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(71) Demandeur/Applicant:
INNOVENT BIOLOGICS (SUZHOU) CO., LTD., CN
(72) Inventeurs/Inventors:
TSUN, ANDY, CN;
BARUAH, HEMANTA, CN;
LIU, XIAOLIN, CN;
CHEN, CHENG, CN;
LIU, JUNJIAN, CN;
CHEN, BINGLIANG, CN;
HUANG, WEIFENG, CN
(74) Agent: GOWLING WLG (CANADA) LLP

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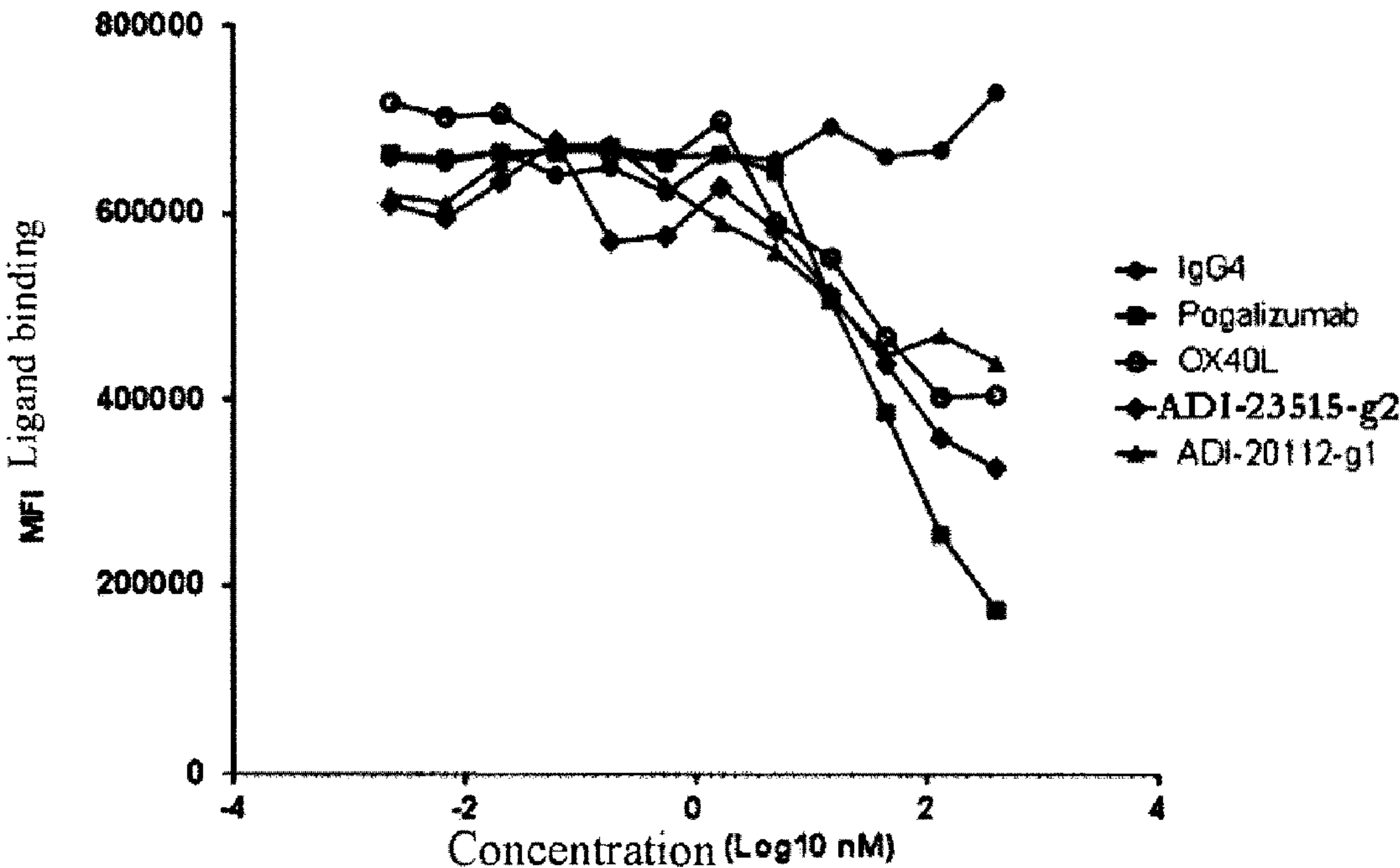


Fig 1

(57) **Abrégé/Abstract:**
Provided are an antibody and an antibody fragment specifically binding to OX40 and a composition comprising the antibody or the antibody fragment. Also provided are a nucleic acid encoding the antibody or the antibody fragment and a host cell comprising the same. Further provided are therapeutic and diagnostic uses of the antibody and the antibody fragment.

Abstract

The present invention relates to a novel antibody and antibody fragment that specifically binds to OX40 and to a composition comprising said antibody or antibody fragment thereof. In addition, the present invention relates to a nucleic acid encoding the antibody or antibody fragment thereof and a host cell comprising the same, and to a related use thereof. In addition, the present invention relates to the use of the antibody and antibody fragment for treatment and diagnosis.

ANTI-OX40 ANTIBODY AND USE THEREOF

The present invention relates to a novel antibody and antibody fragment that specifically binds to OX40 and to a composition comprising said antibody or antibody fragment. In addition, the present invention relates to a nucleic acid encoding the antibody or an antibody fragment thereof and a host cell comprising the same, and to a related use thereof. In addition, the present invention relates to the use of the antibody and antibody fragment for treatment and diagnosis.

Background Technique

OX40 (also known as CD134, TNFRSF4 and ACT35) was originally described as T cell activation markers on rat CD4 T cells (Paterson DJ, Jefferies WA, Green JR, Brandon MR, Cortesy P, Puklavec M, Williams AF. Antigen of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. *Mol Immunol.* 1987; 24: 1281–1290) and subsequently shown to be upregulated in TCR recruitment (Mallett S, Fossum S, Barclay AN. Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes - a molecule related to nerve growth factor receptor. *EMBO J.* 1990; 9: 1063–1068). OX40 was identified on CD4+ T cells, CD8+ T cells, NK cells, NKT cells and neutrophils (D. J. Paterson, W. A. Jefferies, J. R. Green et al., “Antigen of activated Rat T lymphocytes including a molecule of 50,000 M(r) detected only on CD4 positive T blasts,” *Molecular Immunology*, vol. 24, no. 12, pp. 1281-1290, 1987). OX40 signaling can promote costimulatory signals to T cells, leading to enhanced cell proliferation, survival, effector function and migration (Gramaglia I, Weinberg AD, Lemon M, Croft M. Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. *J Immunol.* 1998; 161: 6510-6517; Gramaglia I, Jember A, Pippig SD, Weinberg AD, Killeen N, Croft M. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. *J Immunol.* 2000; 165: 3043-3050).

OX40L, the ligand for OX40, is mainly expressed on antigen presenting cells (APCs) and its expression can be induced by CD40 and mast cell signaling, toll-like receptors (TLR), and inflammatory cytokines. In addition to APC, non-hematopoietic cells such as smooth muscle and vascular endothelial cells can also express OX40L. In transgenic mice overexpressing OX40L, T-cell activation was increased and, when immunized, these mice produced enhanced

T cell responses (Murata K, Nose M, Ndhlovu LC, Sato T, Sugamura K, Ishii N. Constitutive OX40/OX40 ligand interaction induces autoimmune-like diseases. *J Immunol.* 2002; 169: 4628-4636, and Sato T, Ishii N, Murata K, Kikuchi K, Nakagawa S, Ndhlovu LC, Sugamura K. Consequences of OX40-OX40 ligand interactions in langerhans cell function: enhanced contact hypersensitivity responses in OX40L-transgenic mice. *Eur J Immunol.* 2002; 32:3326-3335). This data suggests that OX40L expression is a limiting factor for OX40 signaling in T cells.

In mice bearing tumors, the in vivo ligation of mouse OX40 (by soluble mouse OX40L-immunoglobulin fusion protein or mouse OX40L mimetic, such as anti-mouse CD134 specific antibody) enhances anti-tumor immunity, resulting in no tumor survival in the mouse models of various mouse malignant tumor cell lines such as lymphoma, melanoma, sarcoma, colon cancer, breast cancer and neuroglioma (Sugamura et al. *Nature Rev Imm.* 2004; 4: 420-431).

It has been suggested that the immune response of the mammal to the antigen is enhanced by the use of OX40 binding agent in combination with OX40 (WO99/42585; Weinberg, 2000). Although the literature generally refers to the OX40 binding agent, the emphasis is on the use of OX40L or a portion thereof; the anti-OX40 antibody is disclosed as an equivalent of OX40L. In fact, when the Weinberg group used the study in nonhuman primate studies, they again intentionally selected antibodies that bind OX40L binding sites and generally mimic OX40L.

Al-Shamkhani et al. (*Eur J Chem.* 1996; 26: 1695-1699) used an anti-OX40 antibody called OX86 that did not block OX40L binding, to explore the differential expression of OX40 on activated mouse T cells; Hirschhorn-Cymerman et al. (*J Exp Med.* 2009; 206: 1103-1116) used OX86 and cyclophosphamide as potential chemo-immunotherapy in the mouse model. However, OX86 is expected not to bind to human OX40, and when selecting an antibody that is effective in humans, an antibody that binds to the OX40L binding site will be selected according to Weinberg's study.

In severe combined immunodeficiency (SCID) mice, the in vivo ligation of human OX40 (by anti-human OX40-specific antibodies that interact with the OX40L binding domain on human OX40; US2009/0214560A1) enhances anti-tumor immunity, which results in tumor growth inhibition in a variety of human malignant tumor cell lines such as lymphoma, prostate cancer, colon cancer and breast cancer.

In humans, the exact mechanism of human OX40 ligation-mediated anti-tumor immune response has not been confirmed, but is thought to be mediated by OX40 transmembrane

signaling pathway, which is stimulated by interaction with OX40L. This interaction is mediated by the binding of trimeric OX40L and OX40. In the current anticancer treatment, it is recommended to use trimerized OX40 ligands as a more potent drug than the anti-OX40 antibody (Morris et al. Mol Immunol. 2007; 44: 3112-3121).

In addition, a single anti-OX40 treatment does not provide sufficient anti-tumor immunogenicity in immunologically poor tumors, and it is also desirable to develop a combination of OX40 and other strategies. It has been found that regulating the combination of OX40 signaling and other signaling pathways (such as the angiogenesis pathway, PD-1 pathway) that is dysregulated in tumor cells can further enhance therapeutic efficacy.

However, there remains a need to develop a new anti-OX40 antibody that blocks the binding of OX40 to OX40L less than the anti-OX40 antibodies known in the art to better treat or delay various cancers, immune related diseases and T cell dysfunction diseases.

Summary of the invention

Applicants have surprisingly found that in order to activate T cells and to induce T cell mediated antitumor activity, an antibody or fragment thereof(e.g, antigen-binding fragment) bound to human OX40 is used, wherein the antibody or fragment thereof is capable of binding to human OX40 better while less blocking the binding of human OX40 to OX40 ligand (OX40L) and producing an enhanced immune response.

Preferably, the anti-OX40 antibody or fragment thereof of the present invention is capable of activating T cells, for example enhancing the immunostimulatory/effector function of T-effect cells and/or allowing these cells to proliferate and/or down-regulate the immunosuppressive function of T-regulated cells. More preferably, the antibody is capable of eliciting antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, the OX40 antibody of the present invention can be used to treat or delay various cancers, immune-related diseases and T-cell dysfunction disorders.

The invention thus provides an antibody or fragment thereof (preferably an antigen-binding fragment) that binds human OX40 or cynomolgus monkey OX40, wherein the antibody or fragment thereof less blocks binding of human or cynomolgus OX40 to its ligand OX40L. In some embodiments, the antibody or the fragment thereof of the present invention can bind human OX40, while less blocks binding of human OX40 to its ligand (OX40L)(for

example, in comparison to the known OX40 antibody or OX40L), and produce enhanced immune response.

Preferably, the blocking of the antibody or fragment thereof of the present invention on the binding between human or cynomolgus OX40 and its ligand OX40L is lower than that of OX40L ligand or other anti-OX40 antibody known in the art(e.g., ponalizumab). More preferably, the anti-OX40 antibody or the fragment thereof maintain its strong binding to human or cynomolgus OX40 (e.g., comparable to the known anti-OX40 antibody, e.g., ponalizumab), while less block the binding between human or cynomolgus OX40 and its ligand OX40L (e.g., less than the blocking of OX40L or less than the blocking of ponalizumab).

In some embodiments, the antibody or fragment thereof of the present invention binds to human OX40 or cynomolgus monkey OX40. In some preferable embodiments, the antibody or fragment thereof of the present invention does not bind to murine OX40 or bind to murine OX40 less than binding to human or cynomolgus OX40, the murine OX40 is e.g., rat OX40 or mouse OX40.

In some embodiments, the anti-OX40 antibody of the present invention has agonist activity.

In some embodiments, the anti-OX40 antibody of the present invention or a fragment thereof binds to human OX40 or cynomolgus monkey OX40 with a K_D of less than about 200 nM, preferably less than or equal to about 100nM, about 50nM, about 40nM, about 30nM, about 20nM, about 10 nM, more preferably less than or equal to about 9nM, more preferably less than or equal to about 8 nM, more preferably less than or equal to about 7 nM, 6 nM, 5 nM, 4 nM, 3 nM or 2 nM, and most preferably, the K_D is less than or equal to about 1 nM or 0.8 nM or 0.4nM or 0.3nM or 0.2nM or 0.1nM. In some embodiments, the antibody binding affinity is determined using a Bio-light interferometry (e.g., ForteBio affinity measurement) or an MSD assay.

In some embodiments, the binding to human OX40 or cynomolgus monkey OX40 by the antibodies of the present invention or the fragment thereof is determined using the flow cytometry(e.g.,FACS) assay. In some embodiments, the binding to human OX40 or cynomolgus OX40 in a cell has an EC_{50} of less than or equal to about 10 nM, 9nm or 8nm. In some embodiments, the binding to human OX40 or cynomolgus monkey OX40 has an EC_{50} of less than or equal to about 7 nM or about 6nM or about 5nm or about 4nm or about 3 nM. In some embodiments, in an assay performed by flow cytometry, the antibodies or the antibody

fragments bind to OX40 expressed on the cells with a MFI >1000X fold difference, preferably > 1100 fold difference, 1200 fold difference, 1300 fold difference, 1400 fold difference, 1500 fold difference, 1600 fold difference, 1700 fold difference, 1800 fold difference, 1900 fold difference, 2000 fold difference, 2100 fold difference, 2200 fold difference, 2300 fold difference, 2400 fold difference or 2500 fold difference compared with corresponding control cells that do not express OX40.

In another aspect, the present invention provides an anti-OX40 antibody or fragment thereof having agonist activity capable of activating T cells (e.g., CD4+ T cells). Therefore, in some embodiments, the anti-OX40 antibody or fragment thereof can activate T cells.

In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention enhances CD4+ effector T cell function, for example by increasing CD4+ effector T cell proliferation and/or increasing gamma-interferon production of CD4+ effector T cells (e.g., compared to the proliferation and/or cytokine production prior to the treatment of the anti-OX40 antibody or fragment thereof of the present invention, or compared to the proliferation and/or cytokine production of CD4+ effector T cell treated by the control antibody (e.g., IgG antibody). In some embodiments, the cytokine is a γ -interferon, such as IFN γ or an interleukin, such as IL-2.

In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention increases the number of intratumoral (invasive) CD4+ effector T cells (e.g., the total number of CD4+ effector T cells, or the percentage of CD4+ cells in CD45+ cells, for example), for example, compared to the number of intratumoral (invasive) CD4+ T cells prior to treatment with the anti-OX40 antibody or fragment thereof of the present invention (or after treatment with a control antibody (e.g., an IgG antibody). In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention increases the number of intratumoral (invasive) CD4+ effector T cells expressing γ -interferon (e.g., the total number of CD4+ cells expressing γ -interferon, or a percentage of CD4+ cells expressing γ -interferon in total CD4+ cells), for example, compared to the number of intratumoral (invasive) CD4+ effector T cells expressing γ -interferon prior to treatment with the anti-OX40 antibody or fragment thereof of the present invention (or after treatment with a control antibody (e.g., an IgG antibody).

In some embodiments, the agonist activity of the anti-OX40 antibody is assessed by the level of cytokines released after T cell activation. Accordingly, the present invention provides an anti-OX40 antibody or fragment thereof that can enhance cytokine production of CD4+ T

cells as compared to cytokine production of CD4⁺ T cells treated with IgG control. In some embodiments, the cytokine is an inflammatory cytokine, such as γ -interferon (e.g., IFN γ) or an interleukin (e.g., IL-2).

Preferably, the anti-OX40 antibody or fragment thereof of the present invention is capable of increasing the level of IL-2 secreted by CD4⁺ T cells up to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160 fold or higher as compared to the corresponding control IgG. In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention is capable of increasing the level of IL-2 secreted by CD4⁺ T cells up to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 fold or higher as compared to the corresponding control IgG antibody. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention is capable of increasing IFN γ levels secreted by CD4⁺ T cells by a factor of 1, 2, 3 or more compared to a corresponding control IgG antibody. In some embodiments, the cytokine secretion levels of T cells are determined by ELISA.

In some embodiments, the agonist activity of the anti-OX40 antibody is assessed by OX40 signaling (e.g., monitoring of NF κ B downstream signaling). In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention enhances OX40 signal transduction in target cells expressing OX40. In some embodiments, OX40 signal transduction is detected by monitoring NF κ B downstream signaling.

Accordingly, the present invention provides an anti-OX40 antibody or fragment thereof that enhances NF κ B-mediated transcriptional activity levels as compared to a control IgG antibody. Preferably, the anti-OX40 antibody or fragment thereof of the present invention is capable of increasing the level of NF κ B-mediated transcriptional activity by about 1, 2, 3, 4, 5, 6, 7 fold or higher as compared to the corresponding control IgG antibody.

In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention increases the number of intratumoral (invasive) CD8⁺ effector T cells (e.g., the total number of CD8⁺ effector T cells, or the percentage of CD8⁺ in CD45⁺ cells, for example), for example, compared to the number of intratumoral (invasive) CD8⁺ effector T cells prior to treatment with the anti-OX40 antibody or fragment thereof of the present invention (or after treatment with a control antibody (e.g., an IgG antibody)). In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention increases the number of intratumoral (invasive) CD8⁺ effector T cells expressing γ -interferon (e.g., the percentage of CD8⁺ cells

expressing γ -interferon in total CD8+ cells), for example, compared to the number of intratumoral (invasive) CD8+ T effector cells expressing γ -interferon prior to treatment with the anti-OX40 antibody or fragment thereof of the present invention (or after treatment with a control antibody e.g., an IgG antibody).

In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention enhances memory T cell function, for example by increasing memory T cell proliferation and/or increasing cytokine production in memory cells. In some embodiments, the cytokine is γ -interferon(e.g., IFN γ) or interleukin(e.g., IL-2).

In some preferred embodiments, the anti-OX40 antibody or fragment thereof of the present invention is capable of eliciting antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, the anti-OX40 antibody or fragment thereof of the present invention binds to human effector cells, e.g., binds to Fc γ R (e.g., activated Fc γ R) expressed in human effector cells. In some embodiments, the human effector cells perform (are capable of performing) the ADCC effector function.

In some embodiments, the function of the anti-OX40 antibody or fragment thereof of the present invention requires antibody cross-linking. In some embodiments, the functions of the anti-OX40 antibody or fragment thereof of the present invention are as one or more of the following: increasing CD4+ effector T cell proliferation and/or cytokine production, enhancing CD4+ memory T cell proliferation and/or cytokines generation, and/or reduce the cells by ADCC. In some embodiments, antibody cross-linking is determined by providing anti-human OX40 agonistic antibodies that adhere to a solid surface, such as a cell culture plate. In some embodiments, antibody cross-linking is determined by introducing a mutation (e.g., amino acid mutation) into the IgG Fc portion of the antibody and testing the function of the mutant antibody. In some embodiments, antibody cross-linking is achieved by Fc γ RIIb. In some embodiments, the anti-OX40 antibody or the fragment thereof of the present invention can achieve its function without antibody cross-linking.

In some embodiments, the anti-OX40 antibody or the fragment thereof of the present invention can achieve its function in absence of Fc γ RIIb.

In some embodiments, the anti-OX40 antibody or the fragment thereof of the present invention have better anti-tumor activity. For example, compared to control IgG or known anti-OX40 antibody, the anti-OX40 antibody or the fragment thereof of the present invention can reduce the tumor volume in a subject, preferably do not influence the body weight of the subject

in the meantime.

The IgG antibody as a control described herein includes an IgG1 antibody, or an IgG4 antibody or an IgG2 antibody, for example, an IgG1, IgG2 or IgG4 antibody having a light chain and a heavy chain as shown in Table 7.

In a preferred embodiment, the invention provides an anti-OX40 antibody or fragment thereof having one or more of the properties of an anti-OX40 antibody described above.

In some embodiments, an anti-OX40 antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region (HCVR), wherein the HCVR comprises

(i) three complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 contained in the HCVR of any one of the antibodies listed in Table B, or

(ii) a sequence comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in the three CDR regions relative to the sequence of (i).

In some embodiments, an anti-OX40 antibody or antigen-binding fragment thereof of the invention comprises a light chain variable region (LCVR), wherein the LCVR comprises:

(i) the LCDR1, LCDR2 and LCDR3 sequences contained in the LCVR of anyone of the antibodies listed in Table B; or

(ii) a sequence comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in the three CDR regions relative to the sequence of (i).

In some embodiments, an anti-OX40 antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region HCVR and a light chain variable region LCVR, wherein

(a) the HCVR contains

(i) three complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 contained in the HCVR of anyone of the antibodies listed in Table B, or

(ii) a sequence comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions relative to the sequence of (i); and/or

(b) The LCVR contains:

(i) the LCDR1, LCDR2 and LCDR3 sequences contained in the LCVR of anyone of the antibodies listed in Table B; or

(ii) a sequence comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in the three CDR regions relative to the sequence of (i).

In a preferred embodiment, the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120, or consists of the amino acid sequence.

In a preferred embodiment, the LCVR comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 130, 131, 132, 133, 134, 135, 136, 137 or 138.

In some embodiments, an anti-OX40 antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region (HCVR) and/or a light chain variable region (LCVR), wherein

(i) the HCVR comprises a complementarity determining region (CDR) HCDR1, HCDR2 and HCDR3, wherein HCDR1 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16, or HCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16; HCDR2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33, or HCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33; HCDR3 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, 38, 39 and 40, or HCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, 38, 39 and 40;

and/or

(ii) wherein the LCVR comprises complementarity determining regions (CDRs) LCDR1, LCDR2 and LCDR3, wherein LCDR1 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 42, 43, 44, 45 and 46, or LCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NO: 41, 42, 43, 44, 45 and 46; LCDR2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOS: 47, 48, 49, 50, 51, and 52, or LCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NO: 47, 48, 49, 50, 51, and 52; LCDR3 comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60 and 61, or LCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60 and 61.

In a preferred embodiment, the invention provides an anti-OX40 antibody or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein

(a) the HCVR contains

(i) a combination of HCDR1, HCDR2 and HCDR3 as shown in Table A; or

(ii) a variant of the HCDR combination of (i) comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions;

and/or

(b) the LCVR contains

(i) a combination of LCDR1, LCDR2 and LCDR3 as shown in Table A; or

(ii) a variant of the LCDR combination of (i), said variant comprising at least one and no more than 10 or 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions.

In a preferred embodiment, the present invention provides anti-OX40 antibody or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR comprises a complementarity determining region (CDR) HCDR1, HCDR2 and HCDR3 and the LCVR comprises (CDR) LCDR1, LCDR2 and LCDR3, wherein the combinations of the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprised in said antibody or its antigen-binding fragment are represented as follows (Table A):

Table A: Exemplary combinations of HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 in the antibody or antigen-binding fragment thereof of the present invention:

combination	HCDR1, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	HCDR2, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	HCDR3, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	LCDR1, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	LCDR2, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	LCDR3, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO
(1)	SEQ ID NO:1	SEQ ID NO:17	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(2)	SEQ ID NO:1	SEQ ID NO:17	SEQ ID NO:34	SEQ ID NO:42	SEQ ID NO:48	SEQ ID NO:54
(3)	SEQ ID NO:2	SEQ ID NO:18	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(4)	SEQ ID NO:3	SEQ ID NO:19	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(5)	SEQ ID NO:4	SEQ ID NO:20	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(6)	SEQ ID NO:4	SEQ ID NO:21	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(7)	SEQ ID NO:5	SEQ ID NO:22	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(8)	SEQ ID NO:6	SEQ ID NO:23	SEQ ID NO:34	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:53
(9)	SEQ ID NO:7	SEQ ID NO:24	SEQ ID NO:35	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:55

(10)	SEQ ID NO:8	SEQ ID NO:25	SEQ ID NO:35	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:55
(11)	SEQ ID NO:9	SEQ ID NO:26	SEQ ID NO:35	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:55
(12)	SEQ ID NO:10	SEQ ID NO:27	SEQ ID NO:35	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:55
(13)	SEQ ID NO:11	SEQ ID NO:28	SEQ ID NO:35	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:55
(14)	SEQ ID NO:12	SEQ ID NO:29	SEQ ID NO:36	SEQ ID NO:44	SEQ ID NO:50	SEQ ID NO:56
(15)	SEQ ID NO:13	SEQ ID NO:30	SEQ ID NO:37	SEQ ID NO:45	SEQ ID NO:51	SEQ ID NO:57
(16)	SEQ ID NO:14	SEQ ID NO:31	SEQ ID NO:38	SEQ ID NO:42	SEQ ID NO:52	SEQ ID NO:58
(17)	SEQ ID NO:15	SEQ ID NO:32	SEQ ID NO:39	SEQ ID NO:43	SEQ ID NO:49	SEQ ID NO:59
(18)	SEQ ID NO:15	SEQ ID NO:32	SEQ ID NO:39	SEQ ID NO:46	SEQ ID NO:49	SEQ ID NO:60
(19)	SEQ ID NO:16	SEQ ID NO:33	SEQ ID NO:40	SEQ ID NO:41	SEQ ID NO:47	SEQ ID NO:61

In some embodiments, an anti-OX40 antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region HCVR and/or a light chain variable region LCVR, wherein

(a) heavy chain variable region HCVR

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120; or

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more

preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120, preferably, said amino acid changes do not occur in the CDR regions;

and/or

(b) light chain variable region LCVR

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 130, 131, 132, 133, 134, 135, 136, 137 and 138;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 130, 131, 132, 133, 134, 135, 136, 137 and 138; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 130, 131, 132, 133, 134, 135, 136, 137 and 138, preferably, said amino acid changes do not occur in the CDR regions.

In a preferred embodiment, the present invention provides anti-OX40 antibody or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the combinations of the heavy chain variable region HCVR and light chain variable region LCVR comprised in said antibody or its antigen-binding fragment are represented as follows (Table B):

Table B: Exemplary combinations of the heavy chain variable region HCVR and light chain variable region LCVR in the antibody or antigen-binding fragment thereof of the present invention:

combination	HCVR, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO	LCVR, which comprises or consists of the amino acid sequence as represented in the following SEQ ID NO
(1)	SEQ ID NO:106	SEQ ID NO:130
(2)	SEQ ID NO:106	SEQ ID NO:131

(3)	SEQ ID NO:107	SEQ ID NO:130
(4)	SEQ ID NO:108	SEQ ID NO:130
(5)	SEQ ID NO:109	SEQ ID NO:130
(6)	SEQ ID NO:110	SEQ ID NO:130
(7)	SEQ ID NO:111	SEQ ID NO:130
(8)	SEQ ID NO:112	SEQ ID NO:132
(9)	SEQ ID NO:113	SEQ ID NO:132
(10)	SEQ ID NO:114	SEQ ID NO:132
(11)	SEQ ID NO:115	SEQ ID NO:132
(12)	SEQ ID NO:116	SEQ ID NO:133
(13)	SEQ ID NO:117	SEQ ID NO:134
(14)	SEQ ID NO:118	SEQ ID NO:135
(15)	SEQ ID NO:119	SEQ ID NO:136
(16)	SEQ ID NO:119	SEQ ID NO:137
(17)	SEQ ID NO:120	SEQ ID NO:138

In some embodiments, the anti-OX40 antibody or antigen-binding fragment thereof of the present invention comprises a heavy chain and/or light chain, wherein

(a) heavy chain

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or

167;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or 167; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 20 or 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or 167, preferably, said amino acid changes do not occur in the CDR regions, more preferably, said amino acid changes do not occur in the heavy chain variable region;

and/or

(b) light chain

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 168, 169, 170, 171, 172, 173, 174, 175 or 176;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 168, 169, 170, 171, 172, 173, 174, 175 or 176; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 20 or 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 168, 169, 170, 171, 172, 173, 174, 175 or 176, preferably, said amino acid changes do not occur in the CDR regions, more preferably, said amino acid changes do not occur in the light chain variable region.

In a preferred embodiment, the anti-OX40 antibody or antigen-binding fragment thereof of the present invention comprises a heavy and a light chain, wherein the combinations of the heavy chain and light chain comprised in said antibody or its antigen-binding fragment are as follows (Table C):

Table C: exemplary combinations of the heavy chain and light chain in the antibody or antigen-binding fragment thereof of the present invention:

combination	Heavy chain, which comprises or consists of the amino acid sequence represented by the following SEQ ID NO	Light chain, , which comprises or consists of the amino acid sequence represented by the following SEQ ID NO
(1)	SEQ ID NO:189	SEQ ID NO:168
(2)	SEQ ID NO:139	SEQ ID NO:168
(3)	SEQ ID NO:140	SEQ ID NO:168
(4)	SEQ ID NO:141	SEQ ID NO:168
(5)	SEQ ID NO:142	SEQ ID NO:168
(6)	SEQ ID NO:143	SEQ ID NO:168
(7)	SEQ ID NO:144	SEQ ID NO:168
(8)	SEQ ID NO:145	SEQ ID NO:168
(9)	SEQ ID NO:146	SEQ ID NO:168
(10)	SEQ ID NO:147	SEQ ID NO:168
(11)	SEQ ID NO:148	SEQ ID NO:168
(12)	SEQ ID NO:149	SEQ ID NO:168
(13)	SEQ ID NO:150	SEQ ID NO:169
(14)	SEQ ID NO:151	SEQ ID NO:169
(15)	SEQ ID NO:152	SEQ ID NO:169
(16)	SEQ ID NO:153	SEQ ID NO:169
(17)	SEQ ID NO:154	SEQ ID NO:169
(18)	SEQ ID NO:155	SEQ ID NO:169

(19)	SEQ ID NO:156	SEQ ID NO:169
(20)	SEQ ID NO:157	SEQ ID NO:169
(21)	SEQ ID NO:158	SEQ ID NO:170
(22)	SEQ ID NO:159	SEQ ID NO:170
(23)	SEQ ID NO:160	SEQ ID NO:171
(24)	SEQ ID NO:161	SEQ ID NO:171
(25)	SEQ ID NO:162	SEQ ID NO:172
(26)	SEQ ID NO:163	SEQ ID NO:172
(27)	SEQ ID NO:164	SEQ ID NO:173
(28)	SEQ ID NO:165	SEQ ID NO:173
(29)	SEQ ID NO:164	SEQ ID NO:174
(30)	SEQ ID NO:165	SEQ ID NO:174
(31)	SEQ ID NO:166	SEQ ID NO:175
(32)	SEQ ID NO:167	SEQ ID NO:175
(33)	SEQ ID NO:189	SEQ ID NO:176
(34)	SEQ ID NO:139	SEQ ID NO:176

In some embodiments, the heavy chain of the anti-OX40 antibody or fragment thereof of the present invention further comprises a signal peptide sequence, such as METDTLLLWVLLLLVPGSTG (SEQ ID NO: 182).

In one embodiment of the invention, the amino acid changes described herein include substitutions, insertions or deletions of amino acids. Preferably, the amino acid changes described herein are amino acid substitutions, preferably conservative substitutions.

In some embodiments, an antibody of the invention is capable of binding OX40 and less

blocking the binding of OX40 to its ligand OX40L. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention has a functional Fc region. In some embodiments, the effector function of the functional Fc region is ADCC. In some embodiments, the Fc region is the Fc region of human IgG1 or the Fc region of human IgG2.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention has one or more of the following characteristics:

(i) showing the same or similar binding affinity and/or specificity as anyone of the antibodies listed in Table 5 for OX40 (particularly human OX40);

(ii) inhibiting (e.g, competitive inhibiting) the binding of anyone of the antibodies listed in Table 5 to OX40 (particularly human OX40);

(iii) binding to an epitope that is the same or overlaps with that bound by anyone of the antibodies shown in Table 5;

(iv) competing with anyone of the antibodies shown in Table 5 to bind OX40 (particularly human OX40);

(v) having one or more of the biological properties of anyone of the antibodies listed in Table 5.

In some embodiments, the anti-OX40 antibody of the present invention is an antibody in the form of IgG1 or antibody in IgG2 form or antibody in IgG4 form. In some embodiments, the anti-OX40 antibody is a monoclonal antibody. In some embodiments, the anti-OX40 antibody is humanized. In some embodiments, the anti-OX40 antibody is a human antibody. In some embodiments, at least a portion of the anti-OX40 antibody's framework sequence is a human consensus framework sequence. In one embodiment, the anti-OX40 antibody of the present invention also encompasses an antibody fragment thereof, preferably an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, single chain antibody (e.g., scFv) or (Fab')₂, single domain antibody, diabodies (dAb) or linear antibody .

In certain embodiments, the anti-OX40 antibody molecule is in the form of a bispecific or multispecific antibody molecule. In one embodiment, the bispecific antibody molecule has a first binding specificity for OX40 and a second binding specificity for PD-1, TIM-3, CEACAM (eg, CEACAM-1 and/or CEACAM-5), PD-L1 or PD-L2. In one embodiment, the bispecific antibody molecule binds to OX40 and PD-1. In another embodiment, the bispecific antibody

molecule binds to OX40 and PD-L1. In yet another embodiment, the bispecific antibody molecule binds to OX40 and PD-L2. The multispecific antibody molecule can have any combination of binding specificities for the aforementioned molecules. The multispecific antibody molecule can be, for example, a trispecific antibody molecule comprising a first binding specificity for OX40 and a second and third binding specificity for one or more of the following molecules: PD-1, TIM-3, CEACAM (e.g., CEACAM-1 or CEACAM-5), PD-L1 or PD-L2.

In one aspect, the present invention provides nucleic acids encoding any of the above anti-OX40 antibodies or fragments thereof. In one embodiment, a vector comprising said nucleic acid is provided. In one embodiment, the vector is an expression vector. In one embodiment, a host cell comprising said vector is provided. In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is selected from yeast cells, mammalian cells (e.g., CHO cells or 293 cells), or other cells suitable for the preparation of antibodies or antigen-binding fragments thereof. In another embodiment, the host cell is prokaryotic.

In one embodiment, the present invention provides a method of preparing an anti-OX40 antibody or fragment thereof (preferably an antigen-binding fragment), wherein the method comprises culturing the host cell under conditions suitable for expression of a nucleic acid encoding the antibody or fragment thereof (preferably an antigen-binding fragment), and optionally isolating the antibody or fragment thereof (preferably an antigen-binding fragment). In an embodiment, the method further comprises recovering an anti-OX40 antibody or fragment thereof (preferably an antigen-binding fragment) from the host cell.

In some embodiments, the invention provides an immunoconjugate comprising any of the anti-OX40 antibodies provided herein and other agents, such as a cytotoxic agent. In some embodiments, the immunoconjugate is used to treat cancer, preferably lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In some embodiments, the present invention provides a composition comprising any of the anti-OX40 antibodies or fragments thereof, preferably an antigen-binding fragment thereof, or immunoconjugate as described herein, preferably the composition is a pharmaceutical composition. In one embodiment, the composition further comprises a pharmaceutically acceptable adjuvants. In one embodiment, a composition, e.g., a pharmaceutical composition, comprises a combination of an anti-OX40 antibody or a fragment thereof, or an immunoconjugate thereof, of the invention, and one or more other therapeutic agents (e.g., a

chemotherapeutic agent, an anti-angiogenic agent, an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody). In some embodiments, the pharmaceutical composition is used for the treatment of cancer, preferably lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In one aspect, the present invention relates to a method of activating T cells or inducing T cell mediated antitumor activity or enhancing body immune response in a subject, which comprises administering to said subject an effective amount of any of the anti-OX40 antibodies or fragments thereof, immunoconjugate or pharmaceutical composition as described herein. The present invention also relates to the use of any anti-OX40 antibodies or a fragment thereof described herein for the preparation of a composition or medicament for activating T cells or inducing T cell mediated antitumor activity or enhancing the body immune response of in a subject.

In another aspect, the present invention relates to a method of treating cancer in a subject comprising administering to said subject an effective amount of any anti-OX40 antibody or fragment thereof, immunoconjugate or pharmaceutical composition as described herein. In one embodiment, the cancer is lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like. In another aspect, the invention also relates to the use of any anti-OX40 antibodies or a fragment thereof described herein for the manufacture of a medicament for the treatment of cancer in a subject. In one embodiment, the cancer is lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In some embodiments, the methods described herein further comprise administering to the subject one or more therapies (e.g., a therapeutic manner and/or other therapeutic agent). In some embodiments, the therapeutic manner includes surgery and/or radiation therapy. In some embodiments, the other therapeutic agent is selected from the group consisting of a chemotherapeutic agent, a PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody), or an anti-angiogenic agent (e.g., bevacizumab).

In another aspect, the invention relates to use of the anti-OX40 antibodies or fragments thereof described herein in combination with a PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody) in the manufacture of a medicament for treating cancer in a subject. In one embodiment, the cancer is lung cancer (eg, non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In some embodiments, the subject or individual is a mammal, preferably a human.

In one aspect, the present invention relates to a method of detecting OX40 in a sample comprising (a) contacting a sample with any anti-OX40 antibody or fragment thereof described herein; and (b) detecting the formation of a complex between anti-OX40 antibody or fragment thereof and OX40. In one embodiment, the anti-OX40 antibody is detectably labeled.

In some embodiments, the present invention relates to a kit or a manufacture articles comprising any of the anti-OX40 antibodies or fragments thereof described herein. In some embodiments, the kit or product comprises an anti-OX40 antibody or fragment thereof as described herein and optionally a pharmaceutically acceptable adjuvants, and optionally one or more additional therapeutic agent (such as a chemotherapeutic agent, a PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody), or an anti-angiogenic agent (e.g., bevacizumab). In some embodiments, the kit or manufacture article further comprises instructions for administering a medicament for the treatment of cancer.

The invention also encompasses any combination of any of the embodiments described herein. Any of the embodiments described herein, or any combination thereof, are applicable to any and all of the anti-OX40 antibodies or fragments of the invention as described herein and methods and uses thereof.

Description of Figures

Figure 1 shows the blocking of the binding of OX40L to OX40 expressed on CHO cells by the antibody of the present invention produced in CHO cells along with increasing concentration measured by flow cytometry.

Figure 2 shows IL-2 secretion upon T cell activation in PBMCs activated by PHA plus the antibody of the present invention in the form of IgG1 and IgG2 produced in CHO cells.

Figure 3 shows luciferase reporter detection in Jurkat stably transfected with human OX40 and NFkB promoter-luc by anti-CD3 and anti-CD28 stimulation plus antibodies of the invention in IgG1 or IgG2 format generated in CHO cells.

Figure 4 shows luciferase reporter detection in Jurkat stably transfected with human OX40 and NFkB promoter-luc by anti-CD3 and anti-CD28 stimulation plus antibodies of the invention in IgG2 format generated in CHO cells.

Figure 5 shows luciferase reporter detection in Jurkat stably transfected with human OX40 and NFkB promoter-luc by anti-CD3 and anti-CD28 stimulation plus antibodies of the invention in IgG1 or IgG2 formats generated in CHO cells, wherein the Jurkat is mixed with Raji cells.

Figure 6 shows the luciferase reporter detection in Jurkat stably transfected with human OX40 and NFkB promoter-luc by anti-CD3 and anti-CD28 stimulation plus antibodies of the invention in IgG1 formats generated in CHO cells, wherein the Jurkat is mixed with Raji cells.

Figure 7 shows a DC co-culture assay with SEE (1 ng/ml) and the OX40 antibody of the present invention in IgG1 or IgG2 format produced in CHO cells.

Figure 8 shows the luciferase reporter detection in Jurkat cell stably transfected with human OX40 and NFkB promoter-luc by stimulation of anti-CD3 and anti-CD28 plus recombinant OX40L and antibodies of the invention in IgG1 or IgG2 formats generated in CHO cells or control(IgG4 Ponalizumab, OX40L), mixed with Raji cells.

Figure 9 shows the ADCC activity to CHO cells expressing human OX40 in luciferase reporter based system by the antibody of the invention in IgG1 format generated in HEK293 cells.

Figure 10 shows ADCC activity to CHO cells expressing human OX40 in luciferase reporter based system by the antibody of the invention in IgG2 format generated in HEK293 cells.

Figure 11 shows the tumor growth curve of an individual mouse in the Hu-PBMC LoVo tumor mouse model by administering OX40 antibody ADI-20112-g1 antibodies of the present invention in IgG1 format generated in CHO cells to NOG mice (each curve represents the data of one mouse).

Figure 12 shows the tumor growth curve of an individual mouse in the Hu-PBMC LoVo tumor mouse model by administering OX40 antibody ADI-23515-g2 antibodies of the present invention in IgG2 format generated in CHO cells to NOG mice (each curve represents the data of one mouse).

Figure 13 shows a Hu-PBMC LoVo tumor mouse model by administering OX40 antibody ADI-20112-g1 and ADI-23515-g2 of the present invention in IgG1 and IgG2 format generated in CHO cells to NOG mice. It shows the final tumor volume measurement on day 28 after

tumor implantation.

Figure 14 shows a Hu-PBMC LoVo tumor mouse model by administering OX40 antibody ADI-20112-g1 and ADI-23515-g2 of the present invention in IgG1 and IgG2 format generated in CHO cells to NOG mice. It shows body weight change of the mouse after dosing.

Figure 15 shows the study on efficacy of anti-OX40 antibody in combination with anti-PD-1 antibody in MC38 tumor-bearing OX40 transgenic mice.

Detailed Description

Definition

Before the present invention is described in detail below, it is to be understood that the invention is not limited to the particular methodology, solutions, and reagents described herein, as these may vary. It is also understood that the terminology used herein is for the purpose of describing the particular embodiments and is not intended to limit the scope of the invention, which will only be restricted by the appended claims. All technical and scientific terms used herein have the same meaning as commonly understood by those skilled in the art to which this invention belongs, unless otherwise defined.

For the purpose of interpreting the specification, the following definitions will be used, and the terms used in the singular may also include the plural, vice versa, if appropriate. It is understood that the terminology used herein is for the purpose of describing particular embodiments and is not intended to be restrictive.

The term "about" when used in connection with a numerical value is meant to encompass numerical values within the range between the lower limit of 5% less than the specified numerical value and the upper limit of 5% greater than the specified numerical value.

"Affinity" refers to the strength of the sum of all non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). As used herein, "binding affinity" refers to the intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (e.g., an antibody and an antigen), unless otherwise indicated. The affinity of molecule X for its partner Y is generally expressed by the equilibrium dissociation constant (K_D). Affinity can be measured by conventional methods known in the art, including those known in the art and described herein.

The term "anti-OX40 antibody", "anti- OX40", " OX40 antibody" or "antibody binding to OX40" as used herein refers to an antibody which is capable of binding to (human or Cynomolgus)OX40 protein or a fragment thereof with sufficient affinity such that the antibody can be used as diagnostic and/or therapeutic agent targeting (human or Cynomolgus)OX40. In one embodiment, the anti-OX40 antibody binds to non-(human or Cynomolgus)OX40 protein to an extent lesser than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80% or about 90% or above of the binding of the antibody to (human or Cynomolgus)OX40, as measured, for example, by radioimmunoassay (RIA) or Bio-light interferometry or MSD assay. In some embodiments, the anti-OX40 antibody binds to human or Cynomolgus OX40 with an equilibrium dissociation constant (K_D) of of equilibrium , $\leq$ of equilibrium dissociation 10^{-7} M below, e.g., 10^{-7} M to 10^{-10} M, e.g., 10^{-8} M to 10^{-9} M).

As used herein, "monoclonal antibody" or "mAb" refers to a single copy or cloned antibody derived from, for example, a eukaryotic, a prokaryotic, or a phage clone, while does not refer to a method of producing the same. Monoclonal antibodies or antigen-binding fragments thereof can be produced, for example, by hybridoma technology, recombinant technique, phage display technique, synthetic technique such as CDR grafting, or a combination of such or other techniques known in the art.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises

a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); single domain antibody; and multispecific antibodies formed from antibody fragments.

As used herein, the term "epitope" refers to a portion of an antigen (e.g., OX40) that specifically interacts with an antibody molecule. This portion (referred to herein as an epitope determinant) typically comprises an element such as an amino acid side chain or a sugar side chain or a component thereof. Epitope determinants can be defined according to methods known in the art or disclosed herein (e.g., by crystallography or by hydrogen- deuterium exchange). At least one or some portion of the antibody molecule that specifically interacts with an epitope determinant is generally located within the CDR. Typically, epitopes have specific three dimensional structural characteristics. Typically, epitopes have specific charge characteristics. Some epitopes are linear epitopes, while others are conformational epitopes.

An "antibody that binds to the same or overlapping epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

An antibody that competes with a reference antibody for binding to its antigen refers to an antibody that blocks 50%, 60%, 70%, 80%, 90% or 95% or more of the binding of the reference antibody to its antigen in a competition assay. In other words, the reference antibody blocks 50%, 60%, 70%, 80%, 90% or 95% or more of the binding of the antibody to its antigen in a competition assay. Numerous types of competitive binding assays can be used to determine whether an antigen binding protein competes with another assay such as solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), Sandwich competition assays (see, e.g., Stahli et al, 1983, Methods in Enzymology 9: 242-253).

An antibody that inhibits (e.g., competitively inhibits) binding of a reference antibody to its antigen refers to an antibody that inhibits binding of 50%, 60%, 70%, 80%, 90%, or 95% or more of the reference antibody to its antigen. . Conversely, the reference antibody inhibits binding of the antibody to its antigen by 50%, 60%, 70%, 80%, 90% or 95% or more. The binding of an antibody to its antigen can be measured by affinity (eg, equilibrium dissociation

constant). Methods for determining affinity are known in the art.

An antibody that exhibits the same or similar binding affinity and/or specificity as a reference antibody refers to an antibody that has at least 50%, 60%, 70%, 80%, 90% or 95% or more of the binding affinity and/or specificity of the reference antibody. This can be determined by any method known in the art for determining binding affinity and/or specificity.

There are five major classes of antibodies known in the art: IgA, IgD, IgE, IgG and IgM, and several of these antibodies can be further divided into subclasses (isotypes), for example, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂. The heavy chain constant domains corresponding to different classes of immunoglobulins are referred to as α , δ , ϵ , γ , and μ , respectively. A person skilled in the art can select and obtain the antibody in an appropriate class of the present invention according to the practical desire.

"Antibody in IgG form" refers to the IgG form to which the heavy chain constant region of an antibody belongs. The heavy chain constant regions of all antibodies of the same type are identical, and the heavy chain constant regions differ between different types of antibodies. For example, an antibody in the IgG1 form refers to an Ig domain whose heavy chain constant region Ig domain is IgG1.

The term "dysfunction" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term "dysfunctional", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into downstream T-cell effector functions, such as proliferation, cytokine production (e.g., gamma interferon) and/or target cell killing.

The expression "lower blocking of human OX40 binding to OX40 ligand/OX40L" as used herein means that the antibody of the present invention block the binding of OX40 to OX40L with less extent, compared to the known OX40 antibody (e.g., pogalizumab) or OX40L. In some embodiments, the extent of binding of OX40 to OX40L is reduced less in the presence of an anti-OX40 antibody of the invention compared to the extent of binding of OX40 to OX40L in the presence of a known OX40 antibody (e.g., pogalizumab) or OX40L. In some embodiments, the reduction in the extent of binding of OX40 to OX40L in the presence of an anti-OX40 antibody of the invention is less than about 50%, 40%, 30%, 20%, 10% or less compared to the extent of binding of OX40 to OX40L in the presence of a negative control (e.g., an IgG antibody). In some embodiments, the extent of binding of OX40 to OX40L is determined by a luciferase reporter gene. In some embodiments, the extent of binding of OX40

to OX40L is determined by flow cytometry.

"Activating T cell " means to induce, cause or stimulate an effector or memory T cell to have a renewed, sustained or amplified biological function. Examples of enhancing T-cell function include: increased secretion of γ -interferon(e.g., IFNg) or interleukin(e.g., IL-2) from CD8⁺ effector T cells, increased secretion of γ -interferon(e.g., IFNg) or interleukin(e.g., IL-2) from CD4⁺ memory and/or effector T-cells, increased proliferation of CD4⁺ effector and/or memory T cells, increased proliferation of CD8⁺ effector T-cells, increased antigen responsiveness (e.g. , clearance), relative to such levels before the intervention. In one embodiment, the level of enhancement is at least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160 folds or more. The manner of measuring this enhancement is known to one of ordinary skill in the art.

"Tumor immunity" refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

"Immunogenicity" refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response.

An "agonist activity of an antibody," as used herein, is the activity with which an antibody can activate a biological activity of the antigen it binds.

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. An anti-angiogenic agent may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. In one embodiment, an anti-angiogenic agent is an antibody that binds to vascular endothelial growth factor (VEGF), such as bevacizumab (AVASTIN).

The term "PD-1 axis binding antagonist" refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis - with a result being to restore or enhance T-cell function (e.g., proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist(e.g., PD-1

antibody), a PD-L1 binding antagonist(e.g., PD-L1 antibody) and a PD-L2 binding antagonist(e.g., PD-L2 antibody).

The term "PD-1 binding antagonists" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is, as disclosed in WO2015/095423, MDX-1106(nivolumab), MK-3475(pembrolizumab), CT-011(pidilizumab) or AMP-224.

The term "PD-L1 binding antagonists" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is, as disclosed in WO2015/095423, YW243.55.S70, MDX-1105, MPDL3280A or MEDI4736

The term "PD-L2 binding antagonists" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted immunoglobulin bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. NK cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or U.S. Patent No. 6,737,056 (Presta), may be performed. Useful effector cells for such assays include PBMC and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652-656 (1998). An exemplary assay for assessing ADCC activity is provided in the examples herein.

As used herein, the term "OX40" refers to any natural OX40 from any vertebrate source, including mammals such as primates (eg, humans, cynomolgus monkeys) and rodents (eg, mice and rats), unless otherwise specified. The term encompasses "full length", unprocessed OX40 and any form of OX40 due to processing in the cell. The term also encompasses naturally occurring variants of OX40, such as splice variants or allelic variants.

"OX40 activation" refers to the activation of the OX40 receptor. Typically, OX40 activation results in signal transduction.

The term "cytotoxic agent" as used in the present invention refers to a substance which inhibits or prevents cell function and/or causes cell death or destruction. For examples of cytotoxic agents, see those disclosed in WO 2015/153513.

"Chemotherapeutic agents" include chemical compounds that are useful in the treatment of cancer. For examples of chemotherapeutic agents, see those disclosed in WO 2015/153513, including erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, alkylating agents, such as thiotepa; alkyl sulfonates; aziridines; and the like; and pharmaceutically acceptable salts, acids, or derivatives of any of the above. Chemotherapeutic agents also include dexamethasone, hydrocortisone, interferon, bevacuzimab, bexarotene, and the like, and pharmaceutically acceptable salts thereof. Chemotherapeutic agents also include non-steroidal anti-inflammatory drugs with analgesic, antipyretic and anti-inflammatory effects. For a detailed illustration and introduction of various chemotherapeutic agents that can be used in the present invention, reference is made to those disclosed in PCT International Application WO2015/153513, which is incorporated herein by reference in its entirety.

The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL) and gamma interferon. As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence cytokines, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "diabody" refers to an antibody fragment having two antigen binding sites, and said antibody fragment comprises a heavy chain variable domain (VH) linked to a light chain variable domain (VL) in the same polypeptide chain (VH-VL). The domains are forced to pair with the complementary domains on the other chain to create two antigen binding sites, by using a linker that is too short to make the two domains on the same chain be paired with each other. A diabody can be bivalent or bispecific. Diabodies are more fully described, for example, in EP 404,097; WO 1993/01161; Hudson et al, Nat. Med. 9: 129-134 (2003); and Hollinger et

al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al, Nat. Med. 9: 129-134 (2003).

A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include Clq binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays as disclosed herein.

"Effector function" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotopes. Examples of antibody effector's functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

"Human effector cells" refer to leukocytes that express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells, and neutrophils. The effector cells may be isolated from a native source, e.g., from blood.

The term "effective amount" refers to an amount or dose of an antibody or fragment of the invention that produces the desired effect in a patient to be treated, when administered to the patient in single or multiple doses. An effective amount can be readily determined by the attending physician as a person skilled in the art by considering various factors such as the species of the mammal; its size, age and general health; the particular disease involved; the extent or severity of the disease; the response of an individual patient; the specific antibody to be administered; mode of administration; bioavailability characteristics of the formulation to be administered; selected dosing regimen; and use of any concomitant therapy.

"Antibodies and antigen-binding fragments thereof" suitable for use in the present invention include, but are not limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, recombinant, heterologous, heterozygous, chimeric, humanized (especially grafted with CDRs), deimmunized, or human antibody, Fab fragments, Fab' fragments, F(ab')₂ fragments, fragments produced by Fab expression library, Fd, Fv, disulfide-linked Fv (dsFv), single-chain antibody (e.g., scFv), diabody or tetrabody (Holliger P. et al. (1993) Proc. Natl. Acad. Sci. USA 90 (14), 6444-6448), nanobody (also referred to as

a single domain antibody), anti-idiotypic (anti-Id) antibody (including, for example, an anti-Id antibody against the antibody of the invention), and epitope-binding fragments of any of the above.

The term "Fc region" is used herein to define a C-terminal region of an immunoglobulin heavy chain, and the Fc region comprises at least a portion of the constant region. The term includes native Fc region sequence and Fc region variants. In certain embodiments, the human IgG heavy chain Fc region extends from Cys226 or Pro230 to the carbonyl terminus of the heavy chain. However, the C-terminal lysine (Lys447) of Fc region may or may not be present. Unless otherwise indicated, the amino acid residues in Fc region or constant region are numbered according to the EU numbering system, which is also referred to as the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementary determinant region (CDR). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind to a particular antigen may be isolated using a VH or VL domain from an antibody that binds to the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) (e.g., complementarity determining region) residues. The FR of a variable domain typically consists of four FR domains: FR1, FR2, FR3 and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in the heavy chain variable domain (VH) (or the light chain variable domain (VL)): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

A "complementarity determining region" or "CDR region" or "CDR" is an area that is hypervariable in sequence in an antibody variable domain and that forms a structurally defined loop ("hypervariable loop") and/or contains an antigen contact residue ("antigen contact point"). The CDR is primarily responsible for binding to an epitope. The CDRs of the heavy and light chains are commonly referred to as CDR1, CDR2 and CDR3, numbered sequentially from the

N-terminus. The CDRs located within the antibody heavy chain variable domain are referred to as HCDR1, HCDR2 and HCDR3, while the CDRs located within the antibody light chain variable domain are referred to as LCDR1, LCDR2 and LCDR3. In a given light chain variable region or heavy chain variable region amino acid sequence, the exact amino acid sequence boundaries of each CDR can be determined using any one or combination of a number of well-known antibody CDR assignment systems, including for example: Chothia based on the three-dimensional structure of antibodies and the topology of CDR loops (Chothia et al. (1989) Nature 342: 877-883, Al-Lazikani et al., "Standard conformations for the canonical structures of immunoglobulins", Journal of Molecular Biology, 273, 927-948 (1997)), Kabat based on antibody sequence variability (Kabat et al, Sequences of Proteins of Immunological Interest, 4th edition, US Department of Health and Human Services, National Institutes of Health (1987)) , AbM (University of Bath), Contact (University College London), International ImMunoGeneTics database (IMGT) (imgt.cines.fr/ on the World Wide Web), and North CDR definition based on affinity propagation clustering using a large number of crystal structures. The boundaries of CDRs of the antibodies of the invention can be determined by one of skill in the art in accordance with any aspect of the art, such as different assignment systems or combinations.

However, it should be noted that the boundaries of the CDRs of the variable regions of the same antibody obtained based on different assignment systems may vary. That is, the CDR sequences of the same antibody variable region defined under different assignment systems are different. Thus, where an antibody is defined by a particular CDR sequence as defined by the present invention, the scope of the antibody also encompasses an antibody whose variable region sequence comprises the particular CDR sequence, but due to the application of a different protocol (eg different assignment systems or combinations) have different claimed CDR boundaries than the specific CDR boundaries defined by the present invention.

Antibodies with different specificities (i.e., different binding sites for different antigens) have different CDRs. However, although the CDRs differ among antibodies, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. Using at least two of the Kabat, Chothia, AbM, Contact, and North methods, the minimal overlapping region can be determined to provide a "minimum binding unit" for antigen binding. The minimum binding unit can be a sub-portion of the CDR. As will be apparent to those skilled in the art, residues of the remaining part of the CDR sequences can be determined by the structure of the antibody and protein folding. Accordingly, the invention also contemplates

variants of any of the CDRs presented herein. For example, in a variant of one CDR, the amino acid residues of the minimum binding unit may remain unchanged, while the other CDR residues defined by Kabat or Chothia may be replaced by conservative amino acid residues.

The terms "host cell" refers to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells", which include the primary transformed cell and progeny derived therefrom regardless of the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subtype of variable domain sequences. Generally, the subtype of the sequences is a subtype as disclosed in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In an embodiment, for the VL, the subtype is subtype kappa I as in Kabat et al., *supra*. In some embodiments, for the VH, the subtype is subtype III as in Kabat et al., *supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer

include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer or colon cancer, including metastatic forms of those cancers.

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

The term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre -cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

An "immunoconjugate" is an antibody conjugated to one or more other agents, including but not limited to a cytotoxic agent.

An "individual" or "subject" includes a mammal. Mammals include, but are not limited to, domestic animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments of the present invention, the individual or subject is a human.

An "isolated antibody" is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity, for example, as determined by, e.g., electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatography (e.g., ion exchange or reverse phase HPLC). For a review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).

An "isolated nucleic acid" refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present outside the chromosomes or at a chromosomal location that is different from its natural chromosomal location.

An "isolated nucleic acid encoding an anti-OX40 antibody or fragment thereof" refers to one or more nucleic acid molecules encoding the heavy and light chain of an antibody or antigen-binding fragment thereof, including such nucleic acid molecules contained in a single vector or in separate vectors, as well as such nucleic acid molecules present at one or more locations within a host cell.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithm needed to achieve maximal alignment over the full length of the sequences being compared.

When percentages of sequence identity are referred to in this application, these percentages are calculated relative to the full length of the longer sequence, unless otherwise specifically indicated. The calculation relative to the full length of the longer sequence applies to both the nucleic acid sequence and the polypeptide sequence.

The term "pharmaceutical composition" refers to a formulation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and

which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

The term "pharmaceutically acceptable adjuvants" refers to a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete)), excipient or vehicle co-administered with the therapeutic agent.

As used herein, "treating" refers to slowing, interrupting, arresting, ameliorating, stopping, reducing, or reversing the progression or severity of an existing symptom, condition, disorder, or disease.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

By "subject/patient sample" is meant a collection of cells or fluids obtained from a cancer patient or cancer subject. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebrospinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like. Examples of tumor samples herein include, but are not limited to, tumor biopsy, fine needle aspirate, bronchiolar lavage, pleural fluid, sputum, urine, a surgical specimen, circulating tumor cells, serum, plasma, circulating plasma proteins, ascitic fluid, primary cell cultures or cell lines derived from tumors or exhibiting tumor-like properties, as well as preserved tumor samples, such as formalin-fixed, paraffin-embedded tumor samples or frozen tumor samples.

The "instructions" contained in a kit or article as defined herein are used to refer to instructions generally included in commercial packages of therapeutic products containing information on indications, usage, dosage, administration, combination therapy, contraindications and/or warnings for applications involving such therapeutic products.

The antibody of the present invention

The invention thus provides anti-OX40 antibodies as well as fragments thereof.

In one embodiment of the invention, the amino acid changes described herein include substitutions, insertions or deletions of amino acids. Preferably, the amino acid changes described herein are amino acid substitutions, preferably conservative substitutions.

In a preferred embodiment, the amino acid changes described herein occur in regions outside the CDRs (e.g., in FR). More preferably, the amino acid changes described herein occur in regions outside the heavy chain variable region and/or outside the light chain variable region.

In some embodiments, the substitution is conservative substitution. Conservative substitution means that one amino acid is replaced by another amino acid within the same class, for example, one acidic amino acid is replaced by another acidic amino acid, one basic amino acid is replaced by another basic amino acid, or one neutral amino acid is replaced by another neutral amino acid. Exemplary substitutions are shown in Table D below:

Table D

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg

Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention encompasses an antibody or fragment thereof having a post-translational modification on a light chain variable region, a heavy chain variable region, a light chain or a heavy chain.

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed. In some applications, modifications that remove unwanted glycosylation sites may be useful, for example, modifications that remove fucose modules so as to enhance the antibody-dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277: 26733). In other applications, galactosylation modification may be performed to modify complement-dependent cytotoxicity (CDC).

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant, so as to enhance the efficiency of the antibody, for example, in the treatment of cancer or cell proliferation disease. The Fc region variant may comprise human Fc region sequence (e.g.,

human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues.

In certain embodiments, an antibody provided herein may be further modified to contain additional non-proteinous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly-amino acids (either homopolymers or random copolymers), and glucan or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof.

In some embodiments, the invention encompasses fragments of an anti-OX40 antibody. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabody, linear antibody, single chain antibody molecule (e.g., scFv); and multispecific antibody formed by antibody fragments. Two identical antigen-binding fragments produced by papain digestion on antibody are termed "Fab" fragments, in which each has a single antigen-binding site and a residual "Fc" fragment. Its name reflects the ability susceptible to crystallize. F(ab')₂ fragment is produced by pepsin treatment, and it has two antigen binding sites and is still capable of cross-linking antigen.

In some embodiments, an anti-OX40 antibody of the present invention is a humanized antibody. Different methods for humanizing antibodies are known to those skilled in the art, as reviewed by Almagro & Fransson, the contents of which are incorporated herein by reference in its entirety (Almagro JC and Fransson J (2008) *Frontiers in Bioscience* 13: 1619-1633).

In some embodiments, an anti-OX40 antibody of the invention is a human antibody. Human antibodies may be prepared using a variety of techniques known in the art. Human antibodies are generally described in van Dijk and van de Winkel, *Curr. Opin. Pharmacol* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol* 20: 450-459 (2008).

Antibodies of the present invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In some embodiments, the invention also encompasses an anti-OX40 monoclonal antibody conjugated to other agent, e.g., a therapeutic moiety, such as a cytotoxic agent or an immunosuppressive agent ("immunoconjugates"). Cytotoxic agents include any agent that is harmful to cells. Examples of cytotoxic agents (e.g., chemotherapeutic agents) suitable for forming immunoconjugates are known in the art, see for example WO05/103081. For example, cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleic acid hydrolase; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and various well-known antitumor or anticancer agents. In one embodiment, the cytotoxic agent is a PD-1 axis binding antagonist. In one embodiment, the cytotoxic agent is an antibody, such as an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody. In one embodiment, the cytotoxic agent is an anti-angiogenic agent, such as bevacizumab.

In some embodiments, an antibody of the invention may be monospecific, bispecific or multispecific. A multispecific monoclonal antibody may be specific for various epitopes of a target polypeptide or may contain antigen binding domains specific for more than one target polypeptides. See, for example, Tutt et al. (1991) *J. Immunol.* 147: 60-69. An anti-OX40 monoclonal antibody may be linked to or co-expressed with another functional molecule, such as another peptide or protein. For example, an antibody or fragments thereof may be

functionally linked to one or more other molecules, such as another antibody or antibody fragment(e.g., anti-PD-1 antibody, anti-PD-L1 antibody or anti-PD-L2 antibody or fragments of said antibodies) (e.g., by chemical coupling, genetic fusion, non-covalent association, or otherwise) to produce a bispecific or multispecific antibody with a second or more binding specificities.

The nucleic acid antibodies of the present invention and the host cell comprising the same

In one aspect, the present invention provides nucleic acids encoding any of the above anti-OX40 antibodies or fragments thereof. The nucleic acid may comprise a nucleic acid encoding an amino acid sequence comprising a light chain variable region and/or a heavy chain variable region of an antibody, or a nucleic acid encoding an amino acid sequence comprising a light and/or heavy chain of an antibody. An exemplary nucleic acid sequence encoding a heavy chain variable region of an antibody comprises a nucleic acid sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the nucleic acid sequence selected from SEQ ID NOs: 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105, or comprises the nucleic acid sequence selected from SEQ ID NOs: 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105. An exemplary nucleic acid sequence encoding a light chain variable region of an antibody comprises a nucleic acid sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the nucleic acid sequence selected from SEQ ID NOs: 121, 122, 123, 124, 125, 126, 127, 128 or 129, or comprises the nucleic acid sequence selected from SEQ ID NO: 121, 122, 123, 124, 125, 126, 127, 128 or 129.

In one embodiment, one or more vectors comprising the nucleic acid are provided. In one embodiment, the vector is an expression vector, for example, eukaryotic expression vectors. Vectors include, but are not limited to, viruses, plasmids, cosmids, lambda phage, or yeast artificial chromosomes (YAC). In a preferred embodiment, the expression vector of the invention is a pTT5 expression vector.

In one embodiment, a host cell comprising the vector is provided. Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523.

See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003, pp. 245-254, describing expression of antibody fragments in *E. coli*. After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is selected from the group consisting of a yeast cell, a mammalian cell, or other cells suitable for use in the preparation of an antibody or antigen-binding fragments thereof. For example, eukaryotic microorganisms such as filamentous fungi or yeast are cloning or expression hosts suitable for vectors encoding antibodies. For example, fungi and yeast strains, glycosylation pathways of which have been "humanized", result in the production of antibodies with partial or complete human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22: 1409-1414 (2004), and Li et al, *Nat. Biotech.* 24: 210-215 (2006). Host cells suitable for expression of glycosylated antibodies are also derived from multicellular organisms (invertebrates and vertebrates). Vertebrate cells can also be used as hosts. For example, mammalian cell lines which have been engineered to be suitable for suspension growth may be used. Other examples of useful mammalian host cell lines are the monkey kidney CV1 line (COS-7) transformed with SV40; human embryonic kidney line (293HEK or 293 cells, e.g., as described in such as Graham et al, *J. Gen Virol.* 36:59 (1977)), and so on. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al, *Proc. Natl. Acad. Sci. USA* 77: 216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for producing antibodies, see, for example, Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

In one embodiment, there is provided a method of preparing an anti-OX40 antibody, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody under conditions suitable for antibody expression, as provided above, and optionally recovering the antibody from the host cell (or host cell culture medium). To recombine to produce an anti-OX40 antibody, a nucleic acid encoding an antibody (e.g., an antibody as described above) is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids are readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes capable of specifically binding to genes encoding heavy and light chains of antibodies).

Determination method

The anti-OX40 antibodies provided herein can be identified, screened, or characterized for their physical/chemical properties and/or biological activity by a variety of assays known in the art. In one aspect, the antibody of the present invention is tested for its antigen binding activity, for example, by known methods such as ELISA, Western blot, and the like. Binding to OX40 can be determined using methods known in the art, and exemplary methods are disclosed herein. In some embodiments, Bio-light interferometry (eg, Fortebio affinity measurement) or MSD assay is used.

In another aspect, a competition assay can be used to identify antibodies that compete with any of the anti-OX40 antibodies disclosed herein for binding to OX40. In certain embodiments, such a competitive antibody binds to an epitope (eg, a linear or conformational epitope) that is identical or overlapping with an epitope to which any of the anti-OX40 antibodies disclosed herein bind. A detailed exemplary method for locating epitopes bound by antibodies is found in Morris (1996) "Epitope Mapping Protocols", Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).

The invention also provides assays for identifying biologically active anti-OX40 antibodies. Biological activity can include, for example, binding to OX40 (e.g., binding to human and/or cynomolgus OX40), increasing OX40-mediated signal transduction (e.g., increasing NFkB-mediated transcription), attenuating cells expressing human OX40 by ADCC, enhancing T Effector cell function (e.g., CD4+ effector T cells) (eg, by enhancing effector T cell proliferation and/or increasing cytokine production of effector T cells (e.g., gamma interferon or interleukin)), enhancing memory T cell function (e.g., CD4+ memory T cells) (eg, by increasing memory T cell proliferation and/or increasing cytokine production of memory T cells (eg, gamma interferon or interleukin)), binding to human effector cells. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, the antibodies of the invention are tested for such biological activity.

T cell activation can be assayed using methods known in the art. For example, it is determined by the level of a cytokine released after T cell activation, such as gamma interferon or interleukin. OX40 signaling can also be determined using methods well known in the art to determine activation of T cells. In one embodiment, a transgenic cell expressing human OX40 and a reporter gene comprising an NFkB promoter fused to a reporter gene (eg, beta luciferase)

is generated. Addition of anti-OX40 antibodies to cells results in elevated NFkB transcription, which is detected using an assay for the reporter gene (eg, a luciferase reporter assay).

Cells for use in any of the above in vitro assays include cells or cell lines that naturally express OX40 or that are engineered to express OX40. Such cells include activated T cells that naturally express OX40, Treg cells, and activated memory T cells. Such cells also include cell lines that express OX40 and cell lines that do not normally express OX40 but have been transfected with a nucleic acid encoding OX40.

It will be appreciated that any of the above assays can be performed by replacing or supplementing the anti-OX40 antibody with an immunoconjugate of the invention.

It will be appreciated that any of the above assays can be performed using an anti-OX40 antibody and other active agents.

Pharmaceutical compositions and pharmaceutical preparations

The invention also includes a composition (including pharmaceutical compositions or pharmaceutical preparations) comprising anti-OX40 antibody or immunoconjugate thereof and a composition comprising polynucleotides encoding the anti-OX40 antibody. In certain embodiments, the composition comprises one or more antibodies or fragments thereof that bind to OX40 or one or more polynucleotides encoding one or more antibodies or fragments thereof that bind to OX40. These compositions may also contain suitable pharmaceutically acceptable adjuvants, such as pharmaceutically acceptable carrier, pharmaceutically acceptable excipients known in the art, including buffers.

Pharmaceutically acceptable carriers suitable for use in the present invention may be sterile liquids such as water and oils, including those from petroleum, animal, plant or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solution and aqueous dextrose and glycerol solution can also be used as liquid carriers, especially used as injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glyceryl monostearate, talc, sodium chloride, dried skim milk, glycerin, propylene, glycol, water, ethanol, etc. For excipients and the uses thereof, see also "Handbook of Pharmaceutical Excipients", Fifth Edition, R. C. Rowe, P. J. Seskey and S. C. Owen, Pharmaceutical Press, London, Chicago. If

desired, the composition may also contain minor amounts of wetting or emulsifying agents, or pH buffer. These compositions may be in the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained release preparations and the like. Oral formulations may contain standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, saccharin.

A pharmaceutical formulation comprising an anti-OX40 antibody of the invention can be prepared by mixing the anti-OX40 antibody of the present invention having desired purity with one or more optional pharmaceutically acceptable adjuvants (Remington's Pharmaceutical Sciences, 16th Ed., Osol, A., ed. (1980)), preferably in the form of a lyophilized preparation or an aqueous solution.

Exemplary lyophilized antibody formulations are described in U.S. Patent No. 6,267,958. Aqueous antibody formulations include those described in U.S. Patent No. 6,171,586 and WO2006/044908, and the latter includes histidine-acetate buffer.

The pharmaceutical compositions or formulations of the present invention may also contain one or more active ingredient which is required for a particular indication to be treated, preferably those active ingredients which do not adversely affect each other's complementary activities. For example, it is desirable to further provide other anti-cancer active ingredients, such as chemotherapeutic agents, PD-1 axis binding antagonists (such as anti-PD-1 antibodies or anti-PD-L1 antibodies or anti-PD-L2 antibodies) or anti-angiogenic agents (such as bevacizumab). The active ingredient is suitably present in combination in an amount effective for the intended use.

Sustained release formulations can be prepared. Suitable examples of the sustained release formulations include semipermeable matrices of solid hydrophobic polymers containing antibodies, the matrices are in the form of shaped articles, such as films or microcapsules.

With regard to other components of the pharmaceutical formulation comprising the antibody of the invention, reference is also made to those disclosed in WO2015/153513.

Antibody treatment and use

In one aspect, the invention relates to a method of activating T cell or inducing T cell mediated antitumor activity or enhancing an immune response of the body in a subject

comprising administering to said subject an effective amount of any of the anti-OX40 antibody or fragment thereof, or immunoconjugates or pharmaceutical compositions comprising said antibody or fragments.

In another aspect, the invention relates to a method of treating tumor, e.g., cancer, in a subject comprising administering to said subject an effective amount of any anti-OX40 antibody or fragment thereof as described herein, or immunoconjugates or pharmaceutical compositions comprising said antibody or fragments. In one embodiment, the cancer is lung cancer (e.g., non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In another aspect, the invention relates to a method of causing antibody-dependent cell-mediated cytotoxicity in a subject comprising administering to said subject an effective amount of any anti-OX40 antibody or fragment thereof described herein, or immunoconjugates or pharmaceutical compositions comprising said antibody or fragments.

In another aspect, the invention relates to a method of treating or delaying various cancers, immune-related diseases, and T-cell dysfunction diseases in a subject, comprising administering to said subject an effective amount of any anti-OX40 antibody or fragment thereof described herein, or immunoconjugates or pharmaceutical compositions comprising said antibody or fragments.

In some embodiments, the methods described herein further comprise administering to the subject one or more therapies (e.g., a therapeutic manner and/or other therapeutic agent). In some embodiments, the treatment manner includes surgery and/or radiation therapy. In some embodiments, the other therapeutic agent is selected from the group consisting of a chemotherapeutic agent, a PD-1 axis binding antagonist (eg, an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody), or an anti-angiogenic agent (eg, bevacate) Bead monoclonal antibody).

In some embodiments, the subject or individual is a mammal, preferably a human.

In other aspects, the invention provides the use of an anti-OX40 antibody or fragment thereof in the manufacture or preparation of a medicament for the treatment of a related disease or condition mentioned above.

In some embodiments, an antibody or antibody fragment thereof of the invention may delay the onset of conditions and/or symptoms associated with the condition.

In some embodiments, the cancer suitable for the present invention is selected from the group consisting of lung cancer (eg, non-small cell lung cancer), liver cancer, gastric cancer, colon cancer, and the like.

In some embodiments, examples of cancer further include, but are not limited to, B-cell proliferative disorders, which further include, but are not limited to, lymphomas (e.g., B-Cell Non-Hodgkin's lymphomas (NHL)) and lymphocytic leukemias.

In some embodiments of any of the methods of the invention, the cancer as described herein displays human effector cells (e.g., is infiltrated by human effector cells). Methods for detecting human effector cells are well known in the art, including, e.g., by IHC. In some embodiments, the cancer display high levels of human effector cells. In some embodiments, human effector cells are one or more of NK cells, macrophages, monocytes.

In some embodiments of any of the methods of the invention, the cancer as described herein displays cells expressing FcR (e.g., is infiltrated by cells expressing FcR). Methods for detecting FcR are well known in the art, including, e.g., by IHC. In some embodiments, the cancer display high levels of cells expressing FcR. In some embodiments, FcR is Fc γ R. In some embodiments, FcR is activating Fc γ R. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast carcinoma (e.g. triple-negative breast cancer), gastric cancer, colorectal cancer (CRC), or hepatocellular carcinoma.

In some embodiments of any of the methods of the invention, administration of an anti-OX40 antibody or fragment thereof of the invention having agonist activity is combined with administration of a tumor antigen. In some embodiments, the tumor antigen comprises a protein. In some embodiments, the tumor antigen comprises a nucleic acid. In some embodiments, the tumor antigen is a tumor cell.

In certain embodiments, the methods and uses described herein further comprise administering to the subject one or more therapies(e.g., therapeutic manner and/or other therapeutic agents). Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent.

Such combination therapies encompass combined administration (for example, two or more therapeutic agents are included in the same or separate formulations), and separate

administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-OX40 antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other. Antibodies of the invention can also be used in combination with surgery or radiation therapy.

In some embodiments, the therapeutic manner includes surgery (e.g., tumor resection); radiation therapy (e.g., external particle beam therapy, which involves three-dimensional conformal radiation therapy in which the illumination region is designed), local irradiation (e.g., irradiation pointing to a pre-selected target or organs) or focused irradiation) and the like.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a chemotherapy or chemotherapeutic agent. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a radiotherapy or radiotherapy agent. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a targeted therapy or a targeted therapeutic agent. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an immunotherapy or immunotherapeutic agent, such as a monoclonal antibody.

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a PARP inhibitor (e.g., Olaparib, Rucaparib, Niraparib, Cediranib, BMN673, Veliparib)

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a PD-1 axis binding antagonist. A PD-1 axis binding antagonist includes but is not limited to a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. Alternative names for “PD-1” include CD279 and SLEB2. Alternative names for “PD-L1” include B7-H1, B7-4, CD274, and B7-H. Alternative names for “PD-L2” include B7-DC, Btde, and CD273. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand or binding partners. In a specific aspect the PD-1 ligand are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, the PD-L2 binding

antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab, OPDIVO), Merck 3475 (MK-3475, pembrolizumab, KEYTRUDA) and CT-011 (Pidilizumab). In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. In some embodiments, the PD-L1 binding antagonist is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 binding antagonist is selected from the group consisting of YW243.55.S70, MPDL3280A, MEDI4736 and MDX-1105. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. Antibody YW243.55.S70 (heavy and light chain variable region sequences shown in SEQ ID Nos. 20 and 21, respectively) is an anti-PD-L1 described in WO2010/077634 A1. MDX-1106, also known as MDX-1106-04, ONO-4538, BMS-936558 or nivolumab, is an anti-PD-1 antibody described in WO2006/121168. Merck 3475, also known as MK-3475, SCH-900475 or pembrolizumab, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342. In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for “MDX-1106” include MDX-1106-04, ONO-4538, BMS-936558 or nivolumab. In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4).

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an agonist directed against an activating co-stimulatory molecule. In some embodiments, an activating co-stimulatory molecule may include CD40, CD226, CD28, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, the agonist directed against an activating co-stimulatory molecule is an agonist antibody that binds to CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an antagonist directed against an inhibitory co-stimulatory molecule. In some

embodiments, an inhibitory co-stimulatory molecule may include CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase. In some embodiments, the antagonist directed against an inhibitory co-stimulatory molecule is an antagonist antibody that binds to CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3 (e.g., LAG-3-IgG fusion protein (IMP321)), B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a treatment comprising adoptive transfer of a T cell (e.g., a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR).

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an angiogenesis inhibitor. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention can be administered in combination with bevacizumab (also known as AVASTIN®, Genentech).

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an angiogenesis inhibitor and in combination with a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and MDX-1106 (nivolumab, OPDIVO). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and Merck 3475 (MK-3475, pembrolizumab, KEYTRUDA). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and CT-011 (Pidilizumab). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and YW243.55.S70. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and MPDL3280A. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination

with bevacizumab and MEDI4736. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with bevacizumab and MDX-1105.

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an anti-tumor agent. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an inhibitor of Bruton's tyrosine kinase (BTK).

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with obinutuzumab and a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody).

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a cancer vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., Cancer Sci, 104: 14-21, 2013).

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an adjuvant. In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a treatment comprising a TLR agonist, e.g., Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with a tumor necrosis factor (TNF) α . In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with interleukins (e.g., IL-1, IL-10, IL-4, IL-13, IL-17, etc.). In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with the treatment of targeting CXCL10. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with the treatment of targeting CCL5. In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an LFA-1 or ICAM1 agonist. In some embodiments, an anti-OX40 antibody or fragment thereof

of the invention may be administered in combination with a Selectin agonist.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an inhibitor of B-Raf.

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an inhibitor of B-Raf (e.g., vemurafenib or dabrafenib) and an inhibitor of MEK (e.g., MEK1 and/or MEK2), (e.g., cobimetinib or trametinib).

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an inhibitor of c-Met.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an agent that selectively degrades the estrogen receptor.

In some embodiments, the anti-OX40 antibody or fragment thereof of the invention may be administered in combination with radiation therapy.

In some embodiments, an anti-OX40 antibody or fragment thereof of the invention may be administered in combination with an oncolytic virus.

More therapies or therapeutic agents or pharmaceuticals or active ingredients that can be combined with the anti-OX40 antibody or fragment thereof of the invention are described in WO2015/153513.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies of the invention can also be used in combination with radiation therapy

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments.

Methods and compositions for diagnosis and detection

In certain embodiments, any anti-OX40 antibody or antigen-binding fragment thereof provided herein may be used to detect the presence of OX40 in a biological sample. The term "detection" is used herein to include quantitative or qualitative detection. In certain embodiments, the biological sample is another liquid sample of blood, serum, or biological origin. In certain embodiments, the biological sample comprises cells or tissues.

In one embodiment, an anti-OX40 antibody is provided for a diagnostic or detection method. In another aspect, a method is provided for detecting the presence of OX40 in a biological sample. In certain embodiments, the method comprises detecting the presence of the OX40 protein in a biological sample. In certain embodiments, the OX40 is human OX40. In certain embodiments, the method comprises contacting a biological sample with an anti-OX40 antibody as described herein under conditions that allow binding of the anti-OX40 antibody to OX40 and detecting whether a complex is formed between the anti-OX40 antibody and the OX40. The method may be *in vitro* or *in vivo*. In one embodiment, the anti-OX40 antibody is used to select a subject suitable for treatment with an anti-OX40 antibody, for example wherein the OX40 is a biomarker for selecting a patient.

In one embodiment, the antibody of the present invention may be used to diagnose cancer or tumors.

In certain embodiments, a labeled anti-OX40 antibody is provided. The marker includes, but is not limited to, directly detected markers or portions (such as fluorescent labels, chromophore labels, electronically dense labels, chemiluminescent labels and radiolabeled labels), as well as indirectly detected moieties such as enzymes or ligands, e.g., through enzymatic reactions or molecular interactions. Exemplary markers include, but are not limited

to, radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferase, for example, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), fluorescein, 2,3-dihydrophthalazinedione, horseradish peroxidase (HR), alkaline phosphatase, β -galactosidase, glucoamylase, lyase, carbohydrate oxidase, for example, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, as well as enzymes that oxidize dye precursors using hydrogen peroxide, such as HR, lactoperoxidase, microperoxidase, biotin/avidin, spin label, phage label, stable free radicals, and the like.

In one aspect, the present invention provides diagnostic methods, such as for identifying cancer patients who are likely to respond to anti-OX40 antibody therapy.

In some embodiments, provided is a method for identifying a patient likely to respond to an anti-OX40 antibody treatment or a method for diagnosing cancer, the method comprising (i) determining the presence or absence or amount(e.g., number of each given size of the samples) of FcR-expressing cells from a cancer sample of the patient; (ii) if the sample contains cells expressing FcR (e.g., a high number of cells expressing FcR), the patient is identified as being likely to respond, or diagnose said patient as having cancers comprising FcR(e.g., high FcR). Methods for detecting cells expressing FcR are well known in the art and include, for example, IHC. In some embodiments, FcR is Fc γ R. In some embodiments, FcR is activating Fc γ R. In some embodiments, the cancer is any of the cancers described herein. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast cancer (e.g., triple negative breast cancer), gastric cancer, colorectal cancer (CRC) or hepatocellular carcinoma. In some embodiments, the method is an *in vitro* method.

In some embodiments, provided is a method for identifying a patient likely to respond to an anti-OX40 antibody treatment or a method for diagnosing cancer, the method comprising (i) determining the presence or absence or amount(e.g., number of each given size of the samples) of human effector cells (e.g., invasive effector cells) from the patient's cancer sample, and (ii) if the sample comprises effector cells (e.g., a high number of effector cells), the patient is identified as being likely to respond, or diagnosed as having cancer comprising human effector cell. Methods for detecting invasive human effector cells are well known in the art and include, for example, IHC. In some embodiments, the human effector cells are one or more of NK cells, macrophages, monocytes. In some embodiments, the effector cells express the activating Fc γ R.

In some embodiments, the method is an *in vitro* method. In some embodiments, the cancer is any of the cancers described herein. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast cancer (e.g., triple negative breast cancer), gastric cancer, colorectal cancer (CRC) or hepatocellular carcinoma.

In some embodiments, a method is provided for recommending treatment to a cancer patient comprising the steps of a method for identifying a patient likely to respond to an anti-OX40 antibody treatment or a method for diagnosing cancer as described above, and (iii) when the sample has cells expressing FcR or has human effector cells, it is recommended an anti-OX40 antibody (e.g., anti-OX40 antibody or the fragment thereof of the present invention) treatment.

In some embodiments, a method is provided for treating a cancer patient comprising the steps a method for identifying a patient likely to respond to an anti-OX40 antibody treatment or a method for diagnosing cancer as described above, and (iii) when the sample has cells expressing FcR or has human effector cells, the patient is treated with an anti-OX40 antibody (e.g., an anti-OX40 antibody or the fragment thereof of the present invention).

In some embodiments of any invention provided herein, the sample is obtained prior to treatment with anti-OX40 antibody. In some embodiments, the sample is obtained prior to treatment with the cancer drug. In some embodiments, the sample is obtained after cancer metastasis. In some embodiments, the sample is fixed with formalin and coated with paraffin (FFPE). In some embodiments, the sample is a biopsy (e.g., a core biopsy), a surgical specimen (e.g., from a surgically resected specimen), or a fine needle aspirate.

Sequences of the exemplary anti-OX40 antibody of the present invention

Table 1: FR and CDR sequences of heavy chain variable domain (VH, or heavy chain variable region (HCVR)) of the exemplary antibody of the present invention

Antibody Name	optimized linkage	VH FR1	VH CDR1/HCDR1	VH FR2	VH CDR2/HCDR2	VH FR3	VH CDR3/HCDR3	VH FR4
ADI-20112	Parent antibody	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ ID NO:62)	FTFSSYGMH(SEQ ID NO:1)	WVRQAPGKGLE WVA(SEQ ID NO:183)	VISYDGSNKYYADSV KG(SEQ ID NO:17)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSAF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
ADI-20113	Parent antibody	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ ID NO:62)	FTFSSYGMH(SEQ ID NO:1)	WVRQAPGKGLE WVA(SEQ ID NO:183)	VISYDGSNKYYADSV KG(SEQ ID NO:17)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSAF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
ADI-25650	ADI-20112	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ ID NO:62)	FTFSVYNMH(SEQ ID NO:2)	WVRQAPGKGLE WVA(SEQ ID NO:183)	VIA YDGS AKYYADSV KG(SEQ ID NO:18)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSAF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
ADI-	ADI-	QVQLVESGGGVVQPGRS	FTFSSRNMH(SEQ ID NO:2)	WVRQAPGKGLE	VIA YDGS LKYYADSV	RFTISRDN SKN	ARGRPWY	WGQGT

25651	20112	LRLSCAASG(SEQ NO:62)	ID	ID NO:3)	WVA(SEQ NO:183)	ID	KG(SEQ ID NO:19)	TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	SETGTSF DI(SEQ ID NO:34)	MVTVS S(SEQ ID NO:86)
ADI- 25652	ADI- 20112	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ NO:62)	ID	FTFVSYNMMH(SE QID NO:4)	WVRQAPGKGLE WVA(SEQ NO:183)	ID	VIAVDGSVKYYADSV KG(SEQ ID NO:20)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
ADI- 25653	ADI- 20112	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ NO:62)	ID	FTFVSYNMMH(SE QID NO:4)	WVRQAPGKGLE WVA(SEQ NO:183)	ID	VIMYDGS AKYYADS VKG(SEQ ID NO:21)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
ADI- 25654	ADI- 20112	QVQLVESGGGVVQPGRS LRLSCAASG(SEQ NO:62)	ID	FTFRSYDMH(SE QID NO:5)	WVRQAPGKGLE WVA(SEQ NO:183)	ID	YIAVDGS NKYYADSV KG(SEQ ID NO:22)	RFTISRDN SKN TLYLQMNSLR AEDTAVYYC(S EQ ID NO:68)	ARGRPWY SETGTSF DI(SEQ ID NO:34)	WGQGT MVTVS S(SEQ ID NO:86)
consen sus sequeun				FTFX1X2X3X4M H(wherein X1 is selected from S, V,			X1IX2YDGSX3KY YA DSVKG(wherein X1 is selected from V,Y:X2 is			

ce			R; X2is selected from V,S; X3 is selected from Y,R; X4 is selected from G,N,D)(SEQ ID NO:6)		selected from S,A,M;X3 is selected from N,A,L,V)(SEQ ID NO:23)			
ADI-20078	Parent antibody	QVQLQESGPGGLVKPSQTL SLTCTVSG(SEQ ID NO:63)	GSISSGSYYWS(S EQ ID NO:7)	WIRQHPGKGLE WIG(SEQ ID NO:184)	YIYSGSTYYNPSLKS (SEQ ID NO:24)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SEQ ID NO:69)	ARDVGYP HYYGMD V(SEQ ID NO:35)	WGQGT TVTVSS (SEQ ID NO:87)
ADI-23515	ADI-20078	QVQLQESGPGGLVKPSQTL LSLTCTVSG(SEQ ID NO:63)	GSIRSGAYYWS(S SEQ ID NO:8)	WIRQHPGKGLE WIG(SEQ ID NO:184)	YIYYDGGQTYYNPSLK S(SEQ ID NO:25)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SEQ ID NO:69)	ARDVGYP HYYGMD V(SEQ ID NO:35)	WGQGT TVTVSS (SEQ ID NO:87)
ADI-23518	ADI-20078	QVQLQESGPGGLVKPSQTL LSLTCTVSG(SEQ ID NO:63)	GSISSGASYWS(S EQ ID NO:9)	WIRQHPGKGLE WIG(SEQ ID NO:184)	YIYSGETYYNPSLK S(SEQ ID NO:26)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SEQ ID NO:69)	ARDVGYP HYYGMD V(SEQ ID NO:35)	WGQGT TVTVSS (SEQ ID NO:87)
ADI-23519	ADI-20078	QVQLQESGPGGLVKPSQTL LSLTCTVSG(SEQ ID NO:63)	GSISSGSYYWS(S EQ ID NO:10)	WIRQHPGKGLE WIG(SEQ ID NO:184)	YIYMSGETYYNPSLK S(SEQ ID NO:27)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SEQ ID NO:69)	ARDVGYP HYYGMD V(SEQ ID NO:35)	WGQGT TVTVSS (SEQ ID NO:87)

consensus sequence			GSIX1SGX2X3Y WS(X1 is selected from S,R; X2 is selected from S,A; X3 is selected from Y,S)(SEQ ID NO:11)		YIYX1X2GX3TYYNPS LKS(其中 X1 is selected from Y,M; X2 is selected from D,S; X3 is selected from S,Q,E)(SEQ ID NO:28)				
ADI- 20048	Parent antibody	QVQLQESGPGLVKPSSETL SLTCTVSG(SEQ ID NO:64)	GSISYYWS(SEQ ID NO:12)	WIRQPPGKGGLEW IG(SEQ ID NO:185)	YIYSSGSTNYNPSLKS (SEQ ID NO:29)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SE Q ID NO:69)	ARDAPGG SSYQDYY MDV(SEQ ID NO:36)	WGKGT TVTSS (SEQ ID NO:88)	
ADI- 20096	Parent antibody	EVQLVESGGGLVKGPGS LRLSCAASG(SEQ ID NO:65)	FTFSSYSMN(SEQ ID NO:13)	WVRQAPGKGGLE WVS(SEQ ID NO:186)	SISSSNYYIYADSVK G(SEQ ID NO:30)	RFTISRDNAKN SLYLQMNSLR AEDTAVYYC(S EQ ID NO:70)	ARGPLG YSWEYFD L(SEQ ID NO:37)	WGRGT LVTSS (SEQ ID NO:89)	
ADI- 20051	Parent antibody	QVQLQQWGAGLLKPSET LSLTCAVYG(SEQ ID NO:66)	GSFSGYYWS(SE Q ID NO:14)	WIRQPPGKGGLEW IG(SEQ ID NO:185)	EIDHSGSTNYNPSLKS (SEQ ID NO:31)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SE Q ID NO:69)	ARDGGGG YASPFDY(SEQ ID NO:38)	WGQGT LVTSS (SEQ ID NO:90)	
ADI- 20065	Parent antibody	QLQLQESGPGLVKPSSETL SLTCTVSG(SEQ ID	GSISSSYYWG(S EQ ID NO:15)	WIRQPPGKGGLEW IG(SEQ ID	SIYSSGSTNYNPSLKS (SEQ ID NO:32)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SE	ARDPGYS ASPNWFD P(SEQ ID	WGQGT LVTSS (SEQ ID	

		NO:188)		NO:185)		Q ID NO:69)	NO:39)	NO:90)
ADI-20066	Parent antibody	QLQLQESGPGGLVKPSETL SLTCTVSG(SEQ ID NO:188)	GSISSSSYW(G(S EQ ID NO:15)	WIRQPPGKGLEW IG(SEQ ID NO:185)	SIYYSGSTYYNP SLKS (SEQ ID NO:32)	RVTISVDTSKN QFSLKLSSVTA ADTAVYYC(SEQ ID NO:69)	ARDPGYS ASPNWFD P(SEQ ID NO:39)	WGQGT LVTVSS (SEQ ID NO:90)
ADI-20118	Parent antibody	QVQLVQSGAEVKKPGAS VKVSCKASG(SEQ ID NO:67)	YTFTSYAIS(SEQ ID NO:16)	WVRQAPGQGLE WMG(SEQ ID NO:187)	WISAYNGNTNYAQKL QG(SEQ ID NO:33)	RVTMTTDTSTS TAYMELRSLRS DDTAVYYC(SEQ ID NO:71)	ARSGGS GPNWFD P(SEQ ID NO:40)	WGQGT LVTVSS (SEQ ID NO:90)

Table 2: FR and CDR sequences of light chain variable domain(VL) (or light variable region(LCVR)) of the exemplary antibody of the present invention

Antibody Name	optimized linkage	VL FR1	VL	VL FR2	VL	VL FR3	VL	VL FR4
			CDR1/LCDR1		CDR2/LCDR2		CDR3/LCDR3	
ADI-20066	Parent	DIQMTQSPSSLSASV GDRVITTC(SEQ ID NO:188)	QASQDISNYL N(SEQ ID NO:188)	WYQQKPGKAP KLLIY(SEQ ID NO:185)	DASNLET (SEQ ID NO:185)	GVPSRFSGSGSGTDFTFTI SSLQPEDIATYYC(SEQ ID NO:32)	QQSDHYPT (SEQ ID NO:39)	FGGGTKVEI (SEQ ID NO:90)

201	anti	NO:72)	NO:41)	NO:77)	NO:47)	NO:80)	NO:53)	NO:85)
12	bod							
	y							
AD	Pare	EIVMTQSPATLSVSP	RASQSVSSNL	WYQQKPGKAP	SASTRAT(	GIPARFSGSGSGTEFTLTIS	QQHNVYPPY	FGGGTKVEI
I-	nt	GERATLSC(SEQ ID	A(SEQ ID	RLLIY(SEQ ID	SEQ ID	SLQSEDFAVYYC(SEQ ID	T(SEQ ID	K(SEQ ID
201	anti	NO:73)	NO:42)	NO:78)	NO:48)	NO:81)	NO:54)	NO:85)
13	bod							
	y							
AD	ADI	DIQMTQSPSSLSASV	QASQDISNYL	WYQQKPGKAP	DASNLET(	GVPSRFSGSGSGTDFTFTI	QQSDHYPT(	FGGGTKVEI
I-	-	GDRVITTC(SEQ ID	N(SEQ ID	KLLIY(SEQ ID	SEQ ID	SSLQPEDIATYYC(SEQ ID	SEQ ID	K(SEQ ID
256	201	NO:72)	NO:41)	NO:77)	NO:47)	NO:80)	NO:53)	NO:85)
50	12							
AD	ADI	DIQMTQSPSSLSASV	QASQDISNYL	WYQQKPGKAP	DASNLET(	GVPSRFSGSGSGTDFTFTI	QQSDHYPT(	FGGGTKVEI
I-	-	GDRVITTC(SEQ ID	N(SEQ ID	KLLIY(SEQ ID	SEQ ID	SSLQPEDIATYYC(SEQ ID	SEQ ID	K(SEQ ID
256	201	NO:72)	NO:41)	NO:77)	NO:47)	NO:80)	NO:53)	NO:85)
51	12							
AD	ADI	DIQMTQSPSSLSASV	QASQDISNYL	WYQQKPGKAP	DASNLET(	GVPSRFSGSGSGTDFTFTI	QQSDHYPT(	FGGGTKVEI

I- 256 52	- 201 12	GDRV/TITC(SEQ NO:72)	ID	N(SEQ NO:41)	ID	KLLIY(SEQ NO:77)	ID	SEQ NO:47)	SSLQPEDIATYYC(SEQ NO:80)	ID	SEQ NO:53)	ID	K(SEQ NO:85)	ID
AD I- 256 53	ADI - 201 12	DIQMTQSPSSLSASV GDRV/TITC(SEQ NO:72)	ID	QASQDISNYL N(SEQ NO:41)	ID	WYQQKPGKAP KLLIY(SEQ NO:77)	ID	DASNLET(SEQ NO:47)	GVPSRFSGSGSGTDFTFTI SSLQPEDIATYYC(SEQ NO:80)	ID	QQSDHYPT(SEQ NO:53)	ID	FGGGTKVEI K(SEQ NO:85)	ID
AD I- 256 54	ADI - 201 12	DIQMTQSPSSLSASV GDRV/TITC(SEQ NO:72)	ID	QASQDISNYL N(SEQ NO:41)	ID	WYQQKPGKAP KLLIY(SEQ NO:77)	ID	DASNLET(SEQ NO:47)	GVPSRFSGSGSGTDFTFTI SSLQPEDIATYYC(SEQ NO:80)	ID	QQSDHYPT(SEQ NO:53)	ID	FGGGTKVEI K(SEQ NO:85)	ID
AD I- 200 78	Pare nt anti bod	EIVLTQSPGTLSSLSPG ERATLSC(SEQ NO:74)	ID	RASQSVSSSY LA(SEQ NO:43)	ID	WYQQKPGQAP RLLIY(SEQ NO:78)	ID	GASSRAT(SEQ NO:49)	GIPDRFSGSGSGTDFTLTIS RLEPEDFAVYYC(SEQ NO:82)	ID	QQSERSPFT(SEQ NO:55)	ID	FGGGTKVEI K(SEQ NO:85)	ID

	y											
AD	ADI	EIVLTQSPGTLSSLSPG	RASQSVSSSY	WYQQKPGQAP	GASSRAT(	GIPDRFSGSGGTDFTLTIS	QQSERSPFT(	FGGGTKVEI				
I-	-	ERATLSC(SEQ ID	LA(SEQ ID	RLLIY(SEQ ID	SEQ ID	RLEPEDFAVYYC(SEQ ID	SEQ ID	K(SEQ ID				
235	200	NO:74)	NO:43)	NO:78)	NO:49)	NO:82)	NO:55)	NO:85)				
15	78											
AD	ADI	EIVLTQSPGTLSSLSPG	RASQSVSSSY	WYQQKPGQAP	GASSRAT(	GIPDRFSGSGGTDFTLTIS	QQSERSPFT(	FGGGTKVEI				
I-	-	ERATLSC(SEQ ID	LA(SEQ ID	RLLIY(SEQ ID	SEQ ID	RLEPEDFAVYYC(SEQ ID	SEQ ID	K(SEQ ID				
235	200	NO:74)	NO:43)	NO:78)	NO:49)	NO:82)	NO:55)	NO:85)				
18	78											
AD	ADI	EIVLTQSPGTLSSLSPG	RASQSVSSSY	WYQQKPGQAP	GASSRAT(	GIPDRFSGSGGTDFTLTIS	QQSERSPFT(	FGGGTKVEI				
I-	-	ERATLSC(SEQ ID	LA(SEQ ID	RLLIY(SEQ ID	SEQ ID	RLEPEDFAVYYC(SEQ ID	SEQ ID	K(SEQ ID				
235	200	NO:74)	NO:43)	NO:78)	NO:49)	NO:82)	NO:55)	NO:85)				
19	78											
AD	Pare	EIVLTQSPATLSSLSPG	RASQSVSSYL	WFQQKPGQAPR	DASNRAT(	GIPARFSGSGGTDFTLTIS	QQAFSMPPT	FGGGTKVEI				
I-	nt	ERATLSC(SEQ ID	A(SEQ ID	LLIY(SEQ ID	SEQ ID	SLEPEDFAVYYC(SEQ ID	(SEQ ID	K(SEQ ID				

200 48	anti bod y	NO:75)	NO:44)	NO:79)	NO:50)	NO:83)	NO:56)	NO:85)
AD I- 200 96	Pare nt anti bod y	DIQMTQSPSTLSASV GDRVITITC(SEQ ID NO:76)	RASQSISSWL A(SEQ ID NO:45)	WYQQKPGKAP KLLIY(SEQ ID NO:77)	DASSLES(SEQ ID NO:51)	GVPSRFSGSGSGTEFTLTIS SLQPDDFATYYC(SEQ ID NO:84)	QQFQSYSYT (SEQ ID NO:57)	FGGGTKVEI K(SEQ ID NO:85)
AD I- 200 51	Pare nt anti bod y	EIVMTQSPATLSVSP GERATLSC(SEQ ID NO:73)	RASQSVSSNL A(SEQ ID NO:42)	WYQQKPGQAP RLLIY(SEQ ID NO:78)	GASTRAT(SEQ ID NO:52)	GIPARFSGSGSGTEFTLTIS SLQSEDFAVYYC(SEQ ID NO:81)	QQYHAWPPT (SEQ ID NO:58)	FGGGTKVEI K(SEQ ID NO:85)
AD I- 200 65	Pare nt anti bod	EIVLTQSPGTLSLSPG ERATLSC(SEQ ID NO:74)	RASQSVSSSY LA(SEQ ID NO:43)	WYQQKPGQAP RLLIY(SEQ ID NO:78)	GASSRAT(SEQ ID NO:49)	GIPDRFSGSGSGTDFTLTIS RLEPEDFAVYYC(SEQ ID NO:82)	QQFDSSPT(S EQID NO:59)	FGGGTKVEI K(SEQ ID NO:85)

	y										
AD	Pare	EIVLTQSPGTLSSLSPG	RASQSVSSSF	WYQQKPGQAP	GASSRAT(	GIPDRFSGSGGTDFLTIS	QQYDVFPIT(	FGGGTKVEI			
I-	nt	ERATLSC(SEQ ID	LA(SEQ ID	RLLIY(SEQ ID	SEQ ID	RLEPEDFAVYYC(SEQ ID	SEQ ID	K(SEQ ID			
200	anti	NO:74)	NO:46)	NO:78)	NO:49)	NO:82)	NO:60)	NO:85)			
66	bod										
	y										
AD	Pare	DIQMTQSPSSLSASV	QASQDISNYL	WYQQKPGKAP	DASNLET(	GVPSRFSGSGGTDFFTI	QQPATLPWT(	FGGGTKVEI			
I-	nt	GDRVITITC(SEQ ID	N(SEQ ID	KLLIY(SEQ ID	SEQ ID	SSLQPEDIATYYC(SEQ ID	SEQ ID	K(SEQ ID			
201	anti	NO:72)	NO:41)	NO:77)	NO:47)	NO:80)	NO:61)	NO:85)			
18	bod										
	y										

Table 3: DNA and amino acid sequences of heavy chain variable domain (VH)(or heavy chain variable region (HCVR)) of the exemplary antibody of the present invention

Antibody Name	optimize d linkage	VH DNA sequence	VH amino acid sequence
ADI-20112	Parent antibody	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCAACCTTCAGTAGCTATGGCATGCACTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCGTATGATGGAAGT AATAAATACTATGCAGACTCCGTGAAGGGCCGATTCAACCATCTCCAGAGACAATTCC AAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACACGGCGGTGT ACTACTGCGCCAGAGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTCGAC ATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCA(SEQ ID NO:91)	QVQLVESGGGVVQPGRSLRLSCAASGFT FSSYGMHWVRQAPGKGLEWVAVISYDG SNKYYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCARGRPWYSETGTSF DIWGQGTMTVSS(SEQ ID NO:106)
ADI-20113	Parent antibody	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCAACCTTCAGTAGCTATGGCATGCACTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCGTATGATGGAAGT AATAAATACTATGCAGACTCCGTGAAGGGCCGATTCAACCATCTCCAGAGACAATTCC AAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACACGGCGGTGT ACTACTGCGCCAGAGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTCGAC ATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCA(SEQ ID NO:91)	QVQLVESGGGVVQPGRSLRLSCAASGFT FSSYGMHWVRQAPGKGLEWVAVISYDG SNKYYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCARGRPWYSETGTSF DIWGQGTMTVSS(SEQ ID NO:106)

ADI-25650	ADI-20112	CAGGTGCAGCTGGTGGAGTCTGCGGGAGGCGTGGTCCAGCCTGGGAGGTCCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCACTTTCAGTGTGTATAATATGCACCTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATAGCGTATGATGGAAG TGCGAATACTATGCAGACTCCGTGAAGGGCCGATTCAACATCTCCAGAGACAATT CCAAAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAGACACGGCGGT GTACTACTGCGCCAGAGGCCGCTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTTCG ACATATGGGGTCAGGGTACAATGGTCAACCGTCTCCTCA(SEQ ID NO:92)	QVQLVESGGGVVQPGRSLRLSCAASGFT FSVYNMHWVRQAPGKGLEWVAVIAYD GSAKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARGRPWYSETGTS AFDIWGQGTMTVTVSS(SEQ ID NO:107)
ADI-25651	ADI-20112	CAGGTGCAGCTGGTGGAGTCTGCGGGAGGCGTGGTCCAGCCTGGGAGGTCCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCACTTTCAGTAGCCGGAATATGCACCTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATAGCGTATGATGGAAG TCTTAAATACTATGCAGACTCCGTGAAGGGCCGATTCAACCATCTCCAGAGACAATTC CAAGAAACACGCTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCGGTG TACTACTGCGCCAGAGGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTTCGA CATATGGGGTCAGGGTACAATGGTCAACCGTCTCCTCA(SEQ ID NO:93)	QVQLVESGGGVVQPGRSLRLSCAASGFT FSSRNMHWVRQAPGKGLEWVAVIAYDG SLKYYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCARGRPWYSETGTSAF DIWGQGTMTVTVSS(SEQ ID NO:108)
ADI-25652	ADI-20112	CAGGTGCAGCTGGTGGAGTCTGCGGGAGGCGTGGTCCAGCCTGGGAGGTCCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCACTTTCGTGAGCTATAATATGCACCTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATAGCGTATGATGGAAG	QVQLVESGGGVVQPGRSLRLSCAASGFT FVSYNMHWVRQAPGKGLEWVAVIAYD GSVKYYADSVKGRFTISRDN SKNTLYLQ

		TGTTAAATACTATGCAGACTCCGTGAAGGGCCGATTCCACCATCTCCAGAGACAATTC CAAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACACGGCGGTG TACTACTGCGCCAGAGGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTCGA CATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCTCA(SEQ ID NO:94)	MNSLRAEDTAVYYCARGRPWYSETGTS AFDIWGQGTMTVTVSS(SEQ ID NO:109)
ADI- 25653	ADI- 20112	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCACTTTCGTGAGCTATAATATGCACCTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGTGGCAGTTATAATGTATGATGGAAGT GCTAAATACTATGCAGACTCCGTGAAGGGCCGATTCCACCATCTCCAGAGACAATTCC AAGAACACGCTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCGGTGT ACTACTGCGCCAGAGGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTCGAC ATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCTCA(SEQ ID NO:95)	QVQLVESGGGVVQPGRSLRLSCAASGFT FVSYNMHWVRQAPGKGLEWVAVIMYD GSAKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARGRPWYSETGTS AFDIWGQGTMTVTVSS(SEQ ID NO:110)
ADI- 25654	ADI- 20112	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGA GACTCTCCTGTGCAGCGTCTGGATTCACTTTCAGGAGCTATGATATGCACCTGGGTCC GCCAGGCTCCAGGCAAGGGGCTGGAGTGGTGGCATAATAAGCGTATGATGGAAGT AATAAATACTATGCAGACTCCGTGAAGGGCCGATTCCACCATCTCCAGAGACAATTCC AAGAACACGCTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCGGTGT ACTACTGCGCCAGAGGCCGTCCTTGGTACAGCGAAACTGGTACCTCAGCTTTCGAC	QVQLVESGGGVVQPGRSLRLSCAASGFT FRSYDMHWVRQAPGKGLEWVAVIAYD GSNKYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCARGRPWYSETGTS AFDIWGQGTMTVTVSS(SEQ ID NO:111)

		ATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCTCA(SEQ ID NO:96)	
ADI-20078	Parent antibody	CAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAAGCCTTCACAGACCCCTGT CCCTCACCTGTACTGTCTCTGGTGGCTCCATCAGCAGTGGTAGTACTACTGGAGCT GGATCCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGT GGGAGCACCTACTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACAC GTCTAAGAACCAAGTTCTCCCTGAAGCTGAGTTCTGTGACCCGCCGACACGGCG GTGTACTACTGCGCCAGAGATGTAGGATACCCACACTACTACGGAATGGACGTATGG GGCCAGGGAACAAGTGTACCGTCTCCTCTCA(SEQ ID NO:97)	QVQLQESGPGLVKPSQTLSTCTVSGGSI SSGSYYWSWIRQHHPGKGLEWIGYIYYSG STYYNPSLKSRTISVDTSKNQFSLKLSS VTAADTAVYYCARDVGYPHYYGMDVW GQGTTVTVSS(SEQ ID NO:112)
ADI-23515	ADI-20078	CAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAAGCCTTCACAGACCCCTGT CCCTCACCTGTACTGTCTCTGGTGGCTCCATCCGTTAGTGGTGCTTACTACTGGAGCT GGATCCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACGAT GGGCAGACCTACTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACAC GTCTAAGAACCAAGTTCTCCCTGAAGCTGAGTTCTGTGACCCGCCGACACGGCG GTGTACTACTGCGCCAGAGATGTAGGATACCCACACTACTACGGAATGGACGTATGG GGCCAGGGAACAAGTGTACCGTCTCCTCTCA(SEQ ID NO:98)	QVQLQESGPGLVKPSQTLSTCTVSGGSI RSGAYYWSWIRQHHPGKGLEWIGYIYYD GQTYYNPSLKSRTISVDTSKNQFSLKLS SVTAADTAVYYCARDVGYPHYYGMDV WGQGTTVTVSS(SEQ ID NO:113)
ADI-	ADI-	CAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAAGCCTTCACAGACCCCTGT	QVQLQESGPGLVKPSQTLSTCTVSGGSI

23518	20078	CCCTCACCTGTACTGTCTCTGGTGGCTCCATCAGCAGTGGTGTAGTTACTGGAGCT GGATCCGCCAGCACCCAGGGAAGGCCCTGGAGTGGATTGGGTACATCTATTACAGT GGGAGACCTACTACAACCCGTCCTCAAGAGTCGAGTTACCATATCAGTAGACAC GTCTAAGAACCAAGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGACACGGCG GTGTACTACTGCGCCAGAGATGTAGGATACCCACACTACTACGGAATGGACGTATGG GGCCAGGGAACAACGTGCACCCGTCTCCTCA(SEQ ID NO:99)	SSGASYWSWIRQHPGKGLEWIGYIYSSG ETYYNPSLKSrvTISVDTSKNQFSLKLSS VTAADTAVYYCARDVGYPHYYGMDVW GQGTTVTVSS(SEQ ID NO:114)
ADI- 23519	ADI- 20078	CAGGTGCAGCTGCAGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCCTGT CCCTCACCTGTACTGTCTCTGGTGGCTCCATCAGCAGTGGTTCGTCTCTACTGGAGCT GGATCCGCCAGCACCCAGGGAAGGCCCTGGAGTGGGTACATCTATATGAGT GGGAGACCTACTACAACCCGTCCTCAAGAGTCGAGTTACCATATCAGTAGACAC GTCTAAGAACCAAGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGACACGGCG GTGTACTACTGCGCCAGAGATGTAGGATACCCACACTACTACGGAATGGACGTATGG GGCCAGGGAACAACGTGCACCCGTCTCCTCA(SEQ ID NO:100)	QVQLQESGPGLVKPSQTLSTCTVSGGSI SSGSSYWSWIRQHPGKGLEWIGYIYMSG ETYYNPSLKSrvTISVDTSKNQFSLKLSS VTAADTAVYYCARDVGYPHYYGMDVW GQGTTVTVSS(SEQ ID NO:115)
ADI- 20048	Parent antibody	CAGGTGCAGCTGCAGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGACCCCTGT CCCTCACCTGCACCTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAGCTGGATCC GGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATAGTAGTGGGAGC	QVQLQESGPGLVKPSQTLSTCTVSGGSI SSYYSWIRQPPGKGLEWIGYIYSSGST NYNPSLKSrvTISVDTSKNQFSLKLSSVT

		ACCAACTACAACCCCTCCCTCAAGAGTCGAGTCACCATATATCAGTAGACACGTCCTCAA GAACCAAGTTCTCCCTGAAGCTGAGTTCTGTGACCCGCCGACACACGGCGGTGTACT ACTGCGCCAGAGATGCTCTCTGGCGGATCCTCTACCAGGACTACTACATGGACGTAT GGGCAAGGGTACAACCTGTACCCGTCCTCTCTCA(SEQ ID NO:101)	AADTAVYYCARDAPGGSSYQDYMDV WGKGTTVTVSS(SEQ ID NO:116)
ADI- 20096	Parent antibody	GAGGTGCAGCTGGTGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGTCCCCTGA GACTCTCTGTGCAGCCTCTGGATTCACTTACAGTAGCTATAGCATGAACCTGGGTCC GCCAGGCTCCAGGGAAGGGCTGGAGTGGGTCTCATCCATTAGTAGTAGTAAT TACATATACTACGCAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGC CAAGAACTCACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCGGTG TACTACTGCGCCAGAGGGGCCCCCTCTGGGATACAGCTGGGAGTACTTCGACCTATG GGGAGAGGTACCTTGGTCACCGTCTCTCTCA(SEQ ID NO:102)	EVQLVESGGGLVKPGGSLRLSCAASGFT FSSYSMNWVRQAPGKGLEWVSSISSN YIYADSVKGRFTISRDNAKNSLYLQMN SLRAEDTAVYYCARGAPLGYSWEYFDL WGRGTLVTVSS(SEQ ID NO:117)
ADI- 20051	Parent antibody	CAAGTACAATTACAACAGTGGGGAGCTGGTTTATTAAAGCCTTCAGAAACTTTAAG TTTGACCTGTGCTGTTACGGTGGATCATTTTCTGGTTATTACTGGAGTTGGATTTCGT CAACCACCAAGGCAAAGGATTGGAGTGGATCCGGTGAGATAGACCATTTCAGGCTCCA CTAACTACAATCCAAGTTTAAATCCAGGGTTACTATCTCCGTAGACACGTCCTCAAGA ACCAGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGACACACGGCGGTGTACTAC TGCGCCAGAGATGTTGGCGGAGGATACGCTTCCCCCTTCGACTATTGGGACAGGG	QVQLQQWGAGLLKPSLTSLTCAVYGG SFGYYWSWIRQPPGKGLEWIGIDHSG STNYPNPSLKSRVTISVDTSKNQFSLKLSS VTAADTAVYYCARDGGGGYASPFDYWG QGTLVTVSS(SEQ ID NO:118)

		TACATTGGTCACCGTCTCCTCA(SEQ ID NO:103)	
ADI-20065	Parent antibody	CAGCTGCAGCTGCAGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCCTGT CCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTAGTAGTACTACTGGGGCT GGATCCGCCAGCCCCCAGGGAAAGGGGCTGGAGTGGAGTATCTATTATAGT GGGAGCACCTACTACAACCCGTCCTCAAGAGTCGAGTCACCATATCCGTAGACAC GTCCAAGAACCAGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGACACACGGCG GTGTACTACTGCGCCAGAGATCCAGGATACAGCGCTTCCCCCAATTGGTTTGATCCA TGGGGACAGGGTACATTGGTCACCGTCTCCTCA(SEQ ID NO:104)	QLQLQESGPGLVKPPSETLSLTCTVSGGSI SSSSYYWGWIRQPPGKGLEWIGSIYYS STYYNPSLKSRVTISVDTSKNQFSLKLSS VTAADTAVYYCARDPGYSASPNWFDPW GQGLVTVSS(SEQ ID NO:119)
ADI-20066	Parent antibody	CAGCTGCAGCTGCAGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCCTGT CCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTAGTAGTACTACTGGGGCT GGATCCGCCAGCCCCCAGGGAAAGGGGCTGGAGTGGAGTATCTATTATAGT GGGAGCACCTACTACAACCCGTCCTCAAGAGTCGAGTCACCATATCCGTAGACAC GTCCAAGAACCAGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGACACACGGCG GTGTACTACTGCGCCAGAGATCCAGGATACAGCGCTTCCCCCAATTGGTTTGATCCA TGGGGACAGGGTACATTGGTCACCGTCTCCTCA(SEQ ID NO:104)	QLQLQESGPGLVKPPSETLSLTCTVSGGSI SSSSYYWGWIRQPPGKGLEWIGSIYYS STYYNPSLKSRVTISVDTSKNQFSLKLSS VTAADTAVYYCARDPGYSASPNWFDPW GQGLVTVSS(SEQ ID NO:119)
ADI-20118	Parent antibody	CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGA AGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGCCATCAGCTGGGTGC	QVQLVQSGAEVKKPGASVKVSKASGY TFTSYAISWVRQAPGQGLEWMGWISAY

		GACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGCAGCGCTTACAATGGT AACACAAACTATGCACAGAAAGCTCCAGGGCAGAGTCACCATGACCCACAGACACAT CCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCGGT GTACTACTGCGCCAGATCAGGAGGCGGCAGCGGACCCAAATTGTTGATCCATGGG GACAGGGTACATTGGTCAACCGTCTCCTCA(SEQ ID NO:105)	NGNTNYAQKLQGRVTMTTDTSTAYM ELRSLRSDDTAVYYCARSGGSGPNWF DPWGQGTLLTVSS(SEQ ID NO:120)
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Table 4: DNA and amino acid sequences of light chain variable domain (VL) (or light chain variable region (LCVR)) of the exemplary of the present invention

A nt ib od y N a m e	op ti mi ze d lin ka ge	VL DNA sequence	VL amino acid sequence

A	Pa	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC	DIQMTQSPSSLSASVGDRLVTITCQASQDISNY
D	re	CATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC	LNWYQQKPGKAPKLLIYDASNLETGVPSRF
I-	nt	CAGGGAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCA	SGSGSGTDFTTFTISSLPEDIATYYCQQSDHY
20	an	TCAAGGTTCAGTGGAAGTGATCTGGGACAGATTTTACTTTTACCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
11	tib	GCCTGAAGATAATTGCAACATAATTACTGTACGAGTCCGATCACTACCCCTACTTTTGGCGG	
2	od	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)	
	y		
A	Pa	GAAATAGTGATGACGCAGTCTCCAGCCACCCCTGTCTGTGTCTCCAGGGAAAGAGCCA	EIVMTQSPATLSVSPGERATLSCRASQSVSSN
D	re	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCAGCAGAA	LAWYQQKPGQAPRLIYSASTRATGIPARFS
I-	nt	ACCTGGCCAGGCTCCAGGCTCCTCATCTATAGCGCATCCACCAGGGCCACTGGTATCC	GSGSGTEFTLTISSLQSEDFAVYYCQQHNVY
20	an	CAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCACTCTCACCATCAGCAGCCT	PPYTFGGGTKVEIK(SEQ ID NO:131)
11	tib	GCAGTCTGAAAGATTTTIGCAGTTTATTACTGTCTCAGCAGCACAAATGTCTACCCCTCCTTACAC	
3	od	TTTTGGCGGAGGACCAAGGTTGAGATCAAA(SEQ ID NO:122)	
	y		
A	A	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC	DIQMTQSPSSLSASVGDRLVTITCQASQDISNY
D	DI	CATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC	LNWYQQKPGKAPKLLIYDASNLETGVPSRF
I-	-	CAGGGAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCA	SGSGSGTDFTTFTISSLPEDIATYYCQQSDHY

25	A	20	TCAAGGTT	CAGTGAAGTGGATCTGGGACAGATTTTACTTTTCA	CCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
65	D	11	GCCTGAAGATA	TGCAACATATTACTGT	CAGCAGTCCGATCACTACCCCTACTTTTGGCGG	
0	A	2	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)			
A	D	A	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC			DIQMTQSPSSLSASVGDRVTITCQASQDISNY
I-	D	-	CATCACTTGCCAGGCAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC			LNWYQQKPGKAPKLLIYDASNLETGVPSRF
25	I-	-	CAGGAAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAACAGGGGTCCCA			SGSGSGTDFTTISSLQPEDIAITYYCQQSDHY
25	A	20	TCAAGGTT	CAGTGGAAAGTGGATCTGGGACAGATTTTACTTTTCA	CCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
65	D	11	GCCTGAAGATA	TGCAACATATTACTGT	CAGCAGTCCGATCACTACCCCTACTTTTGGCGG	
1	A	2	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)			
A	D	A	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC			DIQMTQSPSSLSASVGDRVTITCQASQDISNY
D	D	DI	CATCACTTGCCAGGCAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC			LNWYQQKPGKAPKLLIYDASNLETGVPSRF
I-	I-	-	CAGGAAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAACAGGGGTCCCA			SGSGSGTDFTTISSLQPEDIAITYYCQQSDHY
25	A	20	TCAAGGTT	CAGTGGAAAGTGGATCTGGGACAGATTTTACTTTTCA	CCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
65	D	11	GCCTGAAGATA	TGCAACATATTACTGT	CAGCAGTCCGATCACTACCCCTACTTTTGGCGG	
2	A	2	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)			
A	D	A	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC			DIQMTQSPSSLSASVGDRVTITCQASQDISNY
D	D	DI	CATCACTTGCCAGGCAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC			LNWYQQKPGKAPKLLIYDASNLETGVPSRF

I-25	-	CAGGGAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCA	SGSGSGTDFTTFTISSLQPEDIATYYCQQSDHY
65	20	TCAAGGTTCAAGTGGAAGTGGATCTGGGACAGATTTTACTTTTCAACCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
3	11	GCCTGAAGATATTGCAACATATTACTGTTCAGCAGTCCGATCATACTACCCCTACTTTTGGCGG	
	2	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)	
A	A	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC	DIQMTQSPSSLSASVGDRTVTITCQASQDISNY
D	DI	CATCACTTGCCAGGCAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAAC	LNWYQQKPGKAPKLLIYDASNLETGVPSRF
I-25	-	CAGGGAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCA	SGSGSGTDFTTFTISSLQPEDIATYYCQQSDHY
65	20	TCAAGGTTCAAGTGGAAGTGGATCTGGGACAGATTTTACTTTTCAACCATCAGCAGCCTGCA	PTFGGGTKVEIK(SEQ ID NO:130)
4	11	GCCTGAAGATATTGCAACATATTACTGTTCAGCAGTCCGATCATACTACCCCTACTTTTGGCGG	
	2	AGGGACCAAGGTTGAGATCAAA(SEQ ID NO:121)	
A	Pa	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSPGERATLSCRASQSVSSS
D	re	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAG	YLAWYQQKPGQAPRLLIYGASSRATGIPDRF
I-20	nt	AAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCAT	SGSGSGTDFTLTISRLEPEDFAVYQCQSERS
07	an	CCCAGACAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGA	PFTFGGGTKVEIK(SEQ ID NO:132)
8	tib	CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTTCAGCAGTCCGAAACGAAGCCCTTTCAC	
	od	TTTTGGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:123)	

	y		
A	A	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSLSPGERATLSCRASQSVSSS
D	DI	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCCTGGTACCAGCAG	YLAWYQQKPGQAPRLLIYGASSRATGIPDRF
I-	-	AAACCTGGCCAGGCTCCCAAGGCTCCTCATCTATGGTGCATCCAGCAGGGGCCACTGGGCAT	SGSGSGTDFTLTISRLEPEDFAVYYCQQSERS
23	20	CCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGAGA	PFTFGGGTKVEIK(SEQ ID NO:132)
51	07	CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTCCGAAACGAAGCCCTTTTCAC	
5	8	TTTTGGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:123)	
A	A	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSLSPGERATLSCRASQSVSSS
D	DI	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCCTGGTACCAGCAG	YLAWYQQKPGQAPRLLIYGASSRATGIPDRF
I-	-	AAACCTGGCCAGGCTCCCAAGGCTCCTCATCTATGGTGCATCCAGCAGGGGCCACTGGCAT	SGSGSGTDFTLTISRLEPEDFAVYYCQQSERS
23	20	CCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGAGA	PFTFGGGTKVEIK(SEQ ID NO:132)
51	07	CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTCCGAAACGAAGCCCTTTTCAC	
8	8	TTTTGGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:123)	
A	A	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSLSPGERATLSCRASQSVSSS
D	DI	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCCTGGTACCAGCAG	YLAWYQQKPGQAPRLLIYGASSRATGIPDRF
I-	-	AAACCTGGCCAGGCTCCCAAGGCTCCTCATCTATGGTGCATCCAGCAGGGGCCACTGGCAT	SGSGSGTDFTLTISRLEPEDFAVYYCQQSERS
23	20	CCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGAGA	PFTFGGGTKVEIK(SEQ ID NO:132)

51	07	CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTCCGAAACGAAGCCCTTTCAC	
9	8	TTTTGGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:123)	
A	Pa	GAAATTGTGTGACACAGTCTCCAGCCACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCAC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYL
D	re	CCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCCTGGTTCCACAGAAAC	AWFQKPGQAPRLLIYDASN RATGIPARFSG
I-	nt	CTGGCCAGGCTCCCAGGCTCCTCATCTATGATGATGCCAACAGGGCCACTGGCATCCCA	SGSGTDFTLTISSELEPEDFAVYYCQQAFSMP
20	an	GCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAG	TFGGG TKVEIK (SEQ ID NO:133)
04	tib	AGCCTGAAAGATTTTGCAGTTTATTACTGTCAGCAGGCATTCTCCCATGCCCTCCTACTTTTG	
8	od	GCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:124)	
	y		
A	Pa	GACATCCAGATGACCCAGTCTCCTTCCACCCCTGTCTGCATCTGTAGGAGACAGAGTCAC	DIQMTQSPSTLSASVGD RVTITCRASQSISSW
D	re	CATCACTTGCCGGGCCAGTCAGAGTATTAGTGGTTGGCCTGGTATCAGCAGAAAC	LAWYQQKPGKAPKLLIYDASSLES GVP SRFS
I-	nt	CAGGAAAGCCCCCTAAAGCTCCTGAATCTAIGATGCCCTCCAGTTTGAAAGTGGGGTCCCA	GSGSGTEFTLTISSLQPDDFATYYCQQFQSYS
20	an	TCAAGGTTCAGCGGCAGTGGATCTGGGACAGAAATCACTCTCACCATCAGCAGCCTGCA	YTFGGG TKVEIK (SEQ ID NO:134)
09	tib	GCCTGATGATTTTGCAACTTATTACTGCCAGCAGTTCCAAAGTTACTCTTACACTTTTGG	
6	od	CGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:125)	
	y		

A	Pa	GAAATAGTGATGACGCAGTCTCCAGGCCACCCCTGTCTGTGTCTCCAGGGGAAAGAGCCA	EIVMTQSPATLSVSPGERATLSCRASQSVSSN
D	re	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCCTGGTACCAGCAGAA	LAWYQQKPGQAPRLLIYGASTRATGIPARFS
I-	nt	ACCTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCC	GSGSGTEFTLTISLSQSEDFAVYYCQYHAW
20	an	CAGCCAGGTTCA GTGGCAGTGGTCTGGGACAGAGTTCAC TCTCACCATCAGCAGCCT	PPTFGGGTKVEIK(SEQ ID NO:135)
05	tib	GCAGTCTGAAGATTTTGCAGTTTATTACTGTACGAGTACCACGCCCTGGCCTCCTACTTT	
1	od	TGGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:126)	
	y		
A	Pa	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSLSPGERATLSCRASQSVSSS
D	re	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCCTGGTACCAGCAG	YLAWYQQKPGQAPRLLIYGASSRATGIPDRF
I-	nt	AAACCTGGCCAGGCTCCCAAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCAT	SGSGSGTDFTLTISRLEPEDFAVYYCQQFDSS
20	an	CCCAGACAGGTTCA GTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGA	PTFGGGTKVEIK(SEQ ID NO:136)
06	tib	CTGGAGCCCTGAAGATTTTGCAGTGTTACTGTCTCAGCAGTTCGACAGCAGTCCTACTTTT	
5	od	GGCGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:127)	
	y		
A	Pa	GAAATTGTGTGACGCAGTCTCCAGGCACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCA	EIVLTQSPGTLSLSPGERATLSCRASQSVSSF
D	re	CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTTCTTAGCCCTGGTACCAGCAG	LAWYQQKPGQAPRLLIYGASSRATGIPDRFS
I-	nt	AAACCTGGCCAGGCTCCCAAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCAT	GSGSGTDFTLTISRLEPEDFAVYYCQQYDVF

20	an	CCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGA	PITFGGGTKVEIK(SEQ ID NO:137)
06	tib	CTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTACGACGTCTTCCCTATCACT	
6	od	TTTGCGGAGGACCAAGTTGAGATCAAA(SEQ ID NO:128)	
	y		
A	Pa	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC	DIQMTQSPSSLSASVGDRVTITCQASQDISNY
D	re	CATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTAAATTGGTATCAGCAGAAAC	LNWYQQKPGKAPKLLIYDASNLETGVPSRF
I-	nt	CAGGGAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCA	SGSGSGTDFTFTISSLQPEDIATYYCQQPATLP
20	an	TCAAGGTTTCAGTGGAAGTGGATCTGGGACAGATTTTACTTTTCACCATCAGCAGCCTGCA	WTFGGGKVEIK(SEQ ID NO:138)
11	tib	GCCTGAAGATATTGCCAACATATTACTGTTCAGCAGCCCCGCCACCCCTACCTTGGACTTTTGG	
8	od	CGGAGGGACCAAGGTTGAGATCAAA(SEQ ID NO:129)	
	y		

Table 5: Sequences of heavy chain and light chain of the exemplary antibody of the present invention

A	IgG	Amino acid sequence of heavy chain	Amino acid sequence of light chain
nt	Format		
ib			

o d y N a m e			
A D I- 2 0 11 2	IgG1 format; ADI- 20112	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET GTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:189) QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNW YQQKPGKAPKLLIYDASNLETGVPSRFSGSGGTD FTFTISSLQPEDIAITYYCQQSDHYPTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSYSTSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO:168)
A D	IgG2		

I-	format;	GTSAFDIWGQGTMTVTSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV	
2	ADI-	TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPKPSN	
0	20112	TKVDKTVKCCVECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVS	
11		HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY	
2		KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP	
		SDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV	
		MHEALHNHYTQKSLSLSPG(SEQ ID NO:139)	
		QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYNMHWVVRQAPGKGLEWVAVIA	
		YDGS AKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE	
A		TGTSAFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE	
D		PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPS	
I-	IgG1	NTKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCV	
2		VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW	
5	format;	LNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL	
6	ADI-	VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN	
5	25650	VFSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:140)	
0			

	IgG2 format; ADI- 25650	QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYNMHWVVRQAPGKGLEWVAVIA YDGSAKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTTCNV DHKPS NTKVDKTVVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVQFNWYVVDGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:141)
A D I- 2 5 6 5 1	IgG1 format; ADI- 25651	QVQLVESGGGVVQPGRSLRLSCAASGFTFSRNMHWVVRQAPGKGLEWVAVIAY DGLSKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET GTSAFDIWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN HKPSN TKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVF SCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:142)

	IgG2 format; ADI- 25651	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSRNMHWVRQAPGKGLEWVAVIAY DGLSKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET GTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDDHKPSN TKVDKTKVERKCCVECPAPPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTTPMMLDSDGSFFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:143)
A	IgG1 format; ADI- 25652	QVQLVESGGGVVQPGRSLRLSCAASGFTFVSYNMHWVRQAPGKGLEWVAVIA YDGSVKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPS NTKVDDKAEPKCDKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFFLYSKLTVDKSRWQQGN

2		VFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:144)	
	IgG2 format; ADI- 25652	QVQLVESGGGVVQPGRSLRLSCAASGFTFVSYNMHWVRQAPGKGLEWVAVIA YDGSVKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQT YTCNV D HKPS NTKVDKTV ERKCCVECP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS VLT VVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYK TTPPMLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:145)	
A D I- 2 5 6	IgG1 format; ADI- 25653	QVQLVESGGGVVQPGRSLRLSCAASGFTFVSYNMHWVRQAPGKGLEWVAVIM YDGS AKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT YICNV N HKPS NTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLT VV L HQDW	

5		LNGKEYKCKVSNKALPAPIEKTISKAKQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYK TTPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:146)
3		
	IgG2 format; ADI- 25653	QVQLVESGGGVVQPGRSLRLSCAASGFTFVSYNMHWVVRQAPGKGLEWVAVIM YDGSAKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVVDHKPS NTKVDKTVVERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVQFNWYYVDGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYK TTPPM L DSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:147)
A	IgG1	QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYDMHWVVRQAPGKGLEWVAYIA
D	format;	YDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSE
I-	ADI-	TGTSAFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
2	25654	PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN HKPS

5		NTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV	
6		VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW	
5		LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL	
4		VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN	
		VFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:148)	
	IgG2 format; ADI- 25654	QVQLVESGGGVQPGRSLRLSCAASGFTFRSYDMHWVRQAPGKGLEWVAYIA YDGSNKYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARGRPWYSE TGTSAFDIWGQGTMTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:149)	
A	IgG1 format;	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGSYYWSWIRQHPGKGLEWIGYIY	
D		YSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARDVGYPHYYG	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW

I-	ADI- 20078	MDVWGGGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPPVLDSGDGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:150)	YQKPGQAPRLLIYGASSRATGIPDRFSGSGGTD FTLTISRLEPEDFAVYYCQQSERSPFTFGGGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP EAKVQWKVDNALQSGNSQESVTEQDSKDSYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:169)
	IgG2 format; ADI- 20078	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGSYYWSWIRQHPGKGLEWIGYIY YSGSTYYNPSLKSRTISVDTSKNQFSLKLSVTAADTAVYYCARDVGYPHYYG MDVWGGGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVVDHKPSNTKV DKTVERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPPMLDSGDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE ALHNHYTQKSLSLSPG(SEQ ID NO:151)	
A	IgG1	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGSYYWSWIRQHPGKGLEWIGYIY YDGGQTYYNPSLKSRTISVDTSKNQFSLKLSVTAADTAVYYCARDVGYPHYYY	

1-	format;	GMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
2	ADI-	VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
3	23515	VDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
5		VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
1		KEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
5		FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
		SVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:152)
		QVQLQESGPGLVKPSQTLSTCTVSGGSIRSGAYYWSWIRQHPGKGLEWIGYIY
		YDGQTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARDVGYPHYYY
		GMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV
		SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDDHKPSNTK
		VDKTVERRKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
		DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKC
		KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRREEMTKNQVSLTCLVKGFYPSDI
		AVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
		ALHNHYTQKSLSLSPG(SEQ ID NO:153)

A	IgG1 format; ADI- 23518	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGASYWSWIRQHPGKGLEWIGYIY YSGETYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDVGYPHYYG MDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTKVD KKAEPKSCDKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:154)
D	IgG2 format; ADI- 23518	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGASYWSWIRQHPGKGLEWIGYIY YSGETYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDVGYPHYYG MDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDDHKPSNTKV DKTVERKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNNYKTTTPMMLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE

		ALHNHYTQKSLSLSPG(SEQ ID NO:155)	
A	IgG1 format; ADI- 23519	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGSSYWSWIRQHPGKGLEWIGYIY MSGETYYNPSLKS RVTISVDTSKNQFSLKLS SVTAADTAVYYCARDVGYPHYYG MDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHHKPSNTKVD KKAEPKCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:156)	
D			
I-			
2			
3			
5			
1			
9			
	IgG2 format; ADI- 23519	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGSSYWSWIRQHPGKGLEWIGYIY MSGETYYNPSLKS RVTISVDTSKNQFSLKLS SVTAADTAVYYCARDVGYPHYYG MDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVVDH KPSNTKV DKTVERKCCVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCK	

		VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHE ALHNHYTQKSLSLSPG(SEQ ID NO:157)	
A D I- 2 0 0 4 8	IgG1 format; ADI- 20048	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYSSG STNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDAPGGSSYQDYY MDVWGKGTTTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHHKPSNTKVD KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:158)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWF QQKPGQAPRLLIYDASNRAATGIPARFSGSGGTDF TLTISSLEPEDEFAVYYCQQAQAFSMPPTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSYSTLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEN (SEQ ID NO:170)
	IgG2 format; ADI- 20048	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYSSG STNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDAPGGSSYQDYY MDVWGKGTTTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVVDHHPKPSNTKV	

		DKTVKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHE ALHNHYTQKSLSLSPG(SEQ ID NO:159)	
A D I- 2 0 0 9 6	IgG1 format; ADI- 20096	EVQLVESGGGLVKPGGSLRLSCAASGFTSSYSMNWVRQAPGKGLEWVSSISS SNYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGAPLGYSWE YFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVD KKAEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:160)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAW YQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTE FTLTISLQPDDFATYYCQQFQSYSYTFGGGTKEI KRTVAAPSVFIFPPSPDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSITYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:171)
	IgG2 format;	EVQLVESGGGLVKPGGSLRLSCAASGFTSSYSMNWVRQAPGKGLEWVSSISS SNYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGAPLGYSWE	

	ADI- 20096	YFDLWGRGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQYTCNVDPVHKPSNTKV DKTVERKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNNYKTTTPPMLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMIHE ALHNHYTQKSLSLSPG(SEQ ID NO:161)	
A D I- 2 0 0 5 1	IgG1 format; ADI- 20051	QVQLQQWAGALLKPSSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEIDH SGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGGGGYASPF DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KAEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMI HEALHNHYTQKSLSLSPGK(SEQ ID NO:162)	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAW YQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEF TLTISSLSQSEDFAVYYCQQYHAWPPTFGGGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP EAKVQWKVDNALQSGNSQESVTEQDSKDSYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO:172)
	IgG2	QVQLQQWAGALLKPSSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEIDH SGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGGGGYASPF	

	format; ADI- 20051	DYWGQGTLLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKVD KTVERKCCVECPAPCPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPMMLDSGSEFFLYSKLTVDKSRWQQGNVVFSCSVMHE ALHNHYTQKSLSLSPG(SEQ ID NO:163)	
A		QLQLQESGPGLVKPSSETLSLTCTVSGGSISSSYWGWIRQPPGKGLEWIGSIYY SGSTYYNPSSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARDPGYSASP FDPWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVD KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:164)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYCQQFDSSPTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSYSL STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO:173)
I-			
2			
0	IgG1		
0	format;		
6	ADI-		
5	20065		

	IgG2 format; ADI- 20065	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSYYWGWIRQPPGKGLEWIGSIYY SGSTYYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDPGYSASP NW FDPWGQGT LVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNV DCHKPSNTKVD KTV ERKCCVECP PCPAPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYYVDGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHE ALHNHYTQKSLSLSPG(SEQ ID NO:165)	
A D I- 2 0 0 6 6	IgG1 format; ADI- 20066	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSYYWGWIRQPPGKGLEWIGSIYY SGSTYYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDPGYSASP NW FDPWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTKVD KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK(SEQ ID NO:164)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAW YQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYCQQYDVFPIITFGGGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSITYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO:174)

		IgG2 format; ADl- 20066	QLQLQESGPLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSIYY SGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARDPGYSASP NWFDPWGQGTLLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDPKPSNTKVD KTVRKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPMMLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE ALHNHYTQKSLSLSPG(SEQ ID NO:165)
A D I- 2 0 11 8	IgG1 format; ADl- 20118	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAISWVRQAPGQGLEWMGWIS AYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARSGGGSGP NWFDPWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPV VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTK VDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSC	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNW YQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTD FTFTISSLPEDIATYYCQQPATLPWTFGGGGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSITYSL SSITLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC(SEQ ID NO:175)

		SVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:166)	
	IgG2 format; ADI- 20118	QVQLVQSGAEVKKPGASVKVCKASGYTFTSYAISWVRQAPGQGLEWMGWIS AYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARSGGGSGP NWFDPWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPCPAPPVAGPSVFLPPKPKDTLMISRTPEVTCVVVDVSH EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYK CKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVM HEALHNHYTQKSLSLSPG(SEQ ID NO:167)	
A D I- 2 0 11 3	IgG1 format; ADI- 20113	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET GTSAFDIWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSN TKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVF	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAW YQQKPGQAPRLLIYSASTRATGIPARFSGSGSGTEF TLTISSLQSEDFAVYYCQQHNVYPPYTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF

		SCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:189)	NRGEC(SEQ ID NO:176)
	IgG2 format; ADI- 20113	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISY DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGRPWYSET GTSAFDIWGQGTMTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPDHKPSN TKVDKTKVERKCCVECPPCAPPVAGPSVFLPPKPKDTLMISRTPEVTCVVVDVS HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTIVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTTPPMLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPG(SEQ ID NO:139)	

Table 6: Exemplary signal peptide sequence of the present invention

METDTLLWVLLWVPGSTG (SEQ ID NO:182)

Table 7: Heavy chain and light chain sequence of some of antibody control used in the examples of the present invention

IgG1 heavy chain	MGWSLILLFLVAVATRLSEVRLLLESGGGLVQPGGSLRLSCAASGFTFSNYAMGWVRQAPGKGLEWVSAISGGSTYYADSVKGRFTTSR DDSKNALYLQMNSLRAEDTAVYYCARGGPGWYAADVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHN HYTQKSLSLSPGK(SEQ ID NO:177)
IgG4 heavy chain	MGWSLILLFLVAVATRLSEVRLLLESGGGLVQPGGSLRLSCAASGFTFSNYAMGWVRQAPGKGLEWVS AISGGSTYYADSVKGRFTTSRDDSKNALYLQMNSLRAEDTAVYYCARGGPGWYAADVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVNHHKPSNTKVDKRVESKYGPPCPCPAP EFLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLGLK(SEQ ID NO:178)
IgG1&Ig G4 light chain	MDFQVQIISFLLISASVIMSRGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGGTDFTL TISSLQPEDFATYYCQQADLPAFAFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:179)

IgG2 heavy chain	MGWSLILLFLVAVATRVLSQLQLQKSGPGLVKPSETLSLTCTVSGFTFSNYAMGWIRPPGKGLEWIGAISGGSTYYADSVKGRVTISVD TSKNQFSLKLSSVTAADTAVYYCARGGPGWYAADVWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDITLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTQ KLSLSLSPG(SEQ ID NO:180)
IgG2 light chain	MDFQVQIISFLLISASVIMSRGEIVLTQSPATLSLSPGERATLSCRASQISSYLNWYQQKPGQAPRLLIYAASSLQSGIPARFSGSGGTDFLTIT SSLEPEDFAVYYCQQADLPAFAFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:181)

These and other aspects and embodiments of the present invention are described in the drawings (hereinafter briefly described) and the detailed description of the invention and are illustrated in the following examples. Any or all of the features discussed above and throughout this application may be combined in various embodiments of the invention. The invention is further illustrated by the following examples, which are to be understood by way of illustration and not limitation, and the skilled person in the art can make various modifications.

Examples

Example 1. Production and purification of anti-OX40 antibody and control antibody

Anti-OX40 antibodies were identified by the Adimab antibody platform. Libraries and their use in screening procedures are described, for example, in Xu et al, 2013; WO2009036379; WO2010105256; WO2012009568.

The following anti-OX40 antibodies of the present invention were expressed in yeast or CHO-S cell or 293 HEK cells and then purified:

Antibody Name
ADI-20112
ADI-25650 (ADI-20112progeny)
ADI-25651 (ADI-20112progeny)
ADI-25652 (ADI-20112progeny)
ADI-25653 (ADI-20112progeny)
ADI-25654 (ADI-20112progeny)
ADI-20078
ADI-23515 (ADI-20078progeny)
ADI-23518 (ADI-20078progeny)
ADI-23519 (ADI-20078progeny)

ADI-20048
ADI-20096
ADI-20051
ADI-20065
ADI-20066
ADI-20118
ADI-20113

The following control antibodies were expressed in 293HEK cells and then purified:

Control Antibody
pogalizumab
Hu106-222
11D4
tavolixizumab

As used herein, pogalizumab is a human IgG1 OX40 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from Proposed INN: List 114(c.f., <http://www.who.int/medicines/publications/druginformation/innlists/PL114.pdf>). As used herein, Hu106-222 is a humanized IgG1 OX40 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from US patent US9006399. As used herein, 11D4 is a humanized IgG1 OX40 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from US patent US8236930. As used herein, tavolixizumab is a humanized IgG1 OX40 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from Proposed INN: List 115(c.f., <http://www.who.int/medicines/publications/druginformation/innlists/PL115.pdf>).

For the treatment of yeast material:

Yeast clones were grown to saturation and then induced for 48h at 30°C with shaking. After induction, yeast cells were pelleted and the supernatants were harvested for purification. IgGs were purified using a Protein A column and eluted with acetic acid, PH 2.0. Fab fragments were generated by papain digestion and purified over KappaSelect (GE Healthcare LifeSciences).

For the treatment of CHO-S cells:

Expression CHO-S cell lines were generated according to the manufacturer's instructions using the Freedom® CHO-S® Kit (Invitrogen). For mAb expression, DNA sequences of heavy and light chains were inserted into the pCHO 1.0 plasmid with the heavy chain upstream of the light chain. Full length human OX40 CDS sequences (purchased from Sino Biological) were inserted into the pCHO 1.0 vector for the generation of stable overexpression cell lines.

For the treatment of 293HEK cells:

For transient expression of proteins in 293HEK cells, the vector pTT5 was used with heavy and light chains of the antibodies cloned into a standalone vector. Transfection into 293HEK cells was carried out using standard procedures with PEI; supernatants were collected after 7 days of culture and purified on an AKTA system (GE).

Example 2: Binding kinetics and affinity Assay on anti-OX40 antibodies

The kinetics and equilibrium dissociation constant (K_D) for human OX40 is determined for antibodies of the present invention using MSD and bio-light interferometry (ForteBio) assay methods.

1. ForteBio KD assay (Bio-light interferometry)

ForteBio affinity measurements were performed generally as previously described (Estep, P., et al., *High throughput solution-based measurement of antibody-antigen affinity and epitope binning*. MAbs, 2013. 5(2): p. 270-8.). Briefly, ForteBio affinity measurements were performed by equilibrating sensors off-line in assay buffer for 30 min, and then monitoring on-line for 60 seconds for baseline establishment, loading the purified antibodies obtained as described above online onto AHQ sensors (ForteBio). Sensors with loaded antibodies were exposed to 100 nM

OX40 antigens for 5 min, afterwards they were transferred to assay buffer for dissociation of 5 min for off-rate measurement. Kinetics was analysed using the 1:1 binding model.

In experiments performed as described in the above assay, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20112, ADI-20113 and ADI-20118 (Fab format of anti-OX40 antibody in the form of IgG1 expressed in yeast), bind human OX40_Fc(human OX40 binding to antibody Fc moiety, purchased from R&D Systems) with a monovalent K_D in sub micromolar range. When the antibody was on the sensor tip, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118(anti-OX40 antibody in an IgG1 format and expressed in yeast), bind human OX40_Fc(purchased from R&D Systems) with an avid K_D value at a single digit nanomolar to lower range; and bind cyno OX40_Fc(purchased from Acro Biosystems) at double single digit nanomolar or lower range. When the antibody was on the sensor tip, ADI-20048, ADI-20078 and ADI-20096(anti-OX40 antibodies in an IgG1 format and expressed in yeast), bind mouse OX40_Fc(purchased from Acro Biosystems) at single digit nanomolar values (Table 8).

Table 8: Binding by Bio-light interferometry of antibodies of the invention in IgG1 format

Antibody	ForteBio Image: Human OX40-Fc on AHQ tip, antibody in the form of Fab in solution (200 nM) [Monovalent]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Human OX40-Fc in solution (50 nM) [Avid]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Cyno OX40-Fc in solution (100 nM) [Avid]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Mouse OX40-Fc in solution (100 nM) [Avid]
Hu106-222	1.53E-08	2.50E-10	4.96E-09	N.B.
ADI-20048	N.B.	3.68E-09	9.86E-09	3.76E-09
ADI-20051	2.58E-08	7.40E-10	1.18E-08	N.B.
ADI-20065	1.08E-08	3.52E-10	5.77E-09	N.B.
ADI-20066	2.18E-08	9.10E-10	1.43E-08	N.B.
ADI-20078	1.99E-07	1.08E-09	3.35E-09	9.17E-09

ADI-20096	N.B.	3.36E-09	N.B.	5.41E-09
ADI-20112	7.06E-08	7.35E-10	5.98E-09	N.B.
ADI-20113	1.82E-07	1.22E-09	5.52E-09	N.B.
ADI-20118	1.37E-07	5.10E-10	1.14E-08	N.B.

After affinity maturation of ADI-20112 and ADI-20078, its progenies ADI-25650, ADI-25651, ADI-25652, ADI-25653, ADI-25654, ADI-23515, ADI-23518, and ADI-23519 show improved monovalent binding shown by K_D values of the antibodies in Fab format produced from IgG1 expressed in yeast when binding to human OX40_Fc on a sensor chip; improvement of avid binding affinity when the antibody was on the sensor tip, in an IgG1 format and expressed in yeast, binding to human OX40_Fc in solution; and improvement of binding affinity when the antibody was on the sensor tip, in an IgG1 format and expressed in yeast, binding to cyno OX40_Fc in solution (Table 9).

Table 9: Binding by Bio-light interferometry of affinity matured antibodies of the invention in IgG1 format

Antibody	ForteBio Image: Human OX40-Fc on AHQ tip, Antibody in the form of Fab in solution (200 nM) [Monovalent]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Human OX40-Fc in solution (50 nM) [Avid]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Cyno OX40-Fc in solution (100 nM) [Avid]	ForteBio Image: Antibodies in the form of IgG1 on AHQ tip, Mouse OX40-Fc in solution (100 nM) [Avid]
11D4	2.80E-08	6.13E-10	5.95E-09	N.B.
Hu106-222	1.30E-08	4.75E-10	5.17E-09	N.B.
ADI-20112	6.39E-08	7.75E-10	6.82E-09	N.B.
ADI-25650	1.04E-09	2.30E-10	1.60E-09	N.B.
ADI-25651	2.32E-09	2.68E-10	1.37E-09	N.B.
ADI-25652	1.31E-09	2.61E-10	2.11E-09	N.B.

ADI-25653	1.63E-09	2.23E-10	1.15E-09	N.B.
ADI-25654	1.80E-09	2.83E-10	1.53E-09	N.B.
ADI-20078	9.80E-08	1.49E-09	2.85E-09	8.06E-09
ADI-23515	3.79E-09	2.73E-10	1.09E-09	8.98E-09
ADI-23518	7.16E-09	2.97E-10	1.08E-09	P.F.
ADI-23519	5.36E-09	2.55E-10	1.10E-09	P.F.

MSD-SET kinetic assay

Equilibrium affinity measurements see Estep, P., et al., MAbs, 2013. 5(2): p. 270-8. Solution equilibrium titrations (SET) are performed in PBS + 0.1% IgG-Free BSA (PBSF) where antigen (biotin-OX-40 monomer(biotinylated OX40, purchased from Acro Biosystems) is held constant at 10-100 pM and is incubated with 3-to 5-fold serial dilutions of Fab or mAbs starting at 5-100 nM (experimental condition is sample dependent). Antibodies diluted at 20 nM in PBS are coated onto standard bind MSD-ECL plates(Multi-array 96-well plate , Cat#:L15XA-3, <https://www.mesoscale.com/en/products/l15xa-3/>) overnight at 4°C or at room temperature for 30 min. Plates are blocked with BSA for 30 min whilst shaking at 700 rpm. Plates are then washed 3x with wash buffer (PBSF + 0.05% Tween 20). SET samples are applied and incubated on the plates for 150s with shaking at 700 rpm followed by one wash. Antigen captured on a plate is detected with 250 ng/mL sulfo tag-labeled streptavidin in PBSF by incubation on the plate for 3 min. The plates are washed three times with wash buffer and are then read on the MSD Sector Imager 2400 instrument using 1x Read Buffer T(Cat# R92TC-1 <https://www.mesoscale.com/en/products/r92tc-1/>) with surfactant. The percentage free antigen is plotted as a function of titrated antibody in Prism and fit to a quadratic equation to extract the KD. To improve throughput, liquid handling robots are used throughout MSD-SET experiments, including for SET sample preparation.

In experiments performed as described in the above assay, Fab form of ADI-25650, ADI-25651, ADI-25652, ADI-25653, ADI-25654, ADI-23515 and ADI-23519, in an IgG1 format and expressed in yeast, bind human OX40 with detectable sub-nanomolar monovalent K_D values (Table 10).

Table 10: Binding by MSD of antibodies of the invention in IgG1 format

Antibody	MSD KD (M) Human biotinylated OX40-His monomer incubated with Antibody Fab
11D4	N.D.
Hu106-222	N.D.
ADI-20112	N.D.
ADI-25650	1.40E-10
ADI-25651	1.70E-10
ADI-25652	1.50E-10
ADI-25653	8.60E-11
ADI-25654	1.80E-10
ADI-20078	N.D.
ADI-23515	1.60E-10
ADI-23518	N.D.

Example 3: Binding between the anti-OX40 antibody of the present invention and human OX40

The binding of an antibody of the present invention to human OX40 may be measured in a flow cytometry-based assay.

1. Binding to human OX40 on CHO cells

CHO cells overexpressing human OX40 (CHO-hOX40 cells) were generated by transfecting pCHO1.0 vector (Invitrogen) with human OX40 cDNA (Sino Biological, HG10481-G) cloned into the MCS. CHO-hOX40 cells (0.2×10^6 cells) are incubated with the experimental antibody at 100 nM for 30 min in PBS 0.1% BSA on ice. Cells are then washed at least twice, and are incubated with a secondary antibody (PE-labelled, SouthernBiotech, at final concentration of 5 µg/ml) in PBS 0.1% BSA for 30 min on ice (protected from light).

Cells are washed at least twice and analysed via flow cytometry. Flow cytometry is performed on a Canto II system (BD Biosciences) and MFIs are calculated accordingly.

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 (IgG1 format, expressed in yeast) binds OX40 overexpressed on CHO cells with MFI values >1000 fold difference compared with stained wild-type CHO cells (Table 11).

Table 11: Binding of antibodies of the invention in IgG1 format generated in yeast to CHO cells expression human OX40 by flow cytometry

Antibody	CHO binding intensity (MFI)	CHO-OX40 binding intensity (MFI)	Fold difference
Hu106-222	62.48	164239.15	2628.66757362356
ADI-20048	97.8	143461.23	1466.88374233129
ADI-20051	96.17	151191.75	1572.1300821462
ADI-20065	70.1	157294.81	2243.86319543509
ADI-20066	58.39	160635.77	2751.08357595479
ADI-20078	122.4	141543.26	1156.39918300654
ADI-20096	67.67	133399.72	1971.32732377715
ADI-20112	63.68	153432.17	2409.42478015075
ADI-20113	61.79	151897.05	2458.27884770999
ADI-20118	77.18	143967.03	1865.34115055714

In experiments performed as described in the above assay, after affinity maturation of ADI-20112 and ADI-20078, its progenies ADI-25650, ADI-25651, ADI-25652, ADI-25653, ADI-25654, ADI-23515, ADI-23518, and ADI-23519 retain binding strength to CHO cells overexpressing human OX40 (Table 12).

Table 12: Binding of affinity matured antibodies of the invention in IgG1 format generated in yeast to CHO cells expression human OX40 by flow cytometry

Antibody	CHO binding intensity (MFI)	CHO-OX40 binding intensity (MFI)	Fold difference
11D4	103.8	169078.52	1628.88747591522
Hu106-222	107.91	183487.48	1700.375127421
ADI-20112	117.48	244141.56	2078.15423901941
ADI-25650	123.52	251531.8	2036.3649611399
ADI-25651	108.09	252745.63	2338.28874086409
ADI-25652	105.37	252663.66	2397.87093100503
ADI-25653	108.47	251842.51	2321.77108878031
ADI-25654	111.57	251911.27	2257.87640046608
ADI-20078	103.2	140889.45	1365.20784883721
ADI-23515	117.12	181008.63	1545.49718237705
ADI-23518	117.02	173107.61	1479.29935053837
ADI-23519	113.95	177986.07	1561.966388767

In experiments performed as described in the above assay, ADI-20051, ADI-20078, ADI-20112 and ADI-20118, in IgG1 format, expressed in 293HEK cells, bind OX40 overexpressed on CHO cells with EC50 values of 2.734, 2.874, 2.799, and 6.71 nM, respectively (Table 13).

Table 13: Binding of antibodies of the invention in IgG1 format generated in 293HEK cells to CHO cells expressing human OX40 by flow cytometry(MFI)

mAb concentration (nM)	ADI-20051	ADI-20078	ADI-20112	ADI-20118	IgG1 *	Hu106-222	OX40L
200	3,892,625	4,331,942.5	3,968,422.5	3,887,261	2,823	2,641,566.5	2,676,466

100	4,353,62 2	3,749,82 0	3,821,55 6	3,829,76 4.5	1,724. 5	2,501,12 3	2,566,72 9.5
50	3,797,24 5.5	4,177,96 8	4,041,42 3	3,347,59 9.5	1,697	2,337,112	2,718,72 9.5
25	3,888,21 5.5	4,044,85 3.5	3,863,61 8	3,402,19 8	1,564	2,243,31 5	2,800,68 6.5
12.5	3,519,98 9.5	3,086,41 5	3,642,91 0	2,426,69 0	1,629	2,177,78 0	1,990,55 1.5
6.25	3,058,74 0	2,909,22 6	2,635,48 1.5	1,905,83 2	1,599. 5	2,020,45 3.5	1,475,88 8.5
3.125	2,820,88 7	2,260,56 3.5	1,984,98 2	1,424,27 5	1,745. 5	1,769,95 0	932,206
1.5625	976,540. 5	1,278,33 3	1,535,37 5	834,045	1,930	1,253,25 6	553,849
0.78125	603,919	684,834	655,291	529,956	1,681	600,191. 5	316,826
0.390625	493,981	432,702	302,819	191,627	1,710	474,061	118,773.5
0.1953125	293,798	267,094. 5	225,752. 5	131,627. 5	1,505. 5	181,238. 5	83,150
0.09765625	124,618	101,394. 5	80,628.5	75,465	2,383. 5	77,551	35,188.5
0.048828125	63,925	54,087.5	56,293.5	42,682.5	2,016	49,775.5	33,965
0.024414063	43,228.5	35,991.5	36,783.5	18,963.5	2,072	26,693	12,831
EC50	2.734	2.874	2.799	6.71	N/A	1.939	5.325

*IgG1 comprises the heavy chain of SEQ ID NO:177 and the light chain of SEQ ID NO:179, and the same for the IgG1 control in the following content.

In experiments performed as described in the above assay, ADI-20051, ADI-20078, ADI-20112 and ADI-20118, in IgG2 format, expressed in 293HEK cells, bind OX40 overexpressed on CHO cells with EC50 values of 5.49, 4.022, 2.777, and 5.838 nM, respectively (Table 14).

Table 14: Binding of antibodies of the invention in IgG2 format generated in 293HEK cells to CHO cells expressing human OX40 by flow cytometry (MFI)

mAb concentration (nM)	ADI-20051	ADI-20078	ADI-20112	ADI-20118	IgG2*	Hu106-222 (IgG2)	OX40L
200	2,505,57 1.5	2,340,46 1.5	1,794,44 0.5	1,721,94 7.5	216,46 3	2,547,47 3.5	2,079,67 1.5
100	2,352,26 9.5	2,302,55 7.5	1,781,26 7	1,530,37 9	93,016 .5	2,390,47 3.5	2,509,89 1
50	2,097,15 2	1,845,26 9.5	1,552,00 3	1,376,52 3.5	44,135	1,586,63 7	1,075,81 3
25	2,328,71 5	2,331,76 6	1,825,14 3.5	1,500,38 7	22,093 .5	2,158,05 1.5	1,842,25 6
12.5	1,620,69 2	1,504,60 6	1,342,11 4	1,128,28 0.5	11,883	1,524,12 5.5	1,107,92 0.5
6.25	1,331,84 7.5	1,510,03 2.5	1,427,75 7.5	889,093	6,395	1,058,62 6	599,830. 5
3.125	912,960	1,155,51 5.5	985,181	600,036. 5	4,151. 5	660,113	341,966
1.5625	610,550. 5	679,365. 5	602,131. 5	351,749	2,898	333,812	172,319
0.78125	315,041	344,013	313,831	207,156	2,249	188,470	86,024.5
0.390625	155,997. 5	191,290	188,741	119,907	1,892	100,782	49,659.5
0.1953125	80,545.5	91,837.5	94,230	60,444.5	1,685	49,458.5	24,816

0.09765625	40,673.5	44,953.5	48,522	36,842	1,590.5	28,207.5	14,492
0.048828125	19,401.00	26,640.00	25,653.00	18,428.00	1,562.00	15,366	8,354.50
0.024414063	20,873.50	25,589.00	24,560.50	16,484.00	1,511.50	14,635.5	7,974.00
EC50	5.49	4.022	2.777	5.838	N/A	8.367	12.4

*IgG2 comprises the heavy chain of SEQ ID NO:180 and the light chain of SEQ ID NO:181, and the same for the IgG1 control in the following content.

2. Binding to human OX40 on 293HEK cells

The binding of an antibody of the present invention to human OX40 may be measured in a flow cytometry assay. 293HEK cells overexpressing human OX40 (0.2×10^6 cells, prepared by the similar method for CHO cells as described above) are incubated with the experimental antibody at 100 nM for 30 min in PBS 0.1% BSA on ice. Cells are then washed at least twice, and are incubated with a secondary antibody (PE-labelled, SouthernBiotech, at final concentration of 5 µg/ml) in PBS 0.1% BSA for 30 min on ice (protected from light). Cells are washed at least twice and analysed via flow cytometry. Flow cytometry is performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated accordingly.

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 (IgG1 format, expressed in yeast) binds OX40 in a dose-dependent manner, compared to the negative control IgG1 control (Table 15).

Table 15: Binding of antibodies of the invention in IgG1 format generated in yeast cells to 293HEK cells expressing human OX40 by flow cytometry (MFI)

Antibody	Concentration			
	200 nM	40 nM	8 nM	1.6 nM
IgG1 control	11,089.00	25,186.50	5,311.00	5,625.50
11D4	2,405,701.00	2,755,335.00	2,060,113.00	613,085.00

Hu106-222	1,787,941.00	1,798,292.50	1,737,822.00	650,133.50
ADI-20048	1,703,707.00	1,851,598.50	1,726,793.50	976,743.50
ADI-20051	2,465,528.00	2,394,265.00	2,328,569.50	788,317.00
ADI-20065	3,031,816.50	2,424,762.50	2,458,475.00	807,629.00
ADI-20066	2,552,042.00	2,388,745.00	2,823,986.00	625,539.50
ADI-20078	1,943,603.50	1,565,973.00	1,575,529.00	541,760.00
ADI-20096	1,842,613.00	1,816,164.50	1,100,745.50	416,902.00
ADI-20112	2,305,931.00	1,923,515.00	1,975,220.00	894,984.00
ADI-20113	2,409,755.50	2,181,506.00	1,997,251.00	734,281.00
ADI-20118	1,730,309.00	1,979,720.00	1,962,026.00	848,675.50

3. Binding to human OX40 on activated primary CD4+ T cells

The binding of an antibody of the present invention to human OX40 on primary T cells may be measured in a flow cytometry assay.

Primary CD4+ T cells from healthy donors are activated with anti-CD3/CD28 DynaBeads(Invitrogen) for 48 hours and 0.2×10^6 cells are incubated with the experimental antibody at 100 nM for 30 min in PBS 0.1% BSA on ice. Cells are then washed at least twice, and are incubated with a secondary antibody (PE-labelled, SouthernBiotech, at final concentration of 5 µg/ml) in PBS 0.1% BSA for 30 min on ice (protected from light). Cells are washed at least twice and analysed via flow cytometry. Flow cytometry is performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated accordingly.

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 (IgG1 format, expressed in yeast) binds OX40 with a high MFI signal compared to the negative control IgG1 control (Table 16).

Table 16: Binding of antibodies of the invention in IgG1 format generated in yeast cells to activated CD4+ T cells by flow cytometry (MFI)

Antibody	Human OX40 on primary CD4+T cells derived from healthy Donor 1	Human OX40 on primary CD4+T cells derived from healthy Donor 2
IgG1 control	6,309.00	1,707.00
Hu106-222	114,899.50	136,049.50
11D4	196,478.50	242,033.50
ADI-20048	43,129.00	62,592.00
ADI-20051	140,903.50	172,241.50
ADI-20065	152,901.00	208,500.50
ADI-20066	134,167.00	193,030.00
ADI-20078	60,318.50	80,891.00
ADI-20096	54,618.00	75,446.00
ADI-20112	100,101.50	125,179.00
ADI-20113	84,378.50	114,677.00
ADI-20118	78,556.50	107,498.50

Example 4. Blocking of the antibody of the present invention on the interaction between human OX40L and OX40 on CHO cells

The ability of an antibody of the present invention to block binding of human OX40 to OX40L may be measured by flow cytometry.

Method 1:

0.2 x 10⁶ CHO cells expressing human OX40 prepared as described in the above Example 3 are incubated with the experimental antibody (100 nM) for 30 min in PBS 0.1% BSA on ice. Cells are then washed 3X, and are incubated with OX40L-hFc(obtained from AcroBiosystems) (~10 µg/ml) linked with NHS-Fluorescein (Promega) in PBS 0.1% BSA for

30 min on ice (protected from light). Cells are washed 3X. Flow cytometry is performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated on the C6 software.

In the experiment performed by the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 (IgG1 format expressed in yeast) blocked human OX40L-FITC binding to different levels, with only the FITC signal blocking of ADI-20051 is slightly stronger than that of OX40L, with an MFI value of 17,225, compared with 19,344. ADI-20048, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118, show higher MFI values than OX40L, at 34,342, 33,687, 32,813, 28,112, 31,917, 22,525, 24,020.5, and 26,580.5, respectively (Table 17).

Table 17: Blocking of OX40L binding to OX40 expressed on CHO cells by flow cytometry of antibodies of the invention in IgG1 format generated in yeast cells

Antibody	MFI of OX40L signal on CHO-OX40 cells
Blank	5,006
IgG1 control	35090
Hu106-222	25,807
11D4	35,963
OX40L	19,344
ADI-20048	34,342
ADI-20051	17,225
ADI-20065	33,687
ADI-20066	32,813
ADI-20078	28,112
ADI-20096	31,917
ADI-20112	22,525
ADI-20113	24,020.5

ADI-20118	26,580.5
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Method 2:

OX40L fused to murine Fc (OX40L-mFc, obtained from AcroBiosystems) was also used followed by anti-murine FC-FITC secondary antibody(Biolegend), using the staining methods, as described in Method 1. Flow cytometry is performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated on the C6 software.

In experiments performed as described in the above assay, ADI-23515-g2 and ADI-20112-g1 (in IgG2 and IgG1 isoforms, respectively, and expressed in CHO cells) although blocked human OX40L fused to mouse Fc (60 µg/ml) binding to OX40 on CHO cells, showing lower blocking activity compared to Pogalizumab, and at similar levels to OX40L(Figure 1), wherein control IgG4 comprises the heavy chain of SEQ ID NO:178 and the light chain of SEQ ID NO:179 (the same for the IgG4 control in the following content) .

Example 5. Agonist activity of anti-OX40 antibody of the present invention

1. Plate bound antibody T cell activation assay

T cell Isolation is performed as per manufacturer's instructions in the Untouched CD4+ T cell isolation kit (Invitrogen). A magnet fitted with a 1.5 ml tube rack is used to remove unwanted magnetic beads (QIAGEN).

The agonist activity of anti-OX40 antibodies of the present invention may be evaluated by measuring the release of inflammatory cytokines by T cells after T cell activation. 96-well flat-bottom plates (Corning) were coated with suboptimal anti-CD3 (0.25 µg/ml) antibody (Biolegend), and anti-OX40 (6 µg/ml) test antibodies at 37 degrees Celsius for 2 hours or overnight at 4 degrees Celsius. After washing, 100,000 CD4+ primary T cells were added into each well in a total of 200 µl media with 2 µg/ml anti-CD28 antibody (Biolegend) in solution. After 5 days, IL-2 secretion levels were tested by ELISA (Ready-SET-Go!; eBioscience).

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 in IgG1 format generated in yeast cells, increased IL-2 and IFNγ secretion over the IgG4 control, with up to 100-fold increase in IL-2 levels and ~3-fold difference in IFNγ levels in the T cells from

two different healthy donors (Table 18).

Table 18: IL-2 and IFN γ secretion upon T cell activation with suboptimal anti-CD3 activation plus antibody of the invention in IgG1 format generated in yeast cells

Antibody	Fold change in IL-2 or IFN γ secretion over control			
	IL-2 secretion of T cells of Donor 1	IFN γ secretion of T cells of Donor 1	IL-2 secretion of T cells of Donor 1	IFN γ secretion of T cells of Donor 2
IgG4 control	1	1	1	1
Hu106-222	98.799087	3.372629843	27.14136126	2.238952537
11D4	143.68493	3.570898599	150.6753927	2.481314785
OX40L	130.30594	2.25329761	97.4869	1.67649
ADI-20048	143.34703	2.91117065	160.581	2.03546
ADI-20051	157.44292	2.87448475	165.681	1.47095
ADI-20065	142.56621	2.83099753	159.277	1.58129
ADI-20066	142.39269	2.47650453	163.351	1.52032
ADI-20078	152.08219	3.4641385	148.649	2.34984
ADI-20096	143.35616	3.49443528	147.89	2.3868
ADI-20112	150.83562	3.1417972	159.66	2.98882
ADI-20113	141.73059	3.32914262	143.806	1.71399
ADI-20118	147.88128	2.69579555	147.529	1.80742

2. Soluble antibody T cell activation assay

Assay 1:

The agonist activity of anti-OX40 antibodies of the present invention may be evaluated by measuring the release of inflammatory cytokines by T cells after T cell activation. 100,000

CD4⁺ T cells are stimulated by PHA (10µg/ml, Sigma) and 200nM anti-OX40 candidate antibodies for 5 days. IL-2 secretion is tested by ELISA (Ready-SET-Go!; eBioscience).

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 in IgG1 format generated in yeast cells, increased IL-2 secretion over the IgG4 control, with up to 10-fold increases in IL-2 levels from six different healthy donors (Table 19).

Table 19: IL-2 secretion upon T cell activation with PHA activation plus antibodies of the invention in IgG1 format generated in yeast cells

Antibody	Fold change in IL-2 secretion over control					
	IL-2 secretion of T cells of Donor 1	IL-2 secretion of T cells of Donor 2	IL-2 secretion of T cells of Donor 3	IL-2 secretion of T cells of Donor 4	IL-2 secretion of T cells of Donor 5	IL-2 secretion of T cells of Donor 6
IgG4 control	1	1	1	1	1	1
Hu106-222	0.6542	0.602241	0.745803	0.669903	0.541237	0.569652
11D4	1.1542	1.980392	1.551559	0.932039	2.025773	1.514925
OX40L	2.2757	9.509804	2.59952	2.558252	9.384021	3.159204
ADI-20048	11.173	7.358543	4.678657	11.73301	8.515464	3.80597
ADI-20051	2.229	2.515406	1.223022	1.757282	2.646907	1.41791
ADI-20065	2.9626	3.2493	1.484412	2.684466	2.984536	1.679104
ADI-20066	2.2617	2.495798	1.371703	2.495146	2.07732	1.350746
ADI-	10.565	4.719888	6.429257	13.14078	5.10567	7.365672

20078						
ADI-20096	1.8879	2.857143	2.64988	4.728155	2.430412	3.218905
ADI-20112	4.2664	2.753501	2.472422	4.218447	2.780928	2.81592
ADI-20113	4.0654	5.848739	2.285372	4.063107	5.742268	2.365672
ADI-20118	7.0841	10.36975	7.105516	6.65534	10.15722	8.477612

Assay 2:

The agonist activity of anti-OX40 antibodies of the present invention may be evaluated by measuring the release of inflammatory cytokines by T cells after T cell activation. 100,000 CD4+ T cells were stimulated with anti-CD3 antibody(Biolegend) (at the concentration of 1µg/ml), anti-CD28 antibodies (Biolegend) (at the concentration of 2µg/ml) and 10 µg/ml, 20 µg/ml or 40 µg/ml anti-OX40 antibodies for 5 days. IL-2 secretion is tested by ELISA (Ready-SET-Go!; eBioscience).

In experiments performed as described in the above assay, anti-OX40 antibody ADI-2005720051, ADI-20078, ADI-20112 and ADI-20118, in IgG1 format expressed by HEK293 cells, increased IL-2 secretion over the IgG1 control at 20 ug/ml concentrations or above (Table 20), and also anti-OX40 antibody in IgG2 format expressed by HEK293 cells at all concentrations (Table 21).

Table 20: IL-2 secretion upon T cell activation with anti-CD3 antibody, anti-CD28 antibody in solution plus antibody of the invention in IgG1 format generated HEK293 cells

mAb concentration (µg/ml)	ADI-20051	ADI-20078	ADI-200112	ADI-200118	IgG1 control	OX40L
40	1.274526678	2.724612737	2.984509466	1.939759036	1	1.972461274
20	1.4505033	1.9790268	2.7852348	1.4312080	1	1.6879194

	56	46	99	54		63
10	0.8673992 67	1.5465201 47	1.8205128 21	0.9142857 14	1	4.1743589 74

Table 21: IL-2 secretion upon T cell activation with anti-CD3 antibody, anti-CD28 antibody in solution plus antibody of the invention in IgG2 format generated in HEK293 cells

mAb concentration ($\mu\text{g/ml}$)	ADI-20051	ADI-20078	ADI-200112	ADI-200118	IgG1 control	OX40L
40	3.3750759 88	4.9893617 02	8.6990881 46	6.9620060 79	1	8.2158054 71
20	1.1620917 65	3.1885393 71	4.6740132 24	3.8152674 81	1	6.6585854 54
10	14.441301 27	15.826025 46	26.789250 35	22.046676 1	1	3.4908062 23

Assay 3:

The agonist activity of anti-OX40 antibodies of the present invention may be evaluated by measuring the release of inflammatory cytokines by T cells after T cell activation. 100,000 PBMC are stimulated with PHA (5 $\mu\text{g/ml}$; Sigma) and 5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ or 20 $\mu\text{g/ml}$ anti-OX40 antibodies for 5 days before testing IL-2 secretion by ELISA (Ready-SET-Go!; eBioscience).

In experiments performed as described in the above assay, ADI-20078-g1 (IgG1 format), ADI-20078-g2 (IgG2 format), ADI-20112-g1 (IgG1 format), and ADI-20112-g2 (IgG2 format), expressed in CHO cells, , increased IL-2 secretion over the IgG1 control and IgG2 controls at 5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, and 20 $\mu\text{g/ml}$, higher than control antibodies 11D4, Hu106-222, and ponalizumab benchmarks (Figure 2).

3. Luciferase reporter T cell activation assay

The agonist activity of anti-OX40 antibodies of the present invention may be evaluated

by measuring the promotion of NFkB-mediated transcriptional activation in a luciferase reporter assay. Jurkat cells (US, ATCC) overexpressing human OX40(purchased from Sino) and NFkB-luciferase constructs(NFkB promoter-luc, Promega) were activated with PHA (5 µg/ml; Sigma) or anti-CD3 (2 µg/ml; Biolegend) plus anti-CD28 (2 µg/ml; Biolegend) with anti-OX40 antibody in solution (100 nM) for 18h, then tested after cell lysis and addition of substrate and bioluminescence measurement on a detection device (Molecular Devices) that indicates relative luciferase expression induction.

In experiments performed as described in the above assay, ADI-20048, ADI-20051, ADI-20065, ADI-20066, ADI-20078, ADI-20096, ADI-20112, ADI-20113 and ADI-20118 in IgG1 format generated in yeast cells increase luciferase expression greater than the IgG4 control, with up to 4-fold increases and 7-fold increases in signal using PHA (Sigma) or anti-CD3 (Biolegend) activation, respectively (Table 22).

Table 22: Luciferase reporter detection in Jurkat stably transfected with human OX40 and NFkB promoter-luc by PHA or anti-CD3 and anti-CD28 stimulation plus the stimulation of antibodies of the invention in IgG1 format generated in yeast cells

Antibody	NFkB-Luc fold change in Jurkat stimulated by PHA+ anti-CD28	NFkB-Luc fold change in Jurkat stimulated by anti-CD3+ anti-CD28
IgG4 control	1	1
Hu106-222	1.929795	1.774336
11D4	1.955867	1.480546
OX40L	4.823335	4.049893
ADI-20048	3.626118	5.76953
ADI-20051	1.832293	1.289518
ADI-20065	2.112869	1.19408
ADI-	2.085514	1.959262

20066		
ADI-20078	4.623152	7.455066
ADI-20096	4.567301	5.122978
ADI-20112	4.193475	3.972688
ADI-20113	3.794002	3.97635
ADI-20118	3.990324	5.265868

In experiments performed as described in the above assay, using anti-CD3 and anti-CD28 dynabeads (Invitrogen) as an activator, ADI-20078-g2 is a human IgG2 OX40 antibody transiently expressed in CHO cells that increased luciferase expression greater than the IgG control(IgG1 and IgG2 control), ADI-20078-g1 (human IgG1 OX40 antibody expressed in CHO cells), ADI-20112-g1 (human IgG1 OX40 antibody expressed in CHO cells), ADI-20112-g2 (human IgG2 OX40 antibody expressed in CHO cells), ponalizumab and Hu106-222 (Figure 3).

In experiments performed as described in the above assay, ADI-20078-g2 is a human IgG2 OX40 antibody expressed in CHO cells that increases luciferase expression greater than the IgG control(IgG1 control), and ADI-23515-g2 is an affinity matured human IgG2 OX40 antibody expressed in CHO cells that has a lower EC50 value of 0.3904 nM compared to ADI-20078-g2 with an EC50 value of 5.739 nM(Figure 4).

In experiments performed as described in the above assay, adding Raji cells (ATCC) to provide co-stimulation signals and FcγRIIb for IgG cross-linking, ADI-20112-g1 is a human IgG1 OX40 antibody, ADI-20112-g2 is a human IgG2 OX40 antibody, ADI-20078-g1 is a human IgG1 OX40 antibody, ADI-20078-g2 is a human IgG2 OX40 antibody, all expressed in CHO cells, increased luciferase expression through OX40 activation greater than the IgG control (IgG1 control and IgG2 control), with EC50 values of 0.06051 nM, 0.2463 nM, 0.09644 nM, and 1.398 nM, respectively. ADI-20112-g1 is equivalent to or a better agonist compared with ponalizumab, Hu106-222 and 11D4 (Figure 5).

In experiments performed as described in the above assay, ADI-25654-g1, a human IgG1

OX40 antibody generated in CHO cells, is an affinity matured form of ADI-20112-g1, a human IgG1 OX40 antibody generated in CHO cells; and ADI-23515-g1, a human IgG1 OX40 antibody generated in CHO cells, is an affinity matured form of ADI-20078-g1, a human IgG1 OX40 antibody generated in CHO cells. ADI-25654-g1 and ADI-23515-g1 show better agonist activity with EC50 values of 0.02817 nM, and 0.1743 nM respectively, compared with ADI-20112-g1 that has an EC50 of 0.2576 nM (Figure 6).

4. Mixed Lymphocyte Reaction(MLR)/DC T cell co-culture assay

The agonist activity of OX40 signals by antibodies of the present invention may be evaluated by measuring the release of IL-2 during a mixed lymphocyte reaction or DC-T cell co-culture assays. 2×10^6 PBMC are plated per well in a 6 well tissue culture plate or T25 tissue culture flask. Cells are incubated for 2-3 hours, to allow for adherence of monocytes.

Immature myeloid moDCs are generated by culturing monocytes (1×10^6 cells/ml) from PBMC in X-VIVO 15 media containing 1% AB serum, 10mM HEPES, 50 μ M β -Me, IL-4 (1000 U/ml) and GM-CSF (1000 U/ml), or 25-50 ng/ml of each. After 2 days fresh medium supplemented with IL-4 and GM-CSF is added. On Day 5, cells are either frozen or maturation is induced by adding a stimulation cocktail containing rTNFa (1000U/ml), IL-1b (5 ng/ml), IL-6 (10ng/ml) and 1 μ M PGE₂ for 2 days at a cell density of 3×10^5 cells/ml. T cell Isolation is performed as per manufacturer's instructions in the Untouched CD4+ T cell isolation kit (Invitrogen). A magnet fitted with a 1.5 ml tube rack is used to remove unwanted magnetic beads (QIAGEN).

100,000-200,000 isolated T cells (from donor) are mixed with 10,000-20,000 allogeneic moDCs (as above) in a total volume of 200 μ l in 96-round bottom tissue culture plates for 4-5 days at 37°C. SEE (1 ng/ml) was added to increase T cell activation. Test antibodies are added at the beginning of the MLR and incubated throughout the culture period. Detection of IL-2 is carried out as per manufacturer's instructions (eBioscience). OD measurements are determined on a Multiskan FC system (Thermo).

In experiments performed as described in the above assay, ADI-23515-g2, a human antibody in IgG2 format expressed in CHO cells, and ADI-20112-g1, a human IgG1 antibody expressed in CHO cells, increased IL-2 when using at $\sim 13.3 \mu$ g/ml, $\sim 4.4 \mu$ g/ml and $\sim 1.48 \mu$ g/ml concentrations, equally or better than pogalizumab, 11D4, Hu106-222, and tavolixizumab. Pogalizumab had poor agonist activity at all concentrations, and 40 μ g/ml of 11D4, Hu106-222, tavolixizumab, ADI-23515-g1, and ADI-20112-g1 showed lower agonist activity at the

highest concentration of OX40 antibody of 40 µg/ml(Figure 7, wherein IgG control is IgG1 control).

Example 6. Detection of T cell activation of OX40-mediated by OX40L by the antibodies of the present invention based on luciferase reporter

The blocking activity of anti-OX40 antibodies of the present invention may be evaluated by measuring the propensity of the antibody in blocking OX40L-mediated T cell activation of OX40. Activation property of T cells are evaluated by NFkB mediated transcription activity (c.f., Example 5). Jurkat cells(US ATCC) overexpressing human OX40 and NFkB-luciferase constructs (NFkB promoter-luc, US Promega) were activated with anti-CD3 (2 µg/ml; Biolegend), anti-CD28 (2 µg/ml (Biolegend) and recombinant OX40L (60 µg/ml; Acro Biosystems) plus increasing concentrations of anti-OX40 antibody(ADI-0112-g1 in IgG1 format expressed in CHO cells of the present invention, ADI-23515-G2 in IgG2 format expressed in CHO cells of the present invention) and IgG4 control, Pogalizumab, OX40L(obtained AcroBiosystems) in solution for 18h, then tested after cell lysis and addition of substrate and absorbance measurement.

Pogalizumab readily blocked OX40L-based activation at concentrations of more than about 0.08 nM or above, whereas ADI-20112-g1, a human IgG1 OX40 antibody generated in CHO cells, and ADI-23515-g2, a human IgG2 OX40 antibody generated in CHO cells, blocked OX40L-based activation at 20 nM and above (Figure 8).

Example 7. Antibody dependent cell-mediated cytotoxicity of the antibody of the present invention

Luciferase reporter based antibody dependent cell-mediated cytotoxicity assays

Antibody dependent cell-mediated cytotoxicity (ADCC) activity of OX40 antibodies can be measured using a luciferase reporter assay (Promega). Target cells (CHO cells expressing human OX40, prepared as above) are seeded at 1.2×10^6 cells/ml in 25 µl RPMI media(Gibco) containing 10% Ultra-Low IgG FBS(Sigma). 1:3 serial dilutions of 1 µg/ml antibody are made, adding 25 µl/well. 6×10^6 cells/ml ADCC effector cells (Promega) are seeded and incubated for 6 hours at 37 °C with 5% CO₂. 75 µl luciferase assay reagent (Promega) is added and the mixtures are brought to RT for 20 minutes. The plate is centrifuged for 2 minutes at 300 g/min.

120 µl of cell supernatant is carefully transferred to Optiplates. The plate is read in luminometer. Curves are fitting and EC50 of antibody response is determined using GraphPad Prism 6.0.

In experiments performed as described in the above assay, ADI-20051, ADI-20078, ADI-20112, and ADI-20118, in IgG1 format expressed in HEK293 cells, increase luciferase expression with EC50 values of 0.3653, 0.9109, 0.7304, and 0.8867 nM, respectively (Figure 9). ADI-20051, ADI-20078, ADI-20112, and ADI-20118, in IgG2 isotype expressed in HEK293 cells, did not elicit ADCC activity (Figure 10).

Example 8. Anti-tumor activity of the antibodies of the present invention

Anti-tumour efficacy of OX40 antibodies of the invention can be studied in humanized mouse models(NOG hu-PBMC LoVo tumour model).

LoVo human colon cancer cells (ATCC# CCL-229) were cultured according to ATCC instructions (F-12K). Two million LoVo cells suspended in 0.2 mL PBS contained with 0.66 million human PBMCs were implanted in the right flank of female NOG (Beijing Vital River Laboratory Animal Technology Co.).

Tumors and body weights were measured twice a week throughout the study, and mice were euthanized when the tumors reached endpoint or when mice had >20% body weight loss. At 3 days post implantation, mice were randomized into groups of 6-7 each that the mean tumor volume estimated with digital calipers, and tumor volume in mm³ was calculated by the formula: (width)² x length/2 for each group was approximately 50 mm³.

On day 3, 7, 11, and 14 (or 15) post implantation, mice were dosed intraperitoneally (IP) with PBS, 10 mg/kg of relevant IgG isotype control(equitech-Bio), or anti-OX40 antibodies(ADI-20112-g1, human OX40 antibody in IgG1 format expressed in CHO cells or ADI-23515-g2, human OX40 antibody in IgG1 format expressed in CHO cells).

In experiments performed as described in the above assay, ADI-20112-g1, a human OX40 antibody in IgG1 format expressed in CHO cells reduces tumour volume growth compared with IgG control(equitech-Bio) (Figure 11), and compared with ponalizumab and 11D4 (Figure 13). In experiments performed as described in the above assay, ADI-23515-g2, a human OX40 antibody in IgG2 format expressed in CHO cells significantly reduces tumour volume growth compared with IgG control(equitech-Bio) (Figure 12), ponalizumab and 11D4 (Figure 13). ADI-20112-g1 and ADI-23515-g2 do not significantly affect mouse body weight compared

with IgG control, pocalizumab, and 11D4 (Figure 14).

Example 9: Pharmacokinetic assay

Pharmacokinetic data was assessed following ADI-20078-IgG1(IgG1 format, expressed in CHO cells), ADI-20078-IgG2(IgG2 format, expressed in CHO cells), ADI-20112-IgG1(IgG1 format, expressed in CHO cells), ADI-20112-IgG2(IgG2 format, expressed in CHO cells) i.v. doses of 10 mg/kg antibodies to female Balb/C mice. The dose volume was injected into the mouse based on body weight.

Timing for blood collection begun at the beginning of dosing. For i.v. administration, timepoints included 0.083 hours (5 minutes) up to day 21. Blood was collected from three mice for each time point. Approximately 100 ul of blood was collected into microcentrifuge tubes. The blood samples were stored on ice for at least 10 minutes at a speed setting of 3000 RPM to obtain approximately 40-50 ul of serum.

Serum concentrations of antibodies dosed to mice were determined by immunoassay using either a plate-based sandwich ELISA method. Briefly, microtiter plate (Nunc, cat#442404) was coated with OX40 (ACRO, cat #1044-5CIS1-AF) at 1 ug/mL in 0.2 M CBS (PH9.4) at 4°C overnight. After washing, the plate was then blocked with 5% non-fat milk for 1.5 h at ambient temperature, mice serum samples were applied to coated plates for 1.5 h at RT. Anti-OX40 antibody was used to prepare the standard curve in pooled normal mouse serum with a range of 0.315–80 ng/mL, and QC samples.

Bound anti-OX40 antibody was detected using a horseradish peroxidase–labeled goat anti-human Fc antibody (Bethyl, cat#A80-104P-84) diluted at 1:100,000 in 5% BSA in 0.05% PBST (0.05% Tween in PBS). After a wash step, the plate was developed with TMB for 10-15min at room temperature, which was stopped after 5 min by the addition of 2 N sulfuric acid. The optical density was measured at 450 nm with a 620 nm reference wavelength subtraction, using a Thermo ELISA plate reader (Multiskan FC). Quantitation was based on a four-parameter logistic (1/Y2) regression of the prepared standard curves using Skanit Software3.1 (Thermo).

PK parameters (C_{max}, AUC, t_{1/2}, Cl, and V_{ss}) were analyzed by PKSolver software based on a non-compartmental model with means of concentration of each group (Table 23).

Table 23: PK summary of OX40 comparison in female Balb/C mice following a single 10 mg/kg bolus administration							
		Parameters					
Study	Compound	C _{max} (ug/mL)	AUC _{0-t} (ug*hr/mL)	AUC _{0-inf_obs} (ug/mL*hr)	CL (mL/hr/kg)	t _{1/2} (hr)	V _{ss} (mg/kg)/(ug/mL)
PK of OX40	ADI-20078-IgG1	135	24123	39579	0.00025	386	0.13
	ADI-20078-IgG2	168	29502	47639	0.00021	365	0.11
	ADI-20112-IgG1	184	27409	37486	0.00026	264	0.10
	ADI-20112-IgG2	204	39175	55246	0.00018	310	0.07

Example 10. Study on pharmaceutical efficacy of anti-OX40 antibody in combination with anti-PD-1 antibody on MC38 tumor-bearing OX40 transgenic mice

In this study, MC38 (mouse intestinal cancer cells) tumor-bearing OX40 transgenic mice were used to study on the synergistic antitumor efficacy of anti-OX40 antibody ADI-20112-IgG1 (referred as ADI-20112 in this example and in Figure 15) at different doses in combination with anti-PD1 antibody C. Antibody C is a monoclonal antibody against the programmed death receptor 1 (PD-1) (Patent application number: PCT/CN2017/072190).

Human OX40 transgenic mouse

Female human OX40 transgenic mice with C57B1/6 background (approximately 8 weeks old) were purchased from Shanghai Southern Model Biotechnology Co., Ltd. The mice were domesticated for 7 days after arrival and then the study was started.

Cell

MC38 murine colon cancer cells were purchased from Heyuan Biotechnology (Shanghai) Co., Ltd. and routinely subcultured for subsequent in vivo experiments strictly according to the instructions. The cells were collected by centrifugation, resuspended in sterile PBS and adjusted to a cell density of $5 \times 10^6/\text{ml}$. On day 0, 0.2 ml of the cell suspension was subcutaneously inoculated (1×10^6 cells/mouse) into the right abdomen region of human OX40 transgenic mice to establish a MC38-hOX40 tumor-bearing mouse model.

Administration

Six days after tumor cell inoculation, the tumor volume of each mouse was measured, and mice with a tumor volume ranging from 87.4 mm^3 to 228.4 mm^3 were selected and grouped according to the tumor volume on average, so that the average initial volume of each group was about 110 mm^3 .

The mice were divided into 7 groups (7 mice per group), and each group was intraperitoneally injected with the following doses of antibody:

Group 1 (control group): h-IgG (equitech-Bio), 10 mg/kg;

Group 2: anti-OX40 antibody (ADI-20112), 0.1 mg/kg;

Group 3: anti-OX40 antibody (ADI-20112), 1 mg/kg;

Group 4: anti-OX40 antibody (ADI-20112), 10 mg/kg;

Group 5: PD-1 antibody (Antibody C), 0.5 mg/kg;

Group 6: anti-OX40 antibody (ADI-20112) 1 mg/kg + PD-1 antibody (Antibody C) 0.5 mg/kg;

Group 7: Anti-OX40 antibody (ADI-20112) 10 mg/kg + PD-1 antibody (Antibody C) 0.5 mg/kg.

Reagent injection

On the 6th day after inoculation, each group of mice was administered with the above 7 groups of reagents, respectively, by intraperitoneal injection at a frequency of 2 times/week for 2 weeks.

Analysis:

Tumors and body weight were measured twice a week from day 6 (before dosing)

throughout the study and monitored continuously for 4 weeks. The maximum length axis (L) and the largest width axis (W) of the tumor were measured using a vernier caliper, and the tumor volume (V) was calculated as follows: $V = L \times W^2/2$. Tumor size from each group of mice was plotted against time. Analysis of variance (ANOVA) was used to determine statistical significance. P value of <0.05 was considered to be statistically significant in all analyses.

At the end of the experiment at Day 29, the mice were euthanized. The isolated tumor tissues were photographed and weighed, and the tumor weight and volume (tumor terminal volume) of each group were measured, and the relative tumor growth inhibition rate (TGI (%)) was calculated.

Result

The results of the experiment showed that after administration for 1 week, relative to group 1 (h-IgG-10 mg/kg), group 2-4 (ADI-20112-0.1mg/kg, ADI-20112-1mg/kg, ADI-20112-10 mg/kg) inhibited tumor growth in MC38-hOX40 tumor-bearing mice, and the tumor volume and tumor weight decreased, and tumor growth also slowed down, in a dose-dependent manner. The test results also showed that the anti-OX40 antibody ADI-20112 of the present invention exhibited a good synergistic antitumor effect with the anti-PD-1 mAb antibody C. The specific results are as follows.

As shown in Figure 15, after 1 week of administration, relative to group 1 (h-IgG-10 mg/kg group), group 2 (ADI-20112-0.1 mg/kg group) had a slower tumor growth while group 3-7 (ADI-20112-1mg/kg group, ADI-20112-10mg/kg group, antibody C-0.5mg/kg group, ADI-20112-1 mg/kg +antibody C-0.5 mg/kg, ADI-20112-10mg/kg+antibody C-0.5mg/kg) showed significant anti-tumor effects. Tumor growth in mice of group 1 (h-IgG-10 mg/kg administration group) to 500 mm^3 required 14 days, whereas group 2 (ADI-20112-0.1mg/kg administration group) required 19 days, group 3 (ADI-20112-1mg/kg group) required 22 days, group 4 (ADI-20112-10mg/kg group) required 26 days, group 5 (antibody C-0.5 mg/kg group), group 6 (ADI) -20112-1mg/kg +antibody C-0.5 mg/kg group), group 7 (ADI-20112-10mg/kg +antibody C-0.5 mg/kg group) had an average tumor volume of less than 500 mm^3 within 29 days. .

When the experiment was terminated after 29 days, in some experimental groups, the mouse tumor completely disappeared. Table 24 below summarizes the number of mice in each group with complete tumor disappearance.

Table 24: the number of mice in each group with complete tumor disappearance

Group	Administration(Antibody , dose)	The number of mice with complete tumor disappearance
Group 1	h-IgG, 10 mg/kg	0/7
Group 2	ADI-20112, 0.1 mg/kg	0/7
Group 3	ADI-20112, 1 mg/kg	0/7
Group 4	ADI-20112, 10 mg/kg	0/7
Group 5	Antibody C, 0.5 mg/kg	3/7
Group 6	ADI-20112+antibody C, 1+0.5 mg/kg	6/7
Group 7	ADI-20112+antibody C, 10+0.5 mg/kg	7/7

As shown in the above table, in group 5 (antibody C-0.5 mg/kg group), 3 mice had complete tumor disappearance; 6 mice in group 6 (20112-1 mg/kg +antibody C-0.5 mg/kg group) had complete tumor disappearance; 7 mice in group 7 (20112-10 mg/kg + antibody C-0.5 mg/kg) had complete tumor disappearance.

In addition, the present invention also calculated the relative tumor growth inhibition rate in mice of each group at the end of the experiment on Day 29. The relative tumor growth inhibition rate is calculated as follows:

Relative tumor growth inhibition rate $TGI(\%) = 100\% \times (Tvol_{control} - Tvol_{treated}) / (Tvol_{control} - Tvol_{predose})$

wherein $Tvol_{control} - Tvol_{treated}$ = tumor terminal volume after administration in the control group – tumor terminal volume after administration in the drug-administered group; $Tvol_{control} - Tvol_{predose}$ = tumor terminal volume after administration in the control group – tumor volume before administration in the control group (Tumor volume before administration on Day 6).

The calculation results are shown in Table 25 below:

Table 25: Effect of anti-OX40 antibody, anti-PD1 antibody, anti-OX40 antibody + anti-PD1 antibody on tumor tissue growth of tumor-bearing mice (mm³, Mean±SD, n=7;)

Group	Administration(Antibody, dose)	tumor at the end of the experiment (tumor terminal volume)	TGI(%)
Group 1	h-IgG, 10 mg/kg	2531.83±1037.94	N/A
Group 2	ADI-20112, 0.1 mg/kg	1859.82±508.64 [#]	28%
Group 3	ADI-20112, 1 mg/kg	1176.17±897.65	56%
Group 4	ADI-20112, 10 mg/kg	579.38±439.83	81%
Group 5	Antibody C, 0.5 mg/kg	435.07±498.04	86%
Group 6	ADI-20112+antibody C, 1+0.5 mg/kg	66.25±175.27	102%
Group 7	ADI-20112+antibody C, 10+0.5 mg/kg	0.00±0.00	104%

[#]indicated in this statistic analysis, n=6(20112-0.1 group, mice No. 3 died due to tumor rupture)

The results of the experiment showed in group 6 (ADI-20112-1mg/kg + antibody C-0.5 mg/kg group) and group 7 (ADI-20112-10 mg/kg + antibody C-0.5 mg/kg group), tumor growth inhibition rate was 102% and 104%, respectively, and the anti-OX40 antibody showed synergistic anti-tumor effects in combination with the anti-PD1 antibody.

Claims:

1. An anti-OX40 antibody or antigen-binding fragment thereof comprising a heavy chain variable region HCVR and a light chain variable region LCVR, wherein

(a) the HCVR contains

(i) three complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 contained in the HCVR of anyone of the antibodies listed in Table B, or

(ii) a combination of HCDR1, HCDR2 and HCDR3 as shown in Table A; and

(b) the LCVR contains:

(i) the LCDR1, LCDR2 and LCDR3 sequences contained in the LCVR of anyone of the antibodies listed in Table B; or

(ii) a combination of LCDR1, LCDR2 and LCDR3 as shown in Table A.

2. An anti-OX40 antibody or antigen-binding fragment thereof, comprising a heavy chain variable region (HCVR) and/or a light chain variable region (LCVR), wherein

(a) the HCVR comprises

(i) three complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 contained in the HCVR of anyone of the antibodies listed in Table B, or

(ii) a sequence comprising at least one and no more than 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions relative to the sequence of (i); or

(iii) a complementarity determining region (CDR) HCDR1, HCDR2 and HCDR3, wherein HCDR1 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16, or HCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16; HCDR2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33, or HCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32

and 33; HCDR3 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, 38, 39 and 40, or HCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to the amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, 38, 39 and 40; or

(iv) a combination of HCDR1, HCDR2 and HCDR3 as shown in Table A; or

(v) a variant of the HCDR combination of (iv) comprising at least one and no more than 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions.

and/or

(b) the LCVR comprises

(i) the LCDR1, LCDR2 and LCDR3 sequences contained in the LCVR of anyone of the antibodies listed in Table B; or

(ii) a sequence comprising at least one and no more than 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in the three CDR regions relative to the sequence of (i), or

(iii) complementarity determining regions (CDRs) LCDR1, LCDR2 and LCDR3, wherein LCDR1 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 42, 43, 44, 45 and 46, or LCDR1 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NO: 41, 42, 43, 44, 45 and 46; LCDR2 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOS: 47, 48, 49, 50, 51, and 52, or LCDR2 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NO: 47, 48, 49, 50, 51, and 52; LCDR3 comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60 and 61, or LCDR3 comprises an amino acid sequence having one, two or three changes (preferably amino acid substitutions, preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60 and 61, or

(iv) a combination of LCDR1, LCDR2 and LCDR3 as shown in Table A; or

(v) a variant of the LCDR combination of (iv) comprising at least one and no more than 5 amino acid changes (preferably amino acid substitutions, preferably conservative substitutions) in total in said three CDR regions.

3. The antibody or antigen-binding fragment thereof according to claims 1 or 2, which comprises heavy chain variable region HCVR and/or a light chain variable region LCVR, wherein

(a) heavy chain variable region HCVR

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120; or

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 and 120, preferably, said amino acid changes do not occur in the CDR regions;

and/or

(b) light chain variable region LCVR

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 130, 131, 132, 133, 134, 135, 136, 137 and 138;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 130, 131, 132, 133, 134, 135, 136, 137 and 138; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the

group consisting of SEQ ID NOs: 130, 131, 132, 133, 134, 135, 136, 137 and 138, preferably, said amino acid changes do not occur in the CDR regions.

4. An antibody or antigen-binding fragment thereof of anyone of claims 1-3, which comprises a heavy chain and/or light chain, wherein

(a) heavy chain

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or 167;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or 167; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 20 or 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably amino acid conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 189, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166 or 167, preferably, said amino acid changes do not occur in the CDR regions, more preferably, said amino acid changes do not occur in the heavy chain variable region;

and/or

(b) light chain

(i) comprising or consisting of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 168, 169, 170, 171, 172, 173, 174, 175 or 176;

(ii) comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 168, 169, 170, 171, 172, 173, 174, 175 or 176; or

(iii) comprising an amino acid sequence having one or more (preferably no more than 20 or 10, more preferably no more than 5) amino acid changes (preferably amino acid substitutions, more preferably conservative substitutions) compared to an amino acid sequence selected from the group consisting of SEQ ID NOs: 168, 169, 170, 171, 172, 173, 174, 175 or 176, preferably,

said amino acid changes do not occur in the CDR regions, more preferably, said amino acid changes do not occur in the light chain variable region.

5. An anti-OX40 antibody or fragment thereof, which has one or more of the following characteristics:

(i) showing the same or similar binding affinity and/or specificity as anyone of the antibodies listed in Table 5 for OX40 (particularly human OX40);

(ii) inhibiting (e.g, competitive inhibiting) the binding of anyone of the antibodies listed in Table 5 to OX40 (particularly human OX40);

(iii) binding to an epitope that is the same or overlaps with that bound by anyone of the antibodies shown in Table 5;

(iv) competing with anyone of the antibodies shown in Table 5 to bind OX40 (particularly human OX40);

(v) having one or more of the biological properties of anyone of the antibodies listed in Table 5.

6. The anti-OX40 antibody or antigen-binding fragment thereof of anyone of claims 1-5, which has one or more of the following characteristics:

(i) capable of binding to human OX40 better while less blocking the binding of human OX40 to OX40 ligand (OX40L), and producing an enhanced immune response;

(ii) binding to human OX40 or cynomolgus monkey OX40, and preferably while not binding to murine OX40 or binding to murine OX40 less than binding to human or cynomolgus OX40 in the meantime, wherein the murine OX40 is e.g., rat OX40 or mouse OX40;

(iii) binding to human OX40 or cynomolgus monkey OX40 with a K_D of less than about 200 nM, preferably less than or equal to about 20nM, about 10 nM, more preferably less than or equal to about 5 nM, and most preferably, the K_D is less than or equal to about 1 nM;

(iv) binding to human OX40 or cynomolgus OX40 expressed in a cell has an EC_{50} of less than or equal to about 10 nM, 5nm or 3 nM;

(v) capable of activating T cells, for example, enhancing CD4+ effector T cell function, for example by increasing CD4+ effector T cell proliferation and/or increasing gamma-interferon(such as IFN γ or an interleukin, such as IL-2) production of CD4+ effector T cells;

(vi) increasing OX40 signaling transduction in the target cell expressing OX40, preferably,

increasing NFκB-mediated transcriptional activity;

(vii) having better anti-tumor activity, e.g., capable of reducing the tumor volume in a subject, while not influencing the body weight of the subject in the meantime.

7. The anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody is an antibody in IgG1 format or an antibody in IgG2 format or an antibody in IgG4 format or antigen-binding fragment thereof.

8. The anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 7, wherein the antibody is a monoclonal antibody.

9. The anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, wherein the antibody is a humanized antibody or a human antibody.

10. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 9, wherein the antigen-binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, single chain antibody (e.g., scFv) or (Fab')₂, single domain antibody, diabodies (dAb) or linear antibody.

11. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 10, wherein said antibody is a bispecific or multispecific antibody molecule, preferably the bispecific antibody molecule binds to OX40 and PD-1, PD-L1 or PD-L2.

12. An isolated nucleic acid encoding an anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11.

13. A vector comprising the nucleic acid according to claim 11, preferably said vector is an expression vector.

14. A host cell comprising the vector according to claim 13, preferably the host cell is prokaryotic or eukaryotic, more preferably selected from yeast cells, mammalian cells (e.g., CHO cells or 293 cells), or other cells suitable for the preparation of antibodies or antigen-binding fragments thereof.

15. A method of preparing an anti-OX40 antibody or antigen-binding fragment thereof comprising culturing the host cell according to claim 14 under conditions suitable for expression of a nucleic acid encoding an anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11, optionally isolating the antibody or antigen-binding fragment thereof, optionally said method further comprises recovering the anti-OX40 antibody or antigen-binding fragment thereof from the host cell.

16. An immunoconjugate comprising the anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11 and a cytotoxic agent.

17. A pharmaceutical composition comprising the anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11, or the immunoconjugate according to claim 16, and optionally a pharmaceutically acceptable adjuvant, and optionally further comprising one or more other therapeutic agents, e.g., a chemotherapeutic agent, an anti-angiogenic agent(e.g., bevacizumab), an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody.

18. A method of activating T cells or inducing T cell mediated antitumor activity or enhancing the body immune response in a subject comprising administering to said subject an effective amount of the anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11, or an immunoconjugate according to claim 16, or a pharmaceutical composition according to claim 17.

19. A method of treating cancer in a subject comprising administering to said subject an effective amount of the anti-OX40 antibody or antigen-binding fragment thereof according to any one of claims 1 to 11, or the immunoconjugate of claim 16, or the pharmaceutical combination of claim 17, preferably said cancer is lung cancer (e.g., non-small cell lung cancer), liver cancer, stomach cancer, or colon cancer.

20. The method of claim 20, further comprising administering to said subject in combination with one or more therapies, e.g., a therapeutic manner and/or other therapeutic agent, preferably the therapeutic manner includes surgery and/or radiation therapy, and/or the other therapeutic agent is selected from the group consisting of a chemotherapeutic agent, a PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody or an anti-PD-L1 antibody or an anti-PD-L2 antibody), or an anti-angiogenic agent (e.g., bevacizumab).

21. A method of detecting OX40 in a sample, said method comprising:

(A) contacting the sample with any anti-OX40 antibody or antigen binding fragment thereof according to anyone of claims 1 to 11;

(B) detecting the formation of a complex between the anti-OX40 antibody or antigen binding fragment thereof and the OX40; optionally, the anti-OX40 antibody is detectably labeled.

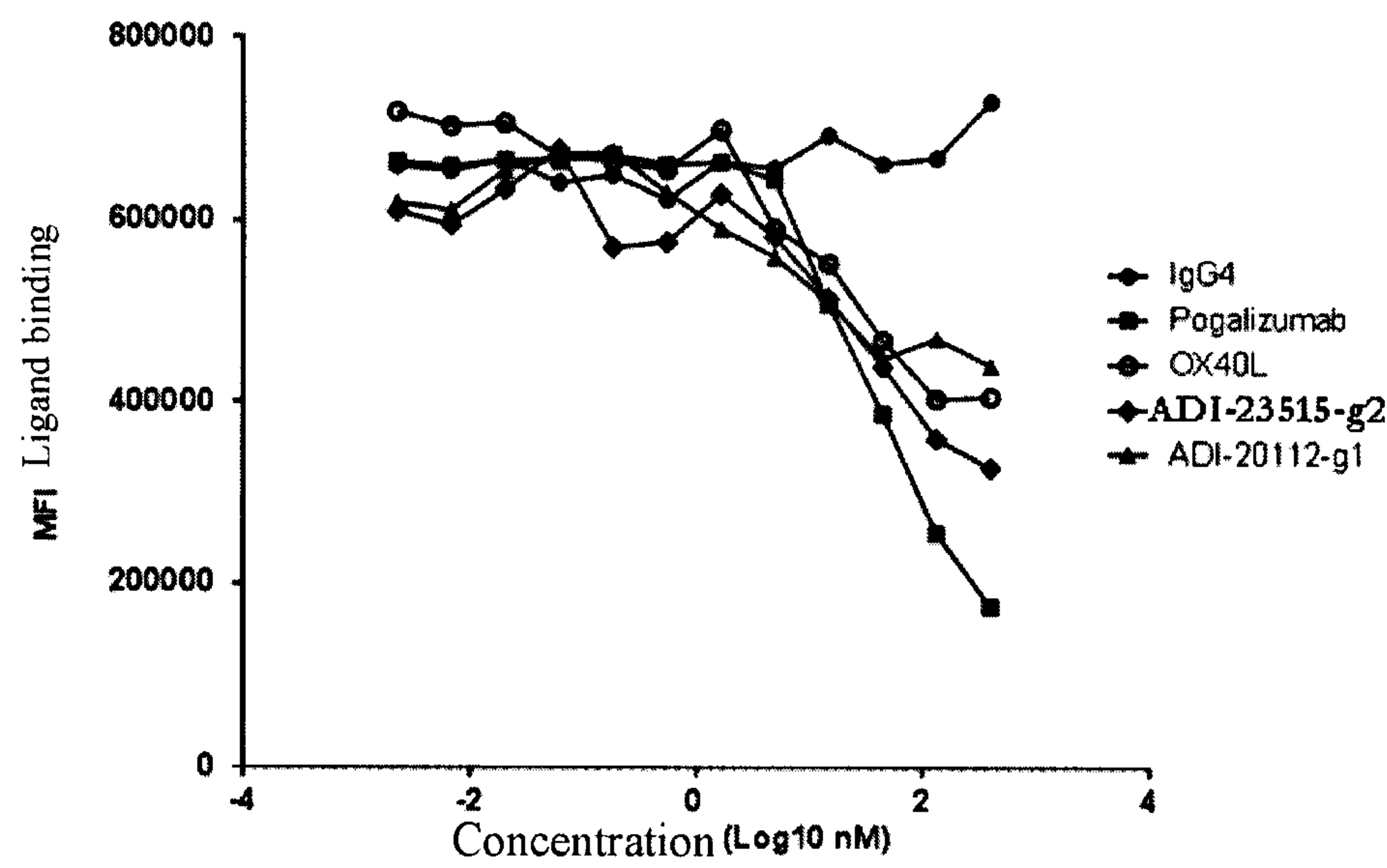


Fig 1

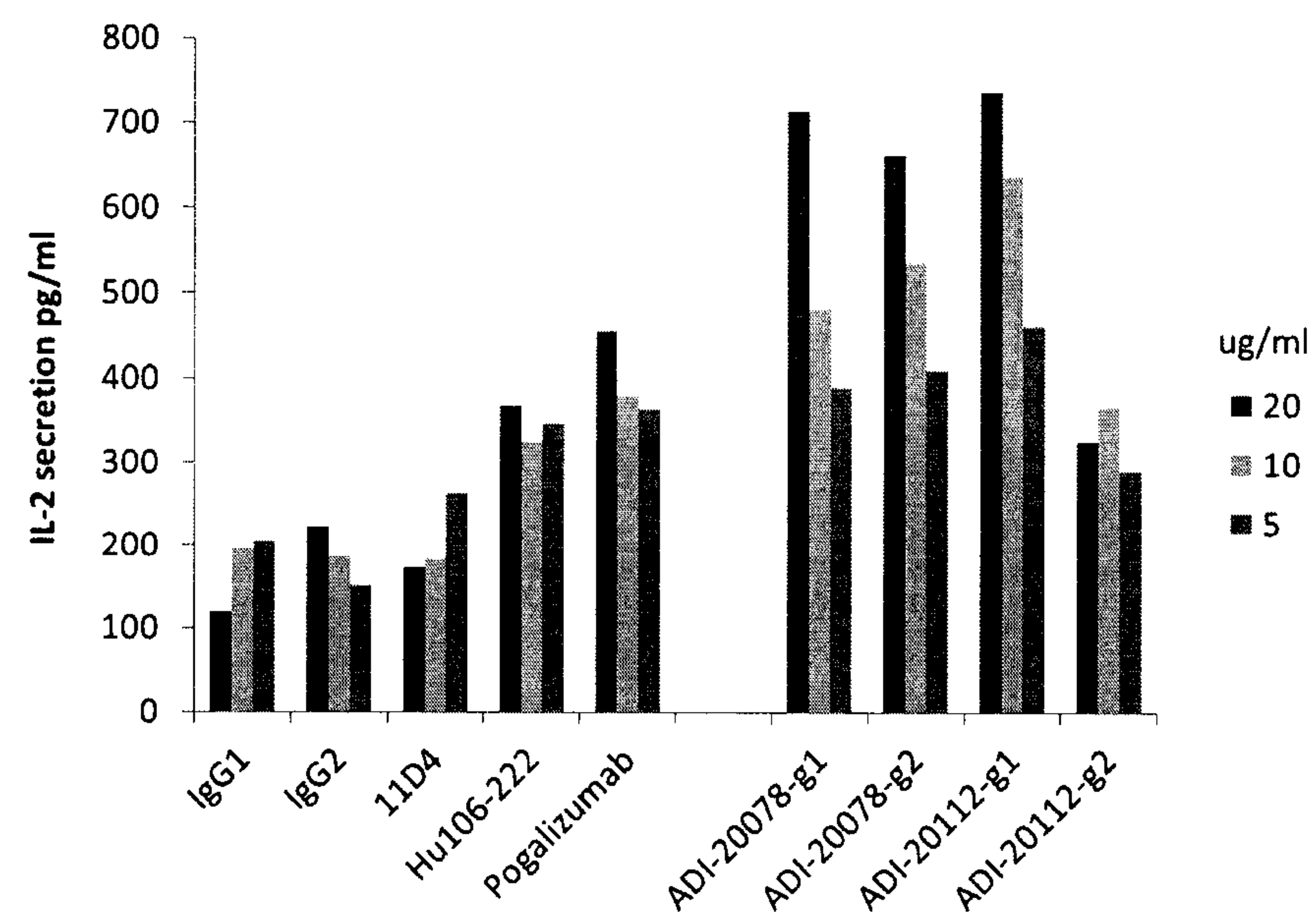


Fig 2

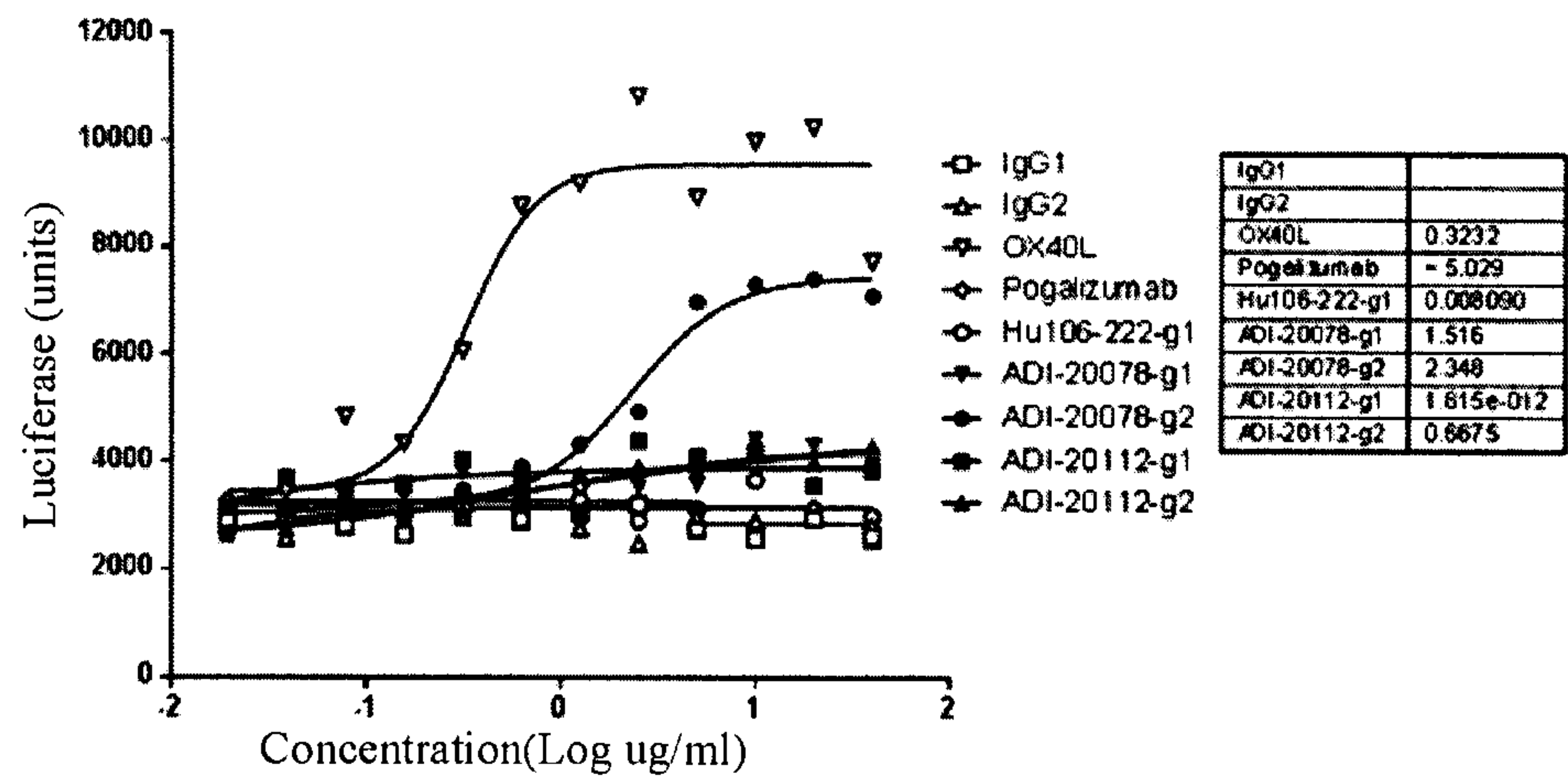


Fig 3

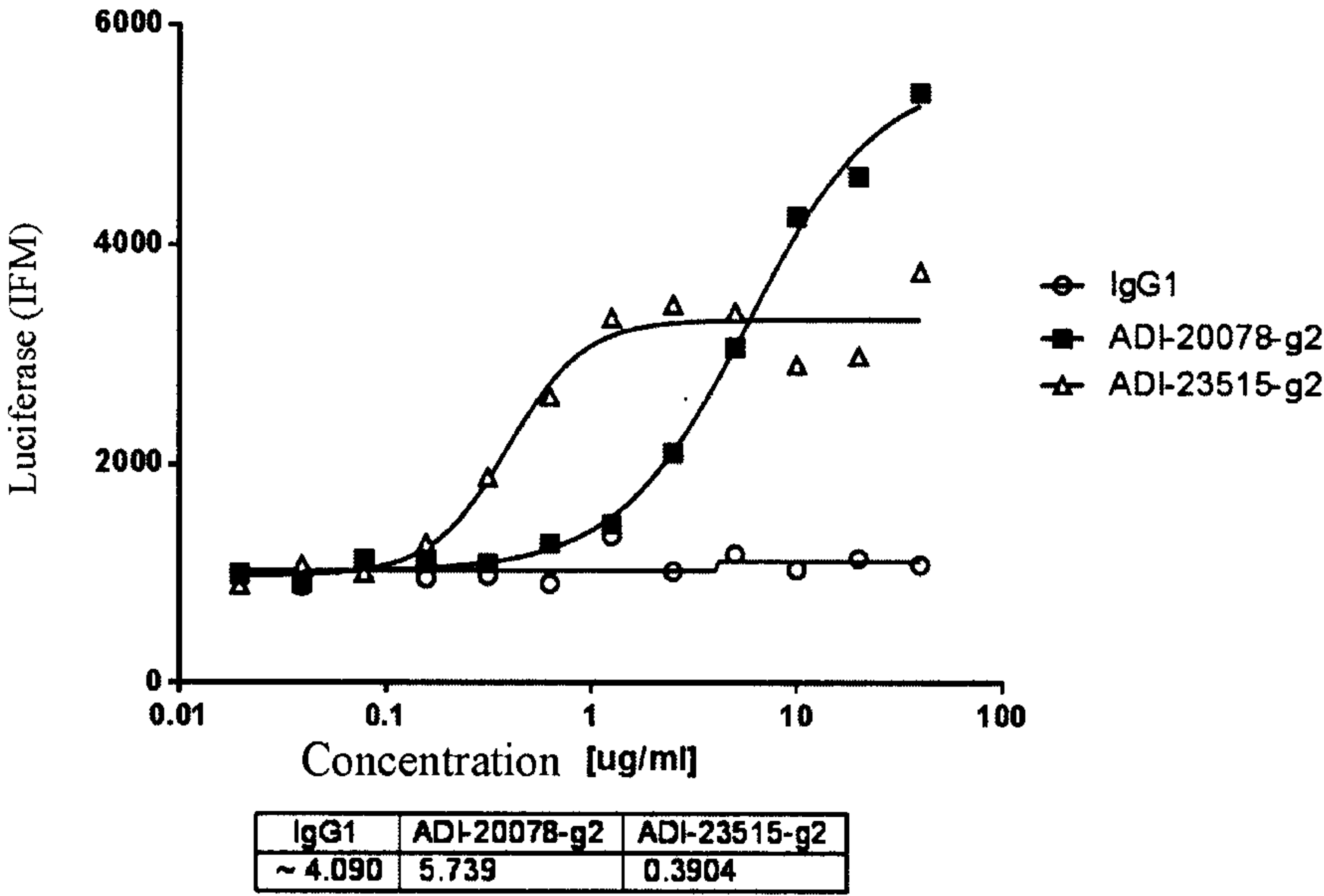


Fig 4

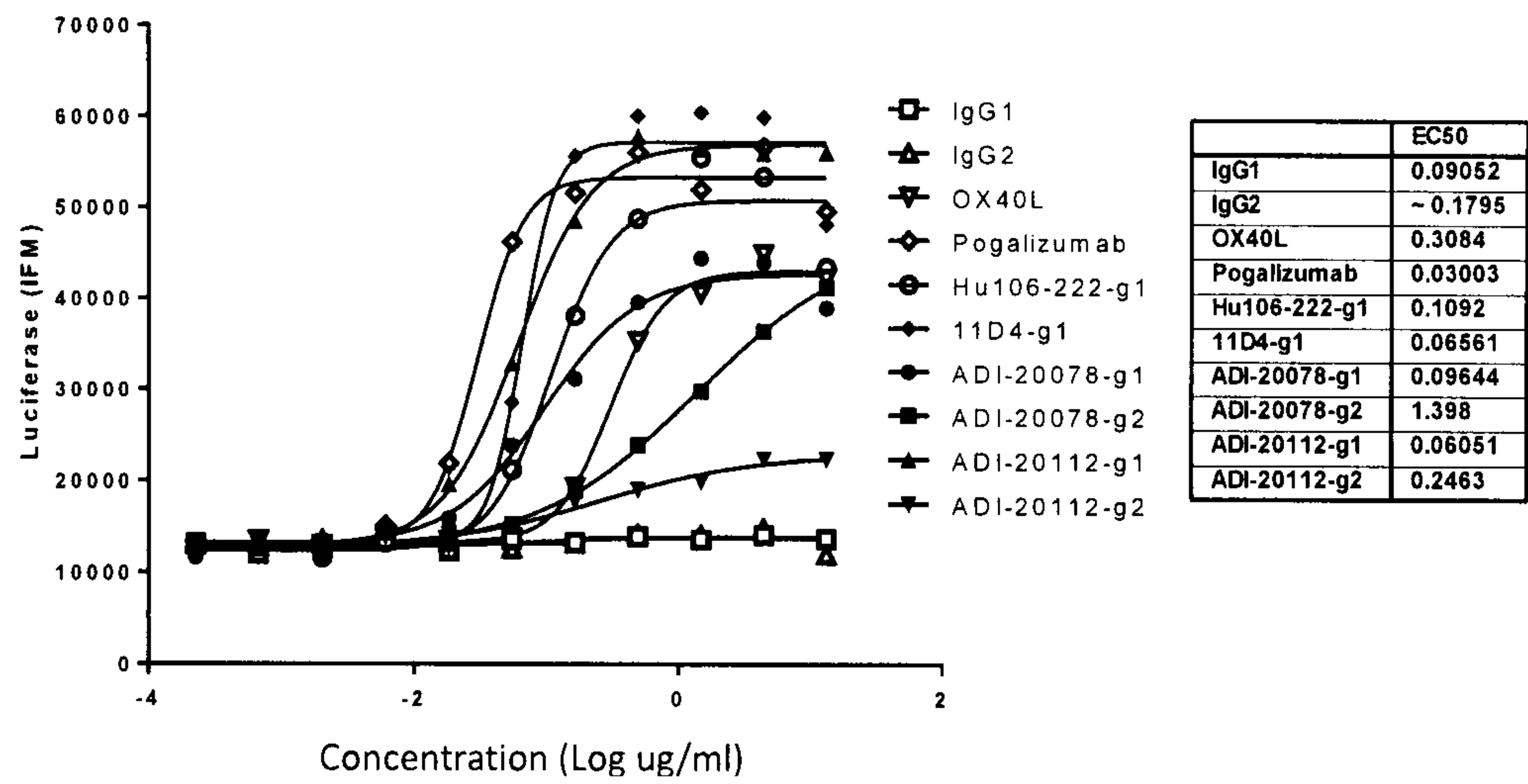


Fig 5

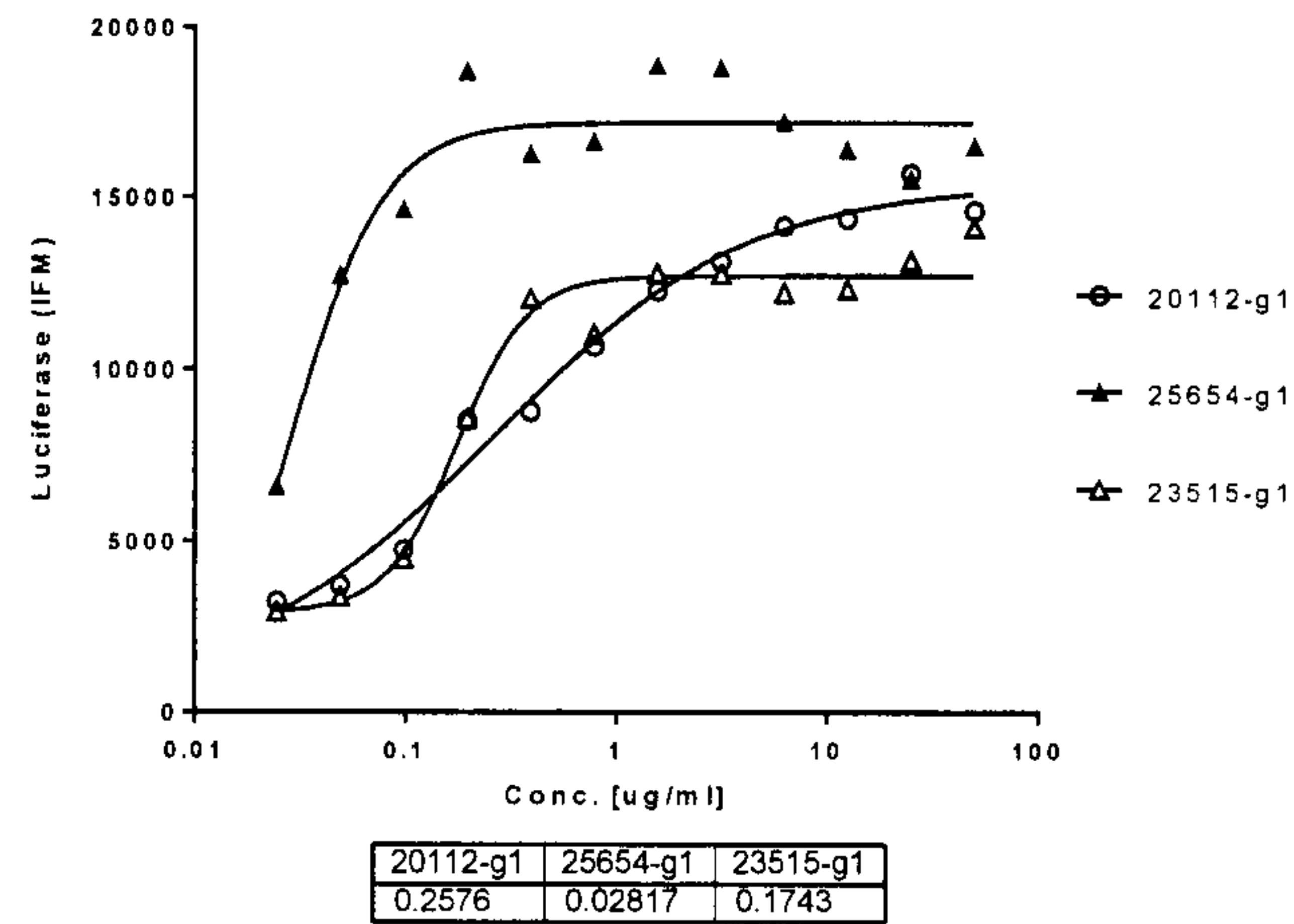


Fig 6

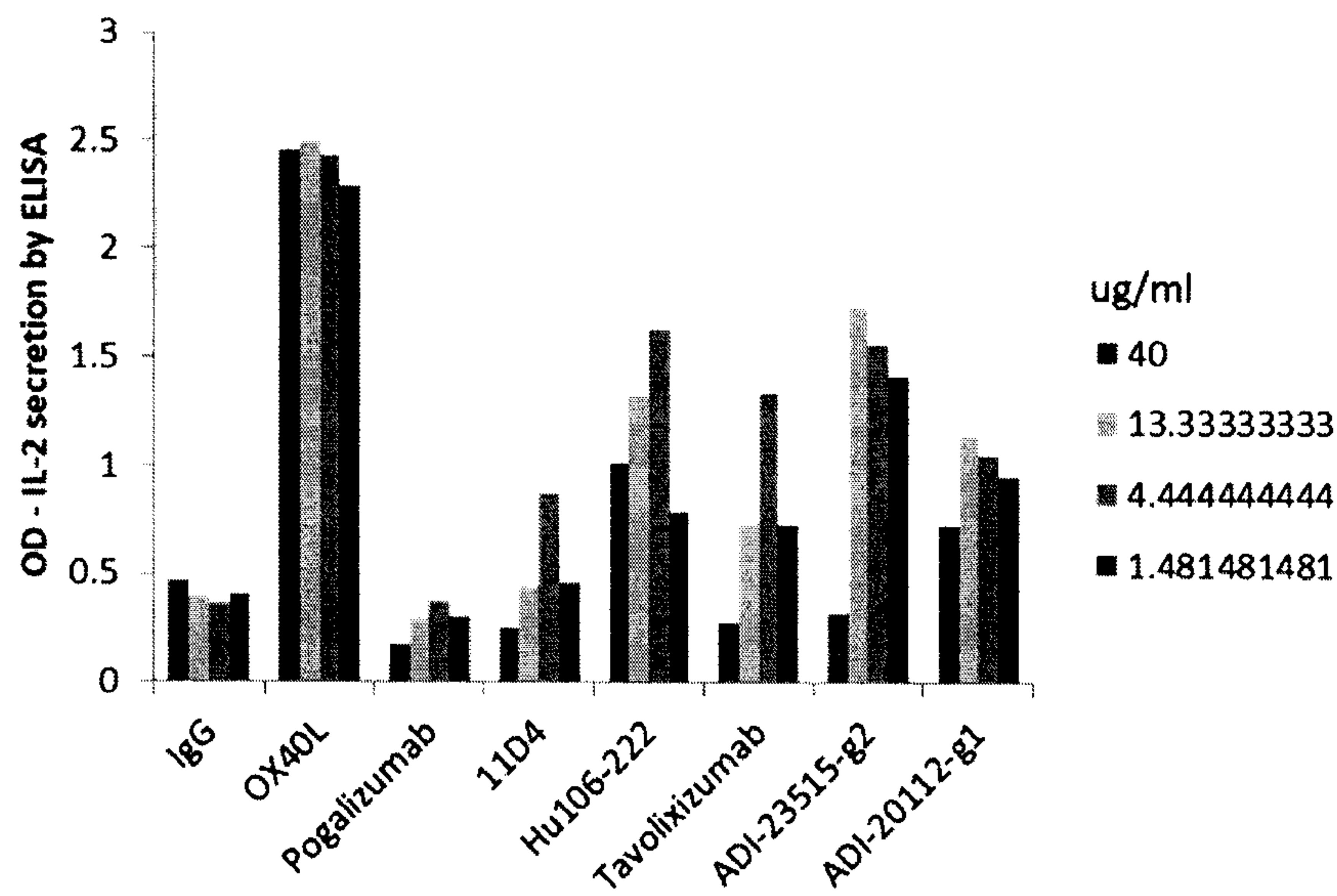


Fig 7

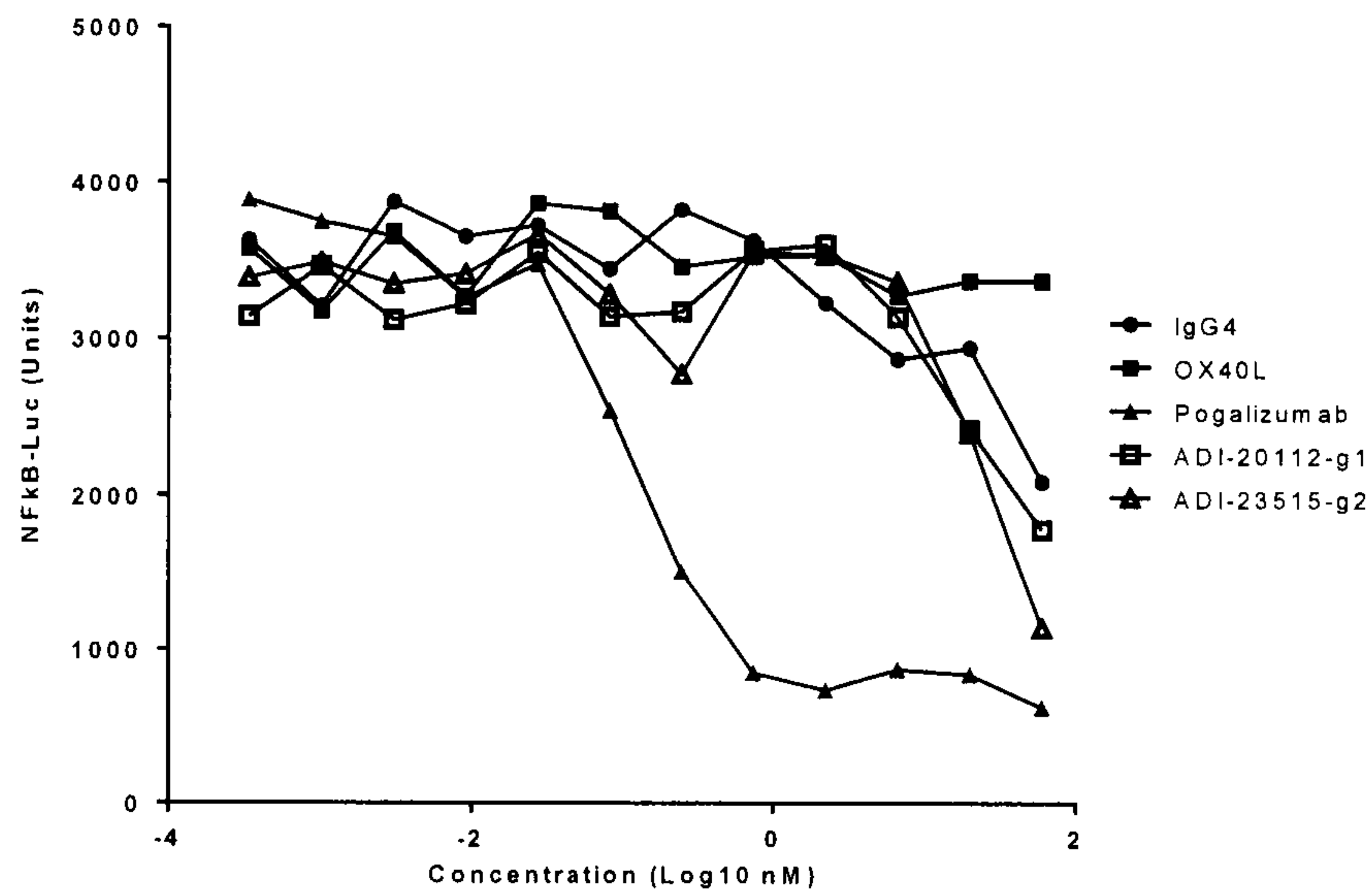


Fig 8

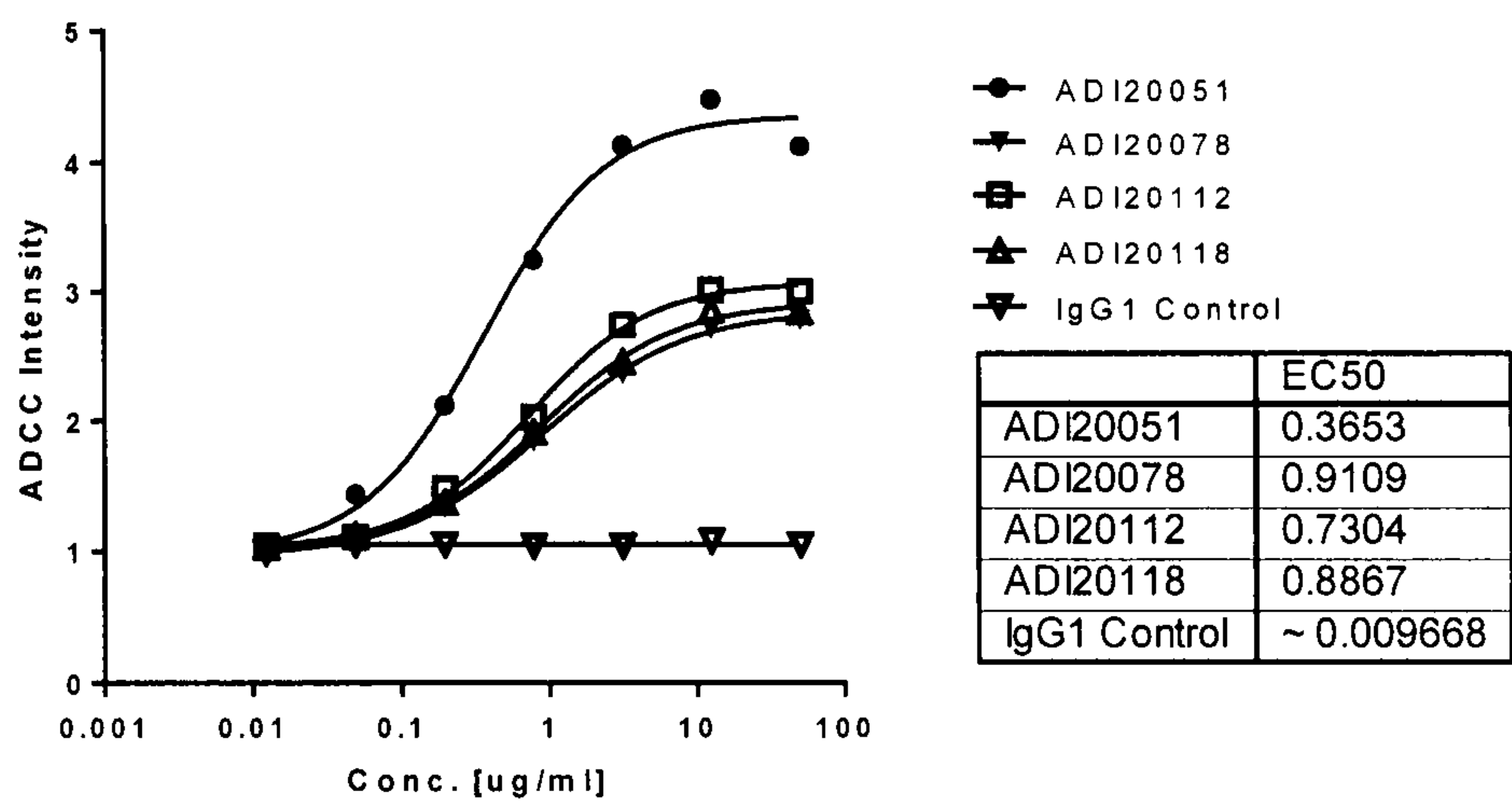


Fig 9

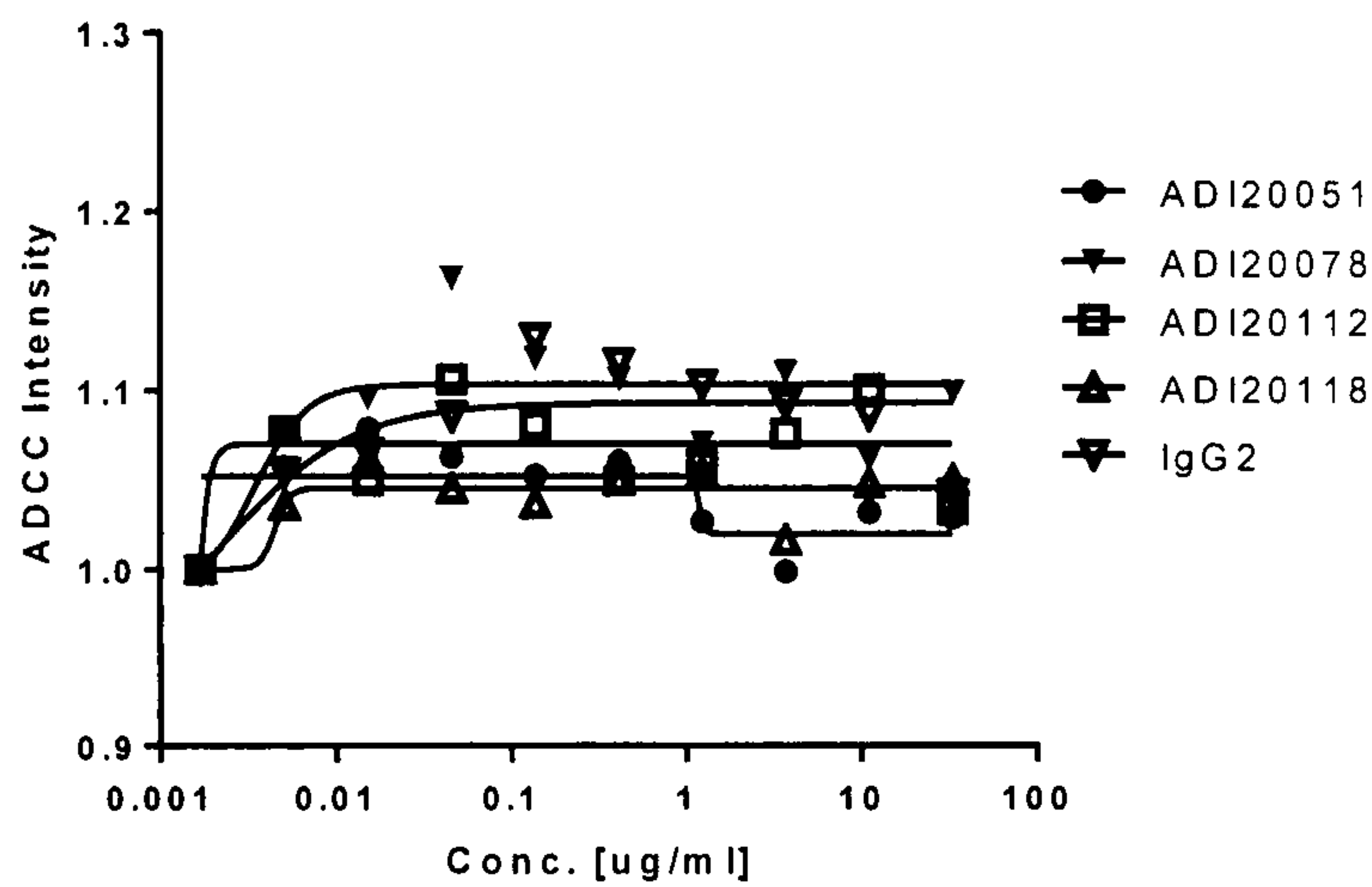


Fig 10

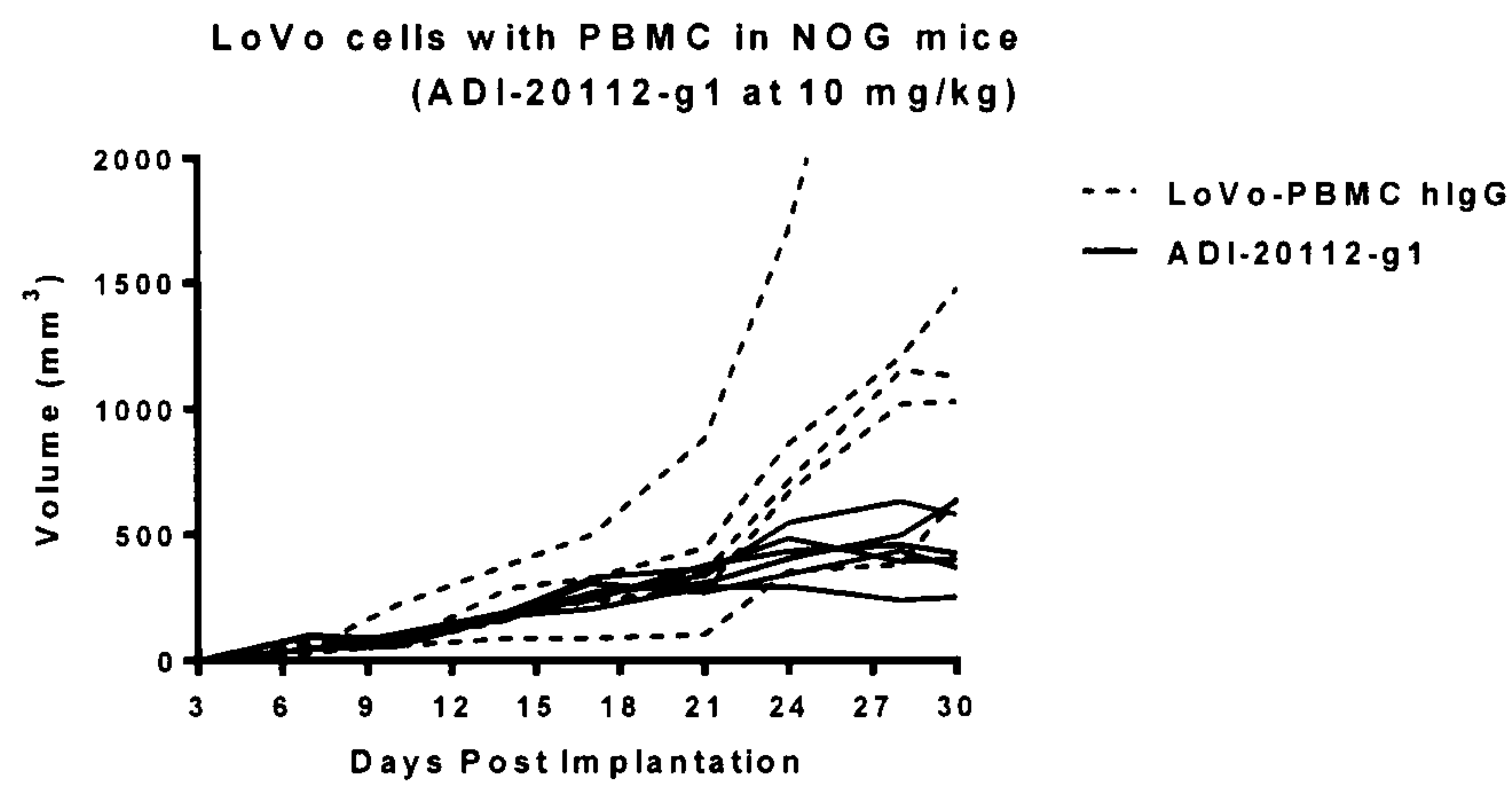


Fig 11

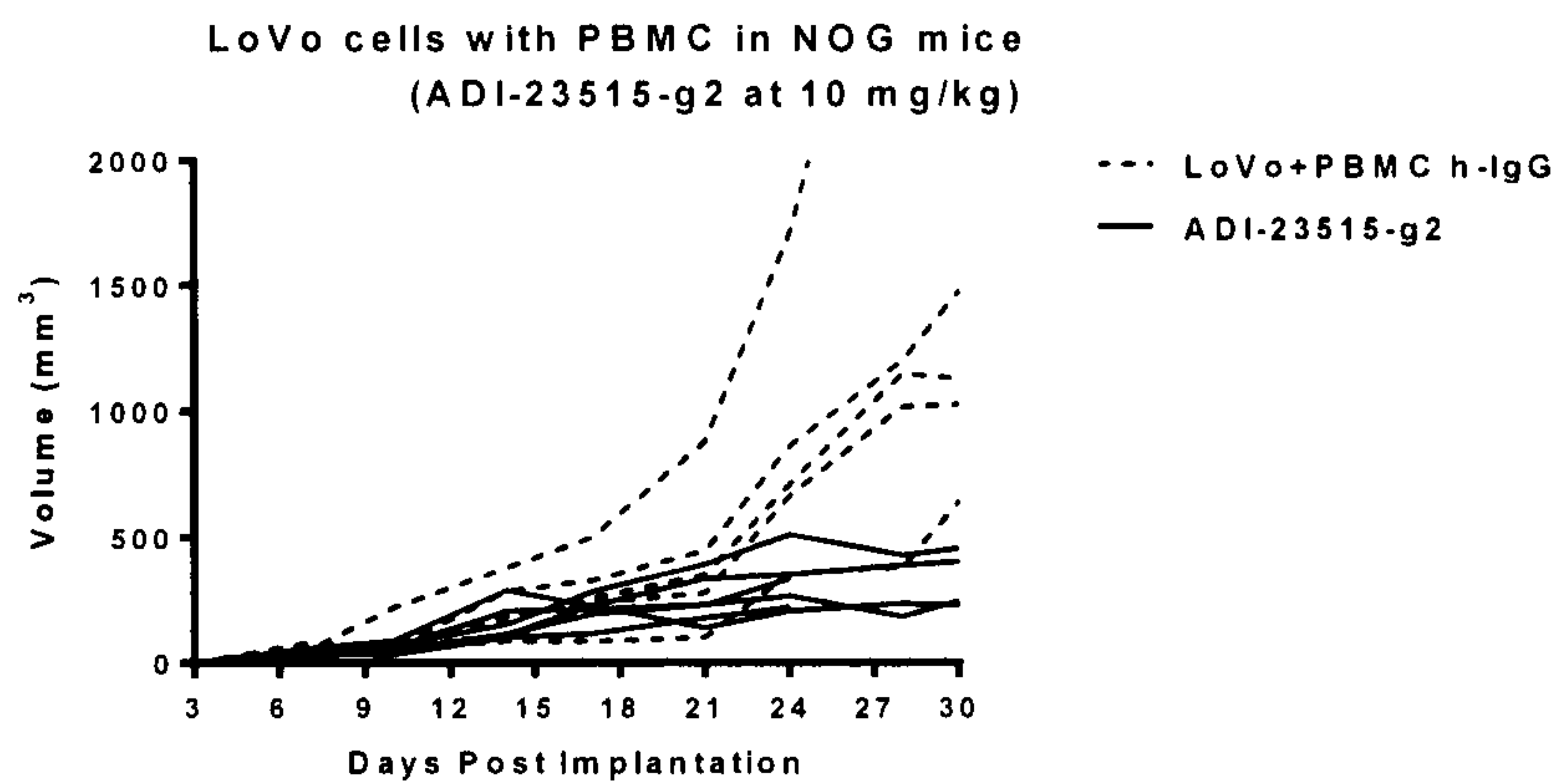


Fig 12

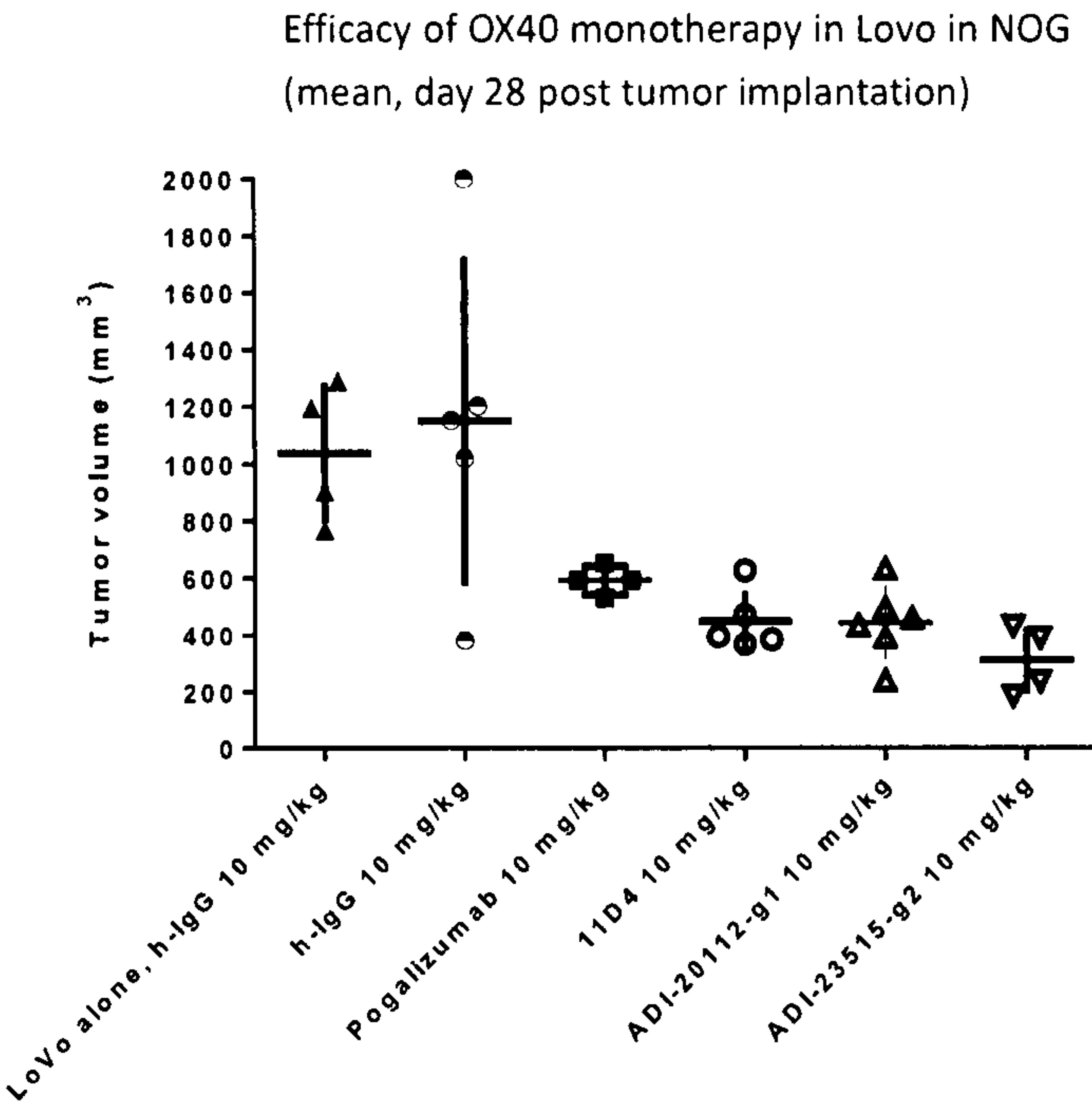


Fig 13

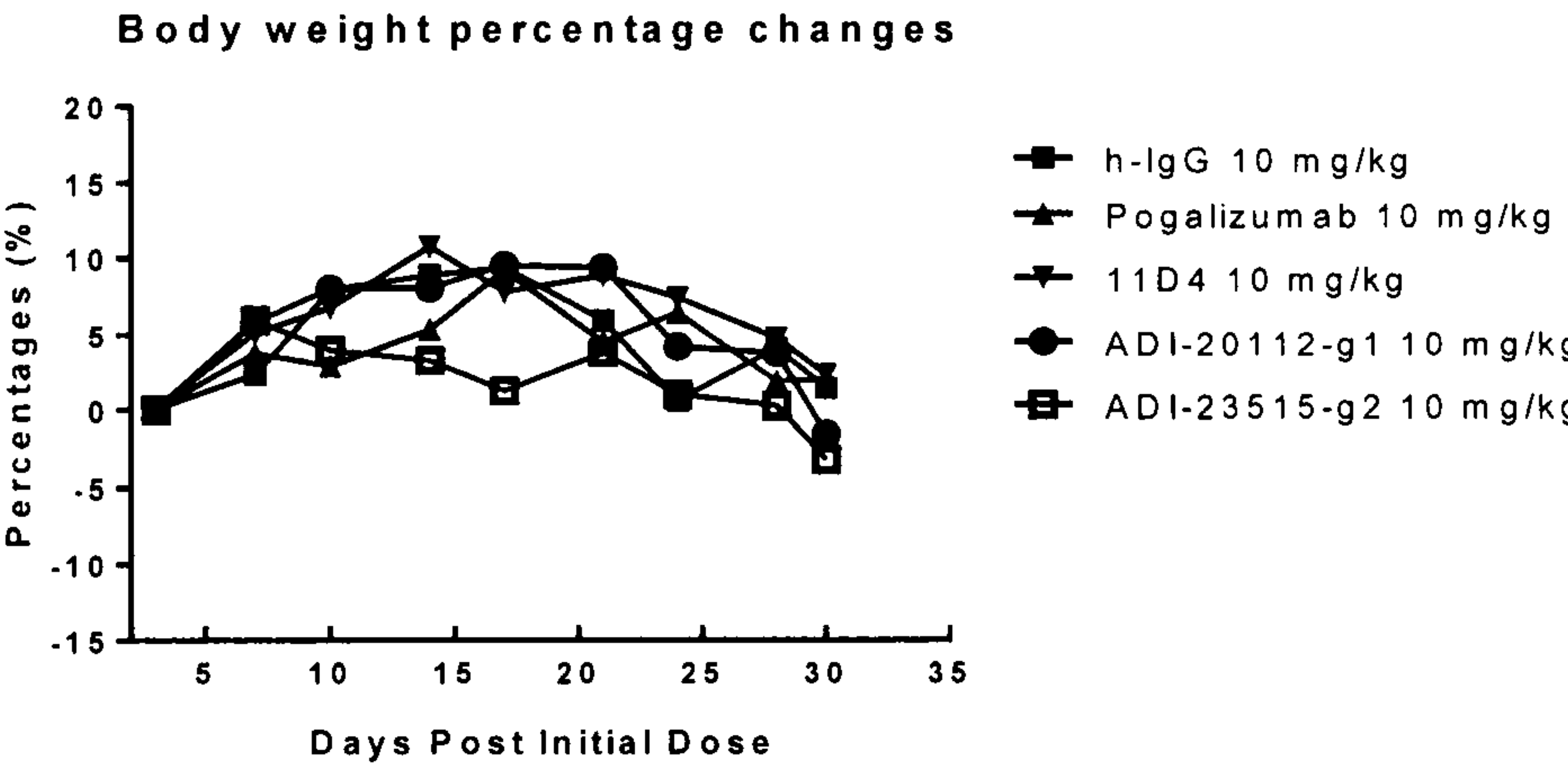


Fig 14

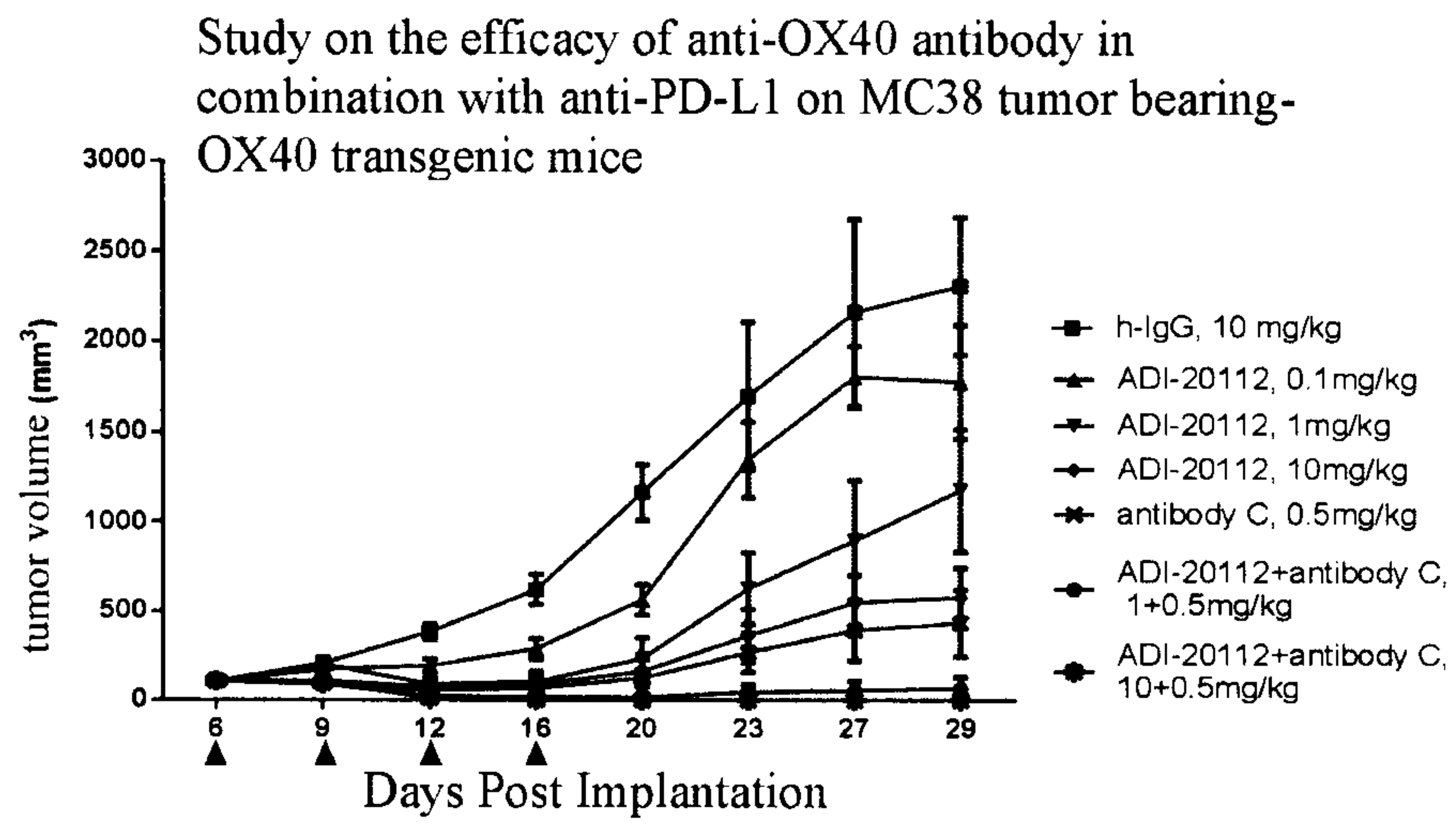
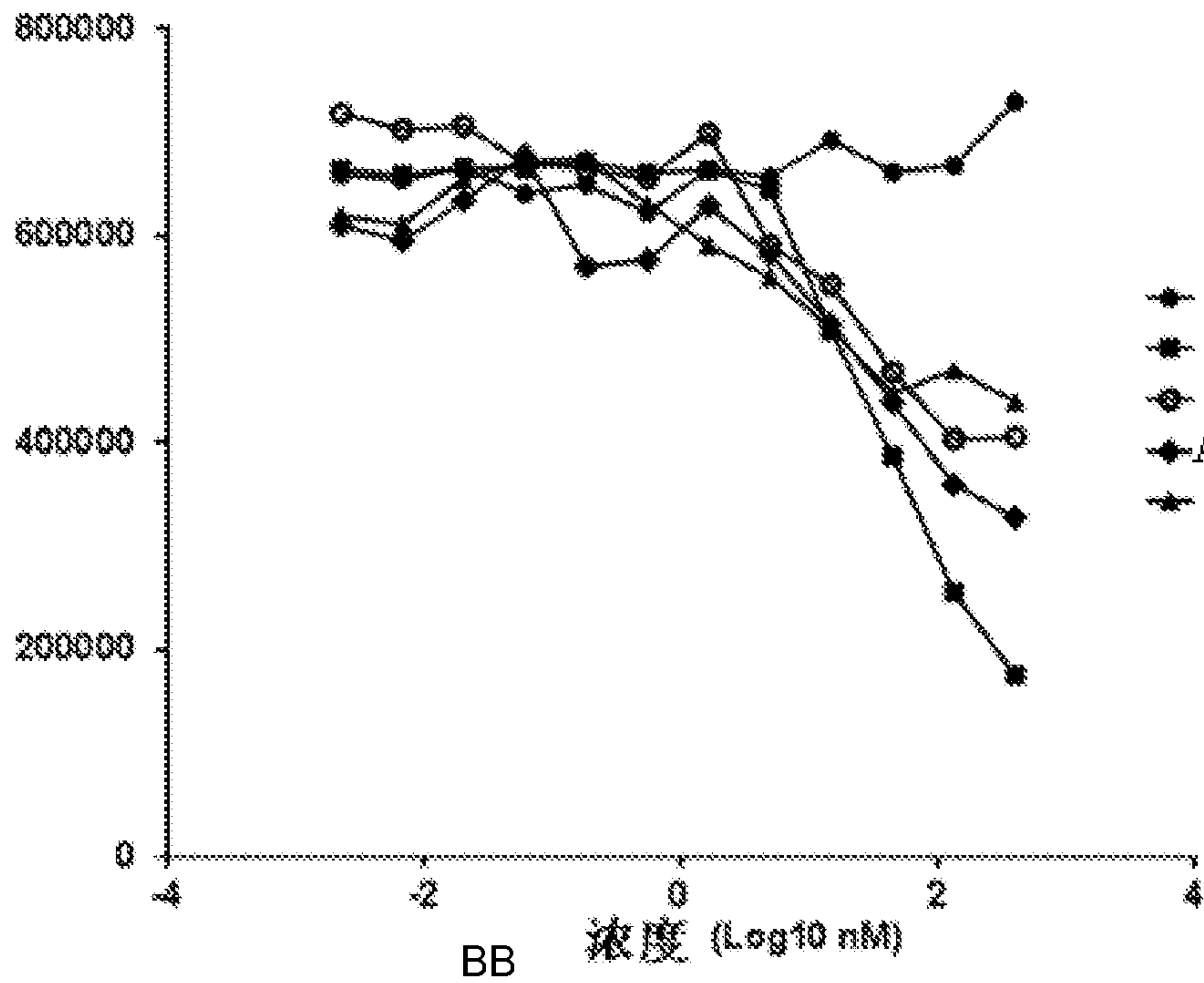


Fig 15

AA

配体结合
RFI



- IgG4
- Pegalizumab
- ON40L
- ADI-23515-g2
- ADI-20112-g1

图 1

AA Ligand binding
BB Concentration