908 / 1909 1910 / 1911 / 1912
AB134_41	P018.S.11.A.F05	1569 / 1570	1913 / 1914 / 1915 1916 / 1917 / 1918

AB134_42	P018.S.11.A.G02	1571 / 1572	1919 / 1920 / 1921 1922 / 1923 / 1924
AB134_43	P018.S.11.A.G03	1573 / 1574	1925 / 1926 / 1927 1928 / 1929 / 1930
AB134_44	P018.S.11.A.G04	1575 / 1576	1931 / 1932 / 1933 1934 / 1935 / 1936
AB134_45	P018.S.11.A.G05	1577 / 1578	1937 / 1938 / 1939 1940 / 1941 / 1942
AB134_46	P018.S.11.A.H02	1579 / 1580	1943 / 1944 / 1945 1946 / 1947 / 1948
AB134_47	P018.S.11.A.H04	1581 / 1582	1949 / 1950 / 1951 1952 / 1953 / 1954
AB134_48	P018.S.11.A.H05	1583 / 1584	1955 / 1956 / 1957 1958 / 1959 / 1960
AB134_49	P018.S.11.A.H06	1585 / 1586	1961 / 1962 / 1963 1964 / 1965 / 1966
AB134_50	P018.S.12.A.A01	1587 / 1588	1967 / 1968 / 1969 1970 / 1971 / 1972
AB134_51	P018.S.12.A.A02	1589 / 1590	1973 / 1974 / 1975 1976 / 1977 / 1978
AB134_52	P018.S.12.A.A03	1591 / 1592	1979 / 1980 / 1981 1982 / 1983 / 1984
AB134_53	P018.S.12.A.A04	1593 / 1594	1985 / 1986 / 1987 1988 / 1989 / 1990
AB134_54	P018.S.12.A.A05	1595 / 1596	1991 / 1992 / 1993 1994 / 1995 / 1996
AB134_55	P018.S.12.A.A06	1597 / 1598	1997 / 1998 / 1999 2000 / 2001 / 2002
AB134_56	P018.S.12.A.B01	1599 / 1600	2003 / 2004 / 2005 2006 / 2007 / 2008
AB134_57	P018.S.12.A.B02	1601 / 1602	2009 / 2010 / 2011 2012 / 2013 / 2014
AB134_58	P018.S.12.A.B03	1603 / 1604	2015 / 2016 / 2017 2018 / 2019 / 2020
AB134_59	P018.S.12.A.B05	1605 / 1606	2021 / 2022 / 2023 2024 / 2025 / 2026
AB134_60	P018.S.12.A.B06	1607 / 1608	2027 / 2028 / 2029 2030 / 2031 / 2032
AB134_61	P018.S.12.A.C01	1609 / 1610	2033 / 2034 / 2035 2036 / 2037 / 2038
AB134_62	P018.S.12.A.C02	1611 / 1612	2039 / 2040 / 2041 2042 / 2043 / 2044
AB134_63	P018.S.12.A.C03	1613 / 1614	2045 / 2046 / 2047 2048 / 2049 / 2050
AB134_64	P018.S.12.A.C04	1615 / 1616	2051 / 2052 / 2053 2054 / 2055 / 2056
AB134_65	P018.S.12.A.C05	1617 / 1618	2057 / 2058 / 2059 2060 / 2061 / 2062
AB134_66	P018.S.12.A.C06	1619 / 1620	2063 / 2064 / 2065 2066 / 2067 / 2068
AB134_67	P018.S.12.A.D02	1621 / 1622	2069 / 2070 / 2071 2072 / 2073 / 2074
AB134_68	P018.S.12.A.D03	1623 / 1624	2075 / 2076 / 2077 2078 / 2079 / 2080
AB134_69	P018.S.12.A.D04	1625 / 1626	2081 / 2082 / 2083 2084 / 2085 / 2086
AB134_70	P018.S.12.A.D05	1627 / 1628	2087 / 2088 / 2089 2090 / 2091 / 2092

AB134_71	P018.S.12.A.D06	1629 / 1630	2093 / 2094 / 2095 2096 / 2097 / 2098
AB134_72	P018.S.12.A.E01	1631 / 1632	2099 / 2100 / 2101 2102 / 2103 / 2104
AB134_73	P018.S.12.A.E02	1633 / 1634	2105 / 2106 / 2107 2108 / 2109 / 2110
AB134_74	P018.S.12.A.E03	1635 / 1636	2111 / 2112 / 2113 2114 / 2115 / 2116
AB134_75	P018.S.12.A.E04	1637 / 1638	2117 / 2118 / 2119 2120 / 2121 / 2122
AB134_76	P018.S.12.A.E05	1639 / 1640	2123 / 2124 / 2125 2126 / 2127 / 2128
AB134_77	P018.S.12.A.E06	1641 / 1642	2129 / 2130 / 2131 2132 / 2133 / 2134
AB134_78	P018.S.12.A.F02	1643 / 1644	2135 / 2136 / 2137 2138 / 2139 / 2140
AB134_79	P018.S.12.A.F03	1645 / 1646	2141 / 2142 / 2143 2144 / 2145 / 2146
AB134_80	P018.S.12.A.F04	1647 / 1648	2147 / 2148 / 2149 2150 / 2151 / 2152
AB134_81	P018.S.12.A.F05	1649 / 1650	2153 / 2154 / 2155 2156 / 2157 / 2158
AB134_82	P018.S.12.A.F06	1651 / 1652	2159 / 2160 / 2161 2162 / 2163 / 2164
AB134_83	P018.S.12.A.G01	1653 / 1654	2165 / 2166 / 2167 2168 / 2169 / 2170
AB134_84	P018.S.12.A.G02	1655 / 1656	2171 / 2172 / 2173 2174 / 2175 / 2176
AB134_85	P018.S.12.A.G03	1657 / 1658	2177 / 2178 / 2179 2180 / 2181 / 2182
AB134_86	P018.S.12.A.G04	1659 / 1660	2183 / 2184 / 2185 2186 / 2187 / 2188
AB134_87	P018.S.12.A.G05	1661 / 1662	2189 / 2190 / 2191 2192 / 2193 / 2194
AB134_88	P018.S.12.A.H01	1663 / 1664	2195 / 2196 / 2197 2198 / 2199 / 2200
AB134_89	P018.S.12.A.H03	1665 / 1666	2201 / 2202 / 2203 2204 / 2205 / 2206
AB134_90	P018.S.12.A.H04	1667 / 1668	2207 / 2208 / 2209 2210 / 2211 / 2212
AB134_91	P018.S.12.A.H05	1669 / 1670	2213 / 2214 / 2215 2216 / 2217 / 2218
AB134_92	P018.S.13.A.G06	1671 / 1672	2219 / 2220 / 2221 2222 / 2223 / 2224

Table 8. Sequence information of agonistic anti-CD134 antibodies (variable domains and CDRs)

Short ID	Anti-CD137 Antibody ID	SEQ ID NOs of Variable Domains VH/VL	SEQ ID NOs of CDRs HCDR1/HCDR2/HCDR3 LCDR1/LCDR2/LCDR3
AB137_1	P018.S.08.A.A04	2225 / 2226	2249 / 2250 / 2251 2252 / 2253 / 2254
AB137_2	P018.S.08.A.E03	2227 / 2228	2255 / 2256 / 2257 2258 / 2259 / 2260
AB137_3	P018.S.08.A.F03	2229 / 2230	2261 / 2262 / 2263 2264 / 2265 /2266
AB137_4	P018.S.13.A.A05	2231 / 2232	2267 / 2268 / 2269

			2270 / 2271 / 2272
AB137_5	P018.S.13.A.A06	2233 / 2234	2273 / 2274 / 2275 2276 / 2277 / 2278
AB137_6	P018.S.13.A.C02	2235 / 2236	2279 / 2280 / 2281 2282 / 2283 / 2284
AB137_7	P018.S.13.A.G03	2237 / 2238	2285 / 2286 / 2287 2288 / 2289 / 2290
AB137_8	P018.S.13.A.G04	2239 / 2240	2291 / 2292 / 2293 2294 / 2295 / 2296
AB137_9	P018.S.14.A.A04	2241 / 2242	2297 / 2298 / 2299 2300 / 2301 / 2302
AB137_10	P018.S.14.A.D06	2243 / 2244	2303 / 2304 / 2305 2306 / 2307 / 2308
AB137_11	P018.S.14.A.G03	2245 / 2246	2309 / 2310 / 2311 2312 / 2313 / 2314
AB137_12	P018.S.14.A.H01	2247 / 2248	2315 / 2316 / 2317 2318 / 2319 / 2320

Table 9. Sequence information of agonistic anti-CD137 antibodies (variable domains and CDRs)

Example 2: Bispecific antibodies targeting CD40 and CD27, CD40 and CD137 or CD40 and CD134

2.1 Reporter assay measuring trans-activation

CD40 is predominantly expressed on antigen-presenting cells (APCs), such as dendritic cells, whereas CD27, CD134 and CD137 are predominantly expressed on T cells. Thus, bispecific antibodies binding to CD40 and CD27, to CD40 and CD137 or to CD40 and CD134 are able to bind simultaneously to APCs and T cells expressing these receptors. Thereby, these bispecific antibodies are able (i) to mediate cell-to-cell interaction between APCs and T cells by receptor binding and (ii) to activate both indicated receptors at once, which is primarily induced by cross-linking and receptor clustering upon cell-to-cell interaction and not necessarily dependent on agonistic activity of the parental monospecific bivalent antibodies. Thus, these trans-activating bispecific antibodies exert costimulatory activity in the context of APC : T cell interactions.

A corresponding reporter assay system was established. To that end, HEK293_NFK_gfp_luc cells were stably transduced with human TNF-receptors, CD27, CD40, CD134 and CD137, respectively. HEK293_NFK_gfp_luc (NF-κB/293/GFP-Luc™ Transcriptional Reporter Cell Line obtained from System Biosciences) is a reporter cell line which allows to monitor the NF-κB pathway by the detection of GFP fluorescence as well as luciferase activity for quantitative transcription activation reporter assays. Additionally, K562 cells were stably transduced with human TNF-receptors, CD27, CD40, CD134 and CD137, as well, in order to generate cell lines

mediating trans-presentation of the indicated receptors. Cell surface expression of the receptors on HEK293_NFK_gfp_luc cells and K562 cells was analyzed by flow cytometry depicted in **Figure 6A** and **Figure 6B**.

The reporter assay measuring trans-activation was set up as follows: HEK293_NFK_gfp_luc cells expressing one of the 4 indicated TNF-receptors were seeded in multi-well culture plates. Bispecific antibodies and corresponding monospecific control antibodies were added in concentrations from 100 ng/ml to 10.000 ng/ml. K562 cells expressing the second TNF-receptor or wildtype K562 (K562_wt) were added to indicated wells. Thus, the bispecific antibodies are able to bind to the first TNF-receptor on the HEK293_NFK_gfp_luc cell line and, at the same time, to the second TNF-receptor on the K562 cell line mediating trans-presentation. Only receptor activation of the first TNF receptor on HEK293_NFK_gfp_luc cells is measured by luciferase activity induced upon NF-κB -signaling. Thus, bispecific antibodies targeting CD40 and CD27, CD40 and CD137 or CD40 and CD134 were analyzed by two reporter assays: (i) the first assay measuring CD27-, CD134- or CD137-activation upon cross-presentation by K562_CD40 and (ii) the second assay measuring CD40-activation upon cross-presentation by K562_CD27, K562_CD134 or K562_CD137, respectively.

2.2 Analysis of CD40xCD137 bispecific antibodies

Bispecific antibodies binding with one arm to CD40 and with the second arm to CD137 were produced and analyzed by the two *Trans*-reporter assays using HEK293_NFK_CD40_gfp_luc + K562_CD137 and HEK293_NFK_CD137_gfp_luc + K562_CD40. Two different bispecific antibodies as well as the control antibodies, the parental monospecific bivalent anti-CD40 and anti-CD137 antibodies and corresponding monovalent antibodies with one irrelevant arm, were tested (**Figure 7A** and **7B**). All antibodies were tested under the indicated trans-presentation conditions using K562 cells expressing the second TNF-receptor and for control without trans-presentation using K562_wt. None of the control antibodies, neither the parental monospecific bivalent antibodies nor the monovalent antibodies with one irrelevant arm, induced luciferase activity in HEK293_NFK_CD40_gfp_luc cells or in HEK293_NFK_CD137_gfp_luc cells. Only the two bispecific CD40xCD137 antibodies resulted in luciferase activity upon addition of antibodies (100 ng/ml and higher concentrations) to HEK293_NFK_CD40_gfp_luc or HEK293_NFK_CD137_gfp_luc cells under trans-presentation conditions. Thus, even if the parental monospecific, bivalent anti-CD40 and anti-CD137 IgG1 antibodies do not show

agonistic activity, the bispecific CD40xCD137 antibody is able to induce activation of both receptors resulting in intracellular signaling and NF-κB pathway activation.

2.3 Analysis of CD40xCD27 bispecific antibodies

A bispecific antibody binding with one arm to CD40 and with the second arm to CD27 was produced and analyzed by the two *Trans*-reporter assays using HEK293_NFK_CD40_gfp_luc + K562_CD27 and HEK293_NFK_CD27_gfp_luc + K562_CD40. The bispecific antibody as well as the control antibodies, the parental monospecific bivalent anti-CD40 and anti-CD27 antibodies and corresponding monovalent antibodies with one irrelevant arm, were tested (**Figure 8**). All antibodies were tested under the indicated trans-presentation conditions using K562 cells expressing the second TNF-receptor and for control without trans-presentation using K562_wt. The bispecific antibody (100 ng/ml and higher concentrations) induced strong luciferase activity in HEK293_NFK_CD40_gfp_luc and in HEK293_NFK_CD27_gfp_luc cell lines, respectively, under trans-presentation conditions. Thus, the bispecific CD40xCD27 antibody is able to induce activation of both receptors resulting in strong intracellular signaling and NF-κB pathway activation.

2.4 Analysis of CD40xCD134 bispecific antibodies

Bispecific antibodies binding with one arm to CD40 and with the second arm to CD134 were produced and analyzed by the two *Trans*-reporter assays using HEK293_NFK_CD40_gfp_luc + K562_CD134 and HEK293_NFK_CD134_gfp_luc + K562_CD40. Two different bispecific antibodies as well as the control antibodies, the parental monospecific bivalent anti-CD40 and anti-CD134 antibodies and the corresponding monovalent antibodies with one irrelevant arm, were tested (**Figure 9A** and **9B**). All antibodies were tested under the indicated trans-presentation conditions using K562 cells expressing the second TNF-receptor and for control without trans-presentation using K562_wt. The parental monospecific bivalent anti-CD134 antibodies induced only very weak luciferase activity. However, the bispecific antibodies (100 ng/ml and higher concentrations) induced strong luciferase activity in HEK293_NFK_CD40_gfp_luc and in HEK293_NFK_CD134_gfp_luc cell lines, respectively, under trans-presentation conditions. Thus, even if the parental monospecific, bivalent anti-CD40 and anti-CD134 IgG1 antibodies show only slight agonistic activity the bispecific CD40xCD134 antibody is able to induce activation of both receptors resulting in strong intracellular signaling and NF-κB pathway activation.

Example 3: Bispecific antibodies targeting CD134 and CD27, CD134 and CD137 or CD27 and CD137

3.1 Reporter assay measuring cis-activation

CD27, CD134 and CD137 are predominantly expressed on T cells. While CD27 is constitutively expressed and CD27 expression density only varies upon T cell activation, CD134 and CD137 expression is only induced upon T cell activation. Thereby, bispecific antibodies binding to CD134 and CD27, to CD134 and CD137 or to CD27 and CD137 most probably target two receptors expressed on the same T cell. However, the activity of these bispecific antibodies depends on the co-expression of the two receptors and thereby on the activation status of the T cells. Activation of these TNF-receptors is dependent on receptor cross-linking and receptor clustering, respectively, which can be achieved by binding agents simultaneously binding to different receptors. If T cells co-express the indicated receptors, these bispecific antibodies can act as costimulatory agents mediating receptor-induced signaling in T cells upon binding of two different TNF-receptors on the same T cell.

A corresponding reporter assay system was established using TNF-receptor double transfectants. To that end, HEK293_NFK_gfp_luc cells were stably transduced with two of the indicated human TNF-receptors, CD27, CD134 and CD137, respectively. Cell surface expression of the receptors was analyzed by flow cytometry depicted in **Figure 10A** and **Figure 10B**.

3.2 Analysis of CD134xCD27 bispecific antibodies

A bispecific antibody binding with one arm to CD134 and with the second arm to CD27 was produced and analyzed by a *Cis*-reporter assay using HEK293_NFK_CD27_CD134_gfp_luc cells. The bispecific antibody as well as the control antibodies, the parental monospecific bivalent anti-CD134 and anti-CD27 antibodies and the corresponding monovalent antibodies with one irrelevant arm, were tested (**Figure 11**). The bispecific antibody induced the strongest luciferase activity in HEK293_NFK_CD27_CD134_gfp_luc cells. Thus, the bispecific CD134xCD27 antibody is able to induce a strong activation of cells expressing both receptors resulting in strong NF-κB signaling.

3.3 Analysis of CD134xCD137 bispecific antibodies

Bispecific antibodies binding with one arm to CD134 and with the second arm to CD137 were produced and analyzed by a *Cis*-reporter assay using the HEK293_NFK_CD134_CD137_gfp_luc cell line. Two different bispecific antibodies as well as the control antibodies, the parental monospecific bivalent anti-CD134 and anti-CD137 antibodies and corresponding monovalent antibodies with one irrelevant arm, were tested (**Figure 12A** and **12B**). None of the control antibodies, neither the parental monospecific bivalent antibodies nor the monovalent antibodies with one irrelevant arm, induced luciferase activity in the HEK293_NFK_CD134_CD137_gfp_luc. Only the bispecific CD134xCD137 antibody resulted in luciferase activity. Thus, even if the parental monospecific, bivalent anti-CD134 and anti-CD137 IgG1 antibodies do not show any agonistic activity, the bispecific CD134xCD137 antibody is able to induce activation in the cell line expressing both receptors resulting in NF-κB pathway activation.

3.3 Analysis of CD27xCD137 bispecific antibodies

Bispecific antibodies binding with one arm to CD137 and with the second arm to CD27 were produced and analyzed by a *Cis*-reporter assay using HEK293_NFK_CD27_CD137_gfp_luc cells. Two different bispecific antibodies as well as the control antibodies, the parental monospecific bivalent anti-CD137 and anti-CD27 antibodies and corresponding monospecific antibodies with one irrelevant arm, were tested (**Figure 13A** and **13B**). In this case, the parental monospecific bivalent anti-CD27 antibodies induced luciferase activity, whereas the parental monospecific bivalent anti-CD137 resulted only in extremely weak maximum activation signals. Both control monovalent antibodies consisting of one irrelevant arm induced very weak activation of HEK293_NFK_CD27_CD137_gfp_luc cells. However, the bispecific antibodies induced stronger luciferase activity in HEK293_NFK_CD27_CD137_gfp_luc cells than the control antibodies with one irrelevant arm.

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Claims

1. A binding agent comprising at least two binding domains, wherein a first binding domain binds to a first receptor of the tumor necrosis factor (TNF) superfamily and a second binding domain binds to a second receptor of the TNF superfamily, wherein the first binding domain binds to CD40 and the second binding domain binds to 4-1BB (CD137), wherein

the binding agent is a bispecific antibody, wherein the binding agent is an agonistic binding agent and activates the first receptor and the second receptor at once,

wherein the first binding domain binding to CD40 is selected from a first group consisting of:

(a) the heavy chain complementarity determining region HCDR1 having the amino acid sequence of SEQ ID NO: 2323, the HCDR2 having the amino acid sequence of SEQ ID NO: 2324 and the HCDR3 having the amino acid sequence of SEQ ID NO: 2325, and

the light chain complementarity determining region LCDR1 having the amino acid sequence of SEQ ID NO: 2326, the LCDR2 having the amino acid sequence of SEQ ID NO: 2327 and the LCDR3 having the amino acid sequence of SEQ ID NO: 2328; and

(b) the heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2321 and the light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2322; and

wherein the second binding domain binding to 4-1BB (CD137) is selected from a second group consisting of:

(a) the heavy chain complementarity determining region HCDR1 having the amino acid sequence of SEQ ID NO: 2297, the HCDR2 having the amino acid sequence of SEQ ID NO: 2298 and the HCDR3 having the amino acid sequence of SEQ ID NO: 2299, and

the light chain complementarity determining region LCDR1 having the amino acid sequence of SEQ ID NO: 2300, the LCDR2 having the amino acid sequence of SEQ ID NO: 2301 and the LCDR3 having the amino acid sequence of SEQ ID NO: 2302;

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(b) the heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2241 and the light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2242;

(c) the heavy chain complementarity determining region HCDR1 having the amino acid sequence of SEQ ID NO: 2255, the HCDR2 having the amino acid sequence of SEQ ID NO: 2256 and the HCDR3 having the amino acid sequence of SEQ ID NO: 2257, and

the light chain complementarity determining region LCDR1 having the amino acid sequence of SEQ ID NO: 2258, the LCDR2 having the amino acid sequence of SEQ ID NO: 2259 and the LCDR3 having the amino acid sequence of SEQ ID NO: 2260;

(d) the heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2227 and the light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2228;

(e) the heavy chain complementarity determining region HCDR1 having the amino acid sequence of SEQ ID NO: 2315, the HCDR2 having the amino acid sequence of SEQ ID NO: 2316 and the HCDR3 having the amino acid sequence of SEQ ID NO: 2317, and

the light chain complementarity determining region LCDR1 having the amino acid sequence of SEQ ID NO: 2318, the LCDR2 having the amino acid sequence of SEQ ID NO: 2319 and the LCDR3 having the amino acid sequence of SEQ ID NO: 2320; and

(f) the heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 2247 and the light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 2248.

2. The binding agent of claim 1, wherein the binding agent is in the format of a full-length antibody.

3. A nucleic acid molecule encoding a binding agent according to claim 1 or 2, wherein, preferably, the nucleic acid molecule is contained in a vector.

4. A cell comprising a nucleic acid molecule according to claim 3.

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5. A pharmaceutical composition comprising, as an active agent, a binding agent according to claim 1 or 2, a nucleic acid molecule according to claim 3, or a cell according to claim 4.

6. The pharmaceutical composition according to claim 5, further comprising a pharmaceutically acceptable carrier and/or excipient.

7. A kit comprising a binding agent according to claim 1 or 2, a nucleic acid molecule according to claim 3, a cell according to claim 4, or a pharmaceutical composition according to claim 5 or 6.

8. A binding agent according to claim 1 or 2, a nucleic acid molecule according to claim 3, a cell according to claim 4, or a pharmaceutical composition according to claim 5 or 6 for use as a medicament.

9. A binding agent according to claim 1 or 2, a nucleic acid molecule according to claim 3, a cell according to claim 4, or a pharmaceutical composition according to claim 5 or 6 for use in the treatment of a disease selected from the group consisting of cancer, infectious diseases, inflammatory diseases, metabolic diseases, autoimmune disorders, degenerative diseases, apoptosis-associated diseases and transplant rejections.