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(54) Title: NOVEL ANTAGONISTIC ANTI TNFR2 ANTIBODY MOLECULES

(57) Abstract: Described are novel antagonistic antibody molecules that specifically bind to TNFR2 on a target cell and thereby block TNF-α binding to TNFR2 and block TNFR2 signaling, wherein the antibody molecules also bind to Fc receptors via the Fc region. Also described is the use of such antibody molecules in treatment of cancer or infections caused by intracellular pathogens.

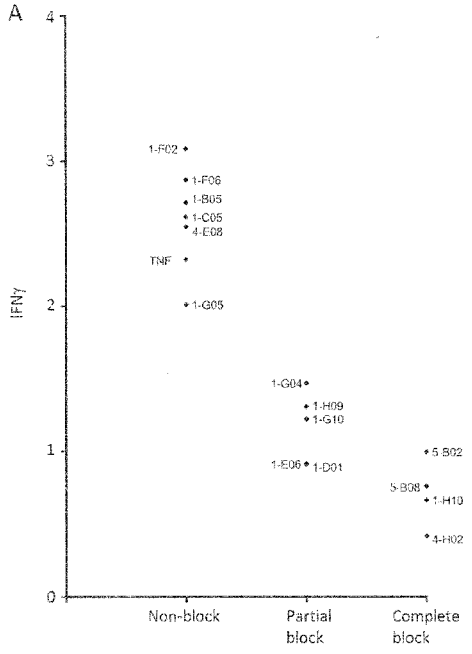

Fig. 8

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NOVEL ANTAGONISTIC ANTI TNFR2 ANTIBODY MOLECULES

FIELD OF THE INVENTION

The present invention relates to novel antagonistic antibody molecules that specifically bind to tumor necrosis factor receptor 2 (TNFR2) on target cells and thereby block the ligand TNF–α from binding to TNFR2 and that also block TNFR2 signaling, which antibody molecules also bind to an Fc receptor via their Fc region. The invention also relates to use thereof in medicine, such as in treatment of cancer or infections caused by an intracellular pathogen.

BACKGROUND OF THE INVENTION

Tumor necrosis factor (TNF) receptor 2 (TNFR2, TNFR-2 or TNFRII), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B) and CD120b, is a membrane receptor that binds tumor necrosis factor-alpha (TNF-α or TNFα). It is found i.e. on the surface of T cells, monocytes and macrophages, and can activate the proliferation of TNFR2 receptor expressing cells through nuclear factor kappa B (NF-κB). Notably, TNFR2 is highly upregulated in cancer and in particular on tumor-infiltrating immune cells, e.g. regulatory T cells (Tregs), $CD8^+$ cytotoxic effector T cells, and different myeloid cell subpopulations.

TNFR2 has been discussed as a promising target for cancer immunotherapy, and has been described to be highly expressed on the surface of i.a. intratumoral Tregs and many human tumor cells (Williams GS et al, Oncotarget. 2016; 7(42): 68278–68291; Vanamee ES et al , Trends in Molecular Medicine, 2017, vol. 23, issue 11, 1037-1046, Frontiers in Immunology , November 2017 | Volume 8 | Article 1482, Sci Signal. 2018 Jan 2;11(511).

Regulatory T cells (which may also be called Treg cells, Tregs or T_{regs}, and which formerly were known as suppressor T cells or suppressive regulatory T cells) constitute a subpopulation of T cells capable of suppressing other immune cells in normal and pathological immune settings. Tregs are CD4 positive cells ($CD4^+$ cells). There are other $CD4^+$ T cells that are not Tregs; however, Tregs can be separated from non-Treg $CD4^+$ cells in that Tregs also are FOXP3 positive ($FOXP3^+$) while the non-Treg $CD4^+$ cells are FOXP3 negative ($FOXP3^-$). Tregs can also be separated from non-Treg $CD4^+$ cells in that Tregs also are $CD25^+CD127^{neg/low}$ while the non-Treg $CD4^+$ cells are either $CD25^-CD127^+$or $CD25^+CD127^+$

TNFR2 has also been discussed in connection with autoimmune diseases (Faustman DL et al, Front Immunol. 2013; 4: 478, Clin Transl Immunology. 2016 Jan

8;5(1):, J Neurosci. 2016 May 4;36(18):5128-43) and inflammatory diseases (Ait-Ali D et al, Endocrinology. 2008 Jun;149(6):2840-52, Sci Rep. 2016 Sep 7;6:32834).

Anti-TNFR2 antibodies of different types and with various characteristics have also been described previously. For example, Williams et al (Oncotarget. 2016 Oct 18;7(42):68278-68291) describes both ligand blocking and ligand non-blocking agonistic antibodies.

WO 2014/124134 discloses the use of a TNFR2 agonist, such as an agonistic anti-TNFR2 antibody) and/or an NF-κB activator for *in vitro* production of a composition enriched in CD4+CD25^{hi} Tregs. The composition is said to be useful in treatment of immunological disorders or infectious diseases in patients. WO 2014/124134 further discloses TNFR2 antagonist antibodies that can bind one or two epitopes of TNFR2. The first of these epitopes included the sequence QTAQMCCSKCSPGQHAKVFC and the second epitope included one specific amino acid in one specific position in the amino acid of human TNFRs; the second epitope may include the sequence RLCAPLRKCRPGF. Such TNFR2 antagonists are said to be useful to produce compositions enriched in lymphocytes and depleted of Tregs. In granted US patent No. 9 821 010, originating from this PCT application, the antagonistic antibody has been specified as selectively binding to an epitope within the sequence KQEGCRLCAPLRKCRPGFGV, such as the epitope comprising the sequence RLCAPLRKCRPGF. The TNFR2 antagonists, and the compositions produced using the TNFR2 antagonists, are said to be useful in treatment of proliferative disorders, such as cancer, or infectious diseases.

WO 2016/187068 discloses antibodies capable of antagonizing tumor necrosis factor receptor superfamily members, such as TNFR2. The antibodies are said to be useful to modulate Tregs, such as in immunotherapy for treatment of proliferative disorders and infectious disease. In particular, it discloses antagonistic TNFR2 antibodies binding to specific epitopes of human TNFR2, and it presents a number of specific CDR sequences of such antibodies. Data in WO 2016/187068 is said to demonstrate that the specific binding of the Fab regions of the antagonistic TNFR2 antibodies to TNFR2 is likely responsible for modulating Treg cell growth, rather than non-specific binding of the Fc regions of these antibodies

WO 2017/040312 discloses anti-TNFR2 antibodies, and in particular agonistic anti-TNFR2 antibodies, that are capable of promoting TNFR2 signaling and having an effect on expansion or proliferation of Tregs. WO 2017/040312 discloses antibodies that bind specifically to an epitope comprising the sequence KCSPG, but not to an epitope comprising the sequence KCRPG, thus excluding the antibodies of US 9 821 010 discussed above, or alternatively not to another TNFR superfamily member. The agonistic

antibodies are said to be useful in treatment of immunological diseases. WO 2017/040312 further sets out the full sequence of human TNFR2.

WO 2017/083525 discusses pharmacological compositions comprising anti-TNFR2 antibodies, and use thereof in treatment of disorders associated with TNF-α and/or TNFR2, such as cancer. WO 2017/083525 further discusses antibodies comprising a human IgG1 Fc domain which is null for binding to an Fcγ receptor, and also suppression of expansion of Tregs.

WO 2017/197331 discloses antagonistic TNFR2 antibodies comprising complementarity determining region-heavy chain 3 having specific sequences, and discusses reducing or inhibiting the proliferation of Tregs and/or promotion of proliferation of T effector cells.

Fc receptors are membrane proteins which are found on the cell surface of immune effector cells including monocytes, macrophages, dendritic cells, neutrophils, mast cells, basophils, eosinophils and Natural Killer cells and B lymphocytes. The name is derived from their binding specificity for the Fc region of antibodies. Fc receptors are found on the cell membrane – otherwise known as the plasma membrane or cytoplasmic membrane. Fc receptors can be subdivided into activating FcγR and inhibitory FcγR, which are known to co-ordinately regulate cellular activation through binding of aggregated immunoglobulin G Fc's, and transmission of activating or inhibitory signals into the cell through intracellular ITAM or ITIM motifs. FcR binding of aggregated immunoglobulin or immune complexes, can mediate antibody internalization into the cell, and can result in antibody-mediated phagocytosis, antibody-dependent cell-mediated cytotoxicity, or antigen presentation or cross-presentation. FcRs are also known to mediate or enhance cross-linking of antibody-bound cell surface receptors. Such cross-linking is known to be required for some (Li et al. 2011. 'Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies', *Science*, 333: 1030-4; White et al. 2011. 'Interaction with FcgammaRIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody', *J Immunol*, 187: 1754-63) but not all (Richman et al. 2014. 'Anti-human CD40 monoclonal antibody therapy is potent without FcR crosslinking', *Oncoimmunology*, 3: e28610) antibodies ability to activate signaling in targeted cells, and may or may not be required to achieve therapeutic effects.

A subgroup of the Fc receptors are Fcγ receptors (Fc-gamma receptors, FcgammaR, FcγR), which are specific for IgG antibodies. There are two types of Fcγ receptors: activating Fcγ receptors (also denoted activatory Fcγ receptors) and inhibitory Fcγ receptors. The activating and the inhibitory receptors transmit their signals via immunoreceptor tyrosine-based activation motifs (ITAM) or immunoreceptor tyrosine-based inhibitory motifs (ITIM), respectively. In humans, FcγRIIb (CD32b) is an inhibitory Fcγ receptor, while

FcγRI (CD64), FcγRIIa (CD32a), FcγRIIc (CD32c) and FcγRIIIa (CD16a) are activating Fcγ receptors. FcγgRIIIb is a GPI-linked receptor expressed on neutrophils that lacks an ITAM motif but through its ability to cross-link lipid rafts and engage with other receptors is also considered activatory. In mice, the activating receptors are FcγRI, FcγRIII and FcγRIV.

It is well-known that antibodies can modulate immune cell activity through interaction with Fcγ receptors. Specifically, how antibody immune complexes modulate immune cell activation is determined by their relative engagement of activating and inhibitory Fcγ receptors. Different antibody isotypes bind with different affinity to activating and inhibitory Fcγ receptors, resulting in different A:I ratios (activation:inhibition ratios) (Nimmerjahn et al; Science. 2005 Dec 2;310(5753):1510-2).

By binding to an inhibitory Fcγ receptor, an antibody can inhibit, block and/or down-modulate effector cell functions. By binding to an inhibitory FcγR, antibodies can further stimulate cell activation through aggregation of antibody-targeted signaling receptors on a target cell (Li et al. 2011. 'Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies', *Science*, 333: 1030-4; White et al. 2011. 'Interaction with FcgammaRIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody', *J Immunol*, 187: 1754-63; White et al. 2014. 'Fcgamma receptor dependency of agonistic CD40 antibody in lymphoma therapy can be overcome through antibody multimerization', *J Immunol*, 193: 1828-35).

By binding to an activating Fcγ receptor, an antibody can activate effector cell functions and thereby trigger mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), cytokine release, and/or antibody dependent endocytosis, as well as NETosis (i.e. activation and release of NETs, Neutrophil extracellular traps) in the case of neutrophils. Antibody binding to an activating Fcγ receptor can also lead to an increase in certain activation markers, such as CD40, MHCII, CD38, CD80 and/or CD86.

Recent data published by i.a. the inventors demonstrate a critical and differential dependence of CD8 T cell agonist and Treg-depleting anti-4-1BB antibodies for binding to activating and inhibitory FcγRs respectively, for therapeutic efficacy (Buchan et al., 'Antibodies to Costimulatory Receptor 4-1BB Enhance Anti-tumor Immunity via T Regulatory Cell Depletion and Promotion of CD8 T Cell Effector Function', *Immunity* 2018 49(5):958-970). Moreover, and critically, simultaneous administration of CD8 T cell agonist and Treg depleting anti-4-1BB antibodies optimized for binding to activating and inhibitory FcγR respectively, impaired therapeutic activity. These data demonstrate the critical importance of developing antibodies with appropriate and tailored engagement of activating and inhibitory FcγRs to maximize therapeutic activity of antibodies with distinct

mechanism-of-action. At the same time, they demonstrate that suboptimal engagement of activating and inhibitory FcγRs may severely reduce therapeutic efficacy.

These data were surprising as they contrasted with findings for antibodies to other TNFSR members, notably immune stimulatory anti-CD40 antibodies, which show an obligate need for engagement of the inhibitory, but not activating, FcγRs (Li et al. 2011. 'Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies', *Science*, 333: 1030-4; White et al. 2011. 'Interaction with FcgammaRIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody', *J Immunol*, 187: 1754-63). Taken together, these results demonstrate that FcγR-dependence can vary between antibodies to different targets of the same receptor superfamily, and even between different types of antibodies to the same target, in a manner that is not easily predictable yet may be critical to understand and harness when developing antibodies for therapeutic use.

SUMMARY OF THE INVENTION

In the work leading to the present invention, and also to a parallel invention, two major different groups of anti-TNFR2 antibodies with powerful therapeutic activity, and different characteristics and mechanism-of-actions were identified.

The inventors first identified a powerful therapeutic activity of antagonistic anti-TNFR2 antibodies that block TNF-α binding to TNFR2 receptor. The activity of such antibodies was shown to be dependent on FcγR-interactions, and in particular binding to activatory FcγR, for in vivo therapeutic activity. This group or category of powerful anti-TNFR2 therapeutic reagents was found to be characterized by 1) pronounced block and inhibition of TNF-α (the ligand) induced TNFR2-signalling, and 2) an activity dependent on FcγR-engagement, benefitting most strongly from engaging activating over inhibitory FcγRs.

The inventors then identified a distinct group of anti-TNFR2 antibodies with equally powerful therapeutic activity in vivo, but whose characteristics in many respects are opposite to those of the antagonistic, blocking type of TNFR2 antibodies constituting the first group and the present invention. The anti-TNFR2 antibodies of this second group do not depend on TNF-α blockade or inhibition of TNFR2-signalling for therapeutic activity, but rather is characterized by strong activation of TNFR2-signalling. Further contrasting with the blocking antibodies of the first group, the agonistic antibodies of the second group do not show obligate dependence on antibody:FcγR-engagement, even though their activity is improved with FcγR:engaging antibody variants. In further contrast to the antagonistic and blocking antibodies of the first group, the agonistic antibodies of the

second group show greatest activity in antibody variants with improved binding to inhibitory vs activating FcγR.

The present invention relates to the first group of anti-TNFR2 antibodies, i.e. to antagonistic antibody molecules that, by binding specifically to TNFR2, block TNF-α from binding to TNFR2 and also block TNFR2 signaling. These antibody molecules also have an Fc region that binds to an Fc receptor, which is useful in conferring FcγR-dependent elimination or functional modulation of TNFR2 positive cells, such as e.g. Treg depletion or modulation of tumor associated macrophages.

Agonistic antibodies belonging to the second group are used in the Examples below for comparison with the antagonistic, blocking TNFR2 antibody molecules of the present invention. In the examples, also other antibodies with some characteristics similar to either those of the first or second group, or both, are used for comparison, as further explained below.

Thus, the present invention relates to antagonistic antibody molecules that specifically bind to TNFR2 on a target cell and thereby block TNF-α binding to TNFR2 and block TNFR2 signaling, and wherein the antibody molecules also bind to an Fcγ receptor via their Fc region.

The present invention also relates to specific examples of such novel antagonistic and blocking anti-TNFR2 antibody molecules.

The present invention also relates to isolated nucleotide sequences encoding at least one of the above antibody molecules.

The present invention also relates to plasmids comprising at least one of the above nucleotide sequences.

The present invention also relates to viruses comprising at least one of the above nucleotide sequences or plasmids.

The present invention also relates to cells comprising one of the above nucleotide sequence, or one of the above plasmids, or one of the above viruses.

The present invention also relates to the above antibody molecules, nucleotide sequences, plasmids, viruses and/or cells for use in medicine.

The present invention also relates to the above antibody molecules, nucleotide sequences, plasmids, a viruses and/or cells for use in the treatment of cancer or infections caused by an intracellular pathogen.

The present invention also relates to the above antibody molecules, nucleotide sequences, plasmids, viruses and/or cells for use in the treatment of cancer or infections caused by an intracellular pathogen.

The present invention also relates to pharmaceutical compositions comprising or consisting of at least one of the above antibody molecules, nucleotide sequences, plas-

mids, viruses and/or cells, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used in the treatment of cancer or infections caused by an intracellular pathogen.

The present invention also relates to methods for treatment of cancer or infections caused by an intracellular pathogen in a patient comprising administering to the patient a therapeutically effective amount of at least one of the above antibody molecule, nucleotide sequences, plasmids, viruses and/or cells.

The present invention also relates to antibody molecules, antibody molecules for use, isolated nucleotide sequences, isolated nucleotide sequences for use, plasmids, plasmids for use, viruses, viruses for use, cells, cells for use, uses, pharmaceutical compositions and methods of treatment as described herein with reference to the accompanying description, examples and/or figures.

DETAILED DESCRIPTION OF THE INVENTION

Thus, the present invention relates to antagonistic antibody molecules that specifically bind to TNFR2 on a target cell and thereby block TNF-α binding to TNFR2 and block TNFR2 signaling, and wherein the antibody molecules also bind to an Fcγ receptor via their Fc region.

The antagonistic antibody molecules disclosed herein block both TNF-α from binding to TNFR2 and TNFR2 signaling. That the antagonistic antibody molecules block TNF-α from binding to TNFR2 means herein that an antibody molecule that binds to the receptor TNFR2 thus prevents the ligand TNF-α from binding to the same receptor. This is demonstrated in more detail in Example 3. That the antagonistic antibody molecules disclosed herein block TNFR2 signaling means that they block TNFR2 mediated cell activation. It has been clearly demonstrated that TNF-α mediated signaling through TNFR2 starts a signaling cascade that ends in activation of the nuclear transcription factor NFκB (Thommesen et al. "Distinct differences between TNF receptor 1- and TNF receptor 2-mediated activation of NFkappaB". J Biochem Mol Biol. 2005 May 31;38(3):281-9; Yang et al. "Role of TNF-TNF Receptor 2 Signal in Regulatory T Cells and Its Therapeutic Implications". Front Immunol. 2018 Apr 19;9:784). This in turn results in activation of the cell and synthesis of several pro-inflammatory factors, one of them being IFN-γ in NK cells (Liu et al. "NF-κB signaling in inflammation". Signal Transduct Target Ther. 2017;2. pii: 17023; Tato et al. "Opposing roles of NF-kappaB family members in the regulation of NK cell proliferation and production of IFN-gamma". Int Immunol. 2006 Apr;18(4):505-13). Herein the terms TNFR2 signaling and TNFR2 activation are used interchangeably.

The antibody molecules bind specifically to TNFR2. It is well known that an antibody specifically binds to or interacts with a defined target molecule or antigen, and that this means that the antibody preferentially and selectively binds its target and not a molecule which is not a target. By "antibody molecule that specifically binds TNFR2" or "TNFR2 specific antibody molecule" we mean an antibody that binds TNFR2 protein in a dose-dependent manner but not to an unrelated protein. In addition, the same antibody binds cells that endogenously express TNFR2, and this binding can be blocked out by pre-incubation of the same cells with a commercially available polyclonal TNFR2 antibody reagent, showing that no non-specific binding can be detected when TNFR2 is masked by a polyclonal reagent. This is shown in example 2.

The antibody molecule that specifically binds TNFR2 (or the anti-TNFR2 antibody molecule) refers to an antibody molecule that specifically binds to at least one epitope in the extracellular domain of TNFR2. Cell surface antigen and epitope are terms that would be readily understood by one skilled in immunology or cell biology.

Methods of assessing protein binding are known to the person skilled in biochemistry and immunology. It would be appreciated by the skilled person that those methods could be used to assess binding of an antibody to a target and/or binding of the Fc region of an antibody to an Fc receptor; as well as the relative strength, or the specificity, or the inhibition, or prevention, or reduction in those interactions. Examples of methods that may be used to assess protein binding are, for example, immunoassays, BIAcore, western blots, radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISAs) and Flow cytometry (FACS) (See Fundamental Immunology Second Edition, Raven Press, New York at pages 332-336 (1989) for a discussion regarding antibody specificity).

The target cells expressing the TNFR2 to which the blocking antibody binds in accordance with the present invention include immune cells and/or tumor cells, as discussed above and below. The effect of the binding of the antagonistic antibody molecules according to the invention to TNFR2 may be a change in composition of the cells in diseased tissue. This change in composition may occur through a change in number and/or frequency of TNFR2-expressing cells in the diseased tissue. For example, the effect in cancer include increased intratumoral T cell numbers, increased ratio of $CD8^+$ T cells/Treg (i.e. the number of $CD8^+$ T cells to the number of Tregs), and/or increased numbers of myeloid cells associated with anti-tumor as opposed to pro-tumor characteristics. This is demonstrated in Example 5. In some embodiments, these effects result in reduced numbers of tissue Tregs, increased numbers of $CD8^+$ effector T cells, and altered composition of tissue myeloid cell subsets. The modulation of TNFR2-expressing cells in tissue following in vivo therapeutic invention with a surrogate (3-F10) to the an-

tagonistic antibody molecules according to the invention is shown detail in Example 5. To test for similar effects of human antagonistic antibody molecules according to the invention, an analogous experiment can be performed in mice that have been deleted in mouse TNFR2 and made transgenic for human TNFR2. Alternatively, and preferably, although requiring significant time and resource, animals transgenic for human TNFR2 and human FcγR can be generated in analogous manner compared to that described for CD40 and hFcγR previously (Dahan et al. 2016. 'Therapeutic Activity of Agonistic, Human Anti-CD40 Monoclonal Antibodies Requires Selective FcgammaR Engagement', *Cancer Cell*, 29: 820-31). Such humanized TNFR2 FcγR mice can then be used analogously to test for similar effects of human antagonistic antibody molecules according to the invention.

Diseased tissue means in this context either tumor tissue (i.e. all cells in the tumor microenvironment, including tumor cells, immune cells, endothelial cells and stromal cells) or tissue infected by an intracellular pathogen.

To decide whether or not an antibody molecule blocks ligand binding to TNFR2, it is possible to use an ELISA assay determining the amount of bound TNF-α ligand to immobilized TNFR2 receptor in the presence of TNFR2 specific antibodies. A blocking antibody will prevent the ligand, TNF-α, from binding to the immobilized receptor TNFR2. This is demonstrated and explained in more detail in Example 3 below.

A blocking antibody molecule according to the invention is a complete blocker, which additionally is capable of antagonizing TNFR2 signaling.

A complete blocker is defined herein as an antibody molecule that reduces the TNF-α binding to TNFR2 by more than 98 %, i.e. up to 100%, compared to TNF-α binding in the presence of only an isotype control antibody molecule. An isotype control antibody is an antibody raised towards a protein or other structure that is not present in any form in the assay under study. The isotype control ideally has the same framework but at least the same Fc part as the comparing antibodies. This is well known to the skilled person. In the examples described herein, the isotype control had the same framework, the same Fc part, and was specific for Fluorescein isothiocyanate (FITC). In some embodiments the complete blocker reduces the TNF-α binding with more than 99.5 %.

Other types of blockers are partial blockers and weak blockers. As used herein, a partial blocker is an antibody molecule that reduces the TNF-α binding to TNFR2 by 60-98% (e.g. by 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97 or 98 % and all decimal numbers in between) compared to TNF-α binding in the presence of only an isotype control antibody molecule, and a weak blocker is an antibody molecule that reduces the TNF-α binding to TNFR2 by less than 60%, such as 50-59.9 % (or 50, 51, 52, 53, 54, 55,

56, 57, 58, 59 or 59.9 % and all decimal numbers in between), compared to TNF-α binding in the presence of only an isotype control antibody molecule.

On the contrary, a non-blocking TNFR2 antibody molecule is an antibody molecule that reduces the TNF-α binding to TNFR2 by less than 50 % compared to TNF-α binding in the presence of only an isotype control antibody molecule. In some embodiments, this is determined in high-dose, one-point ELISA as or a dose-titration ELISA as shown in Example 3 and Figures 6 and 7.

Partially blocking, weak blocking and non-blocking antibodies are used in the examples for comparison with the antagonistic blocking antibody molecules of the present invention.

Several properties and features can underlie and (co-)determine the biological activity of antibodies. Besides ability to block ligand from binding to receptor, important such properties include antibody ability to modulate receptor signaling i.e. agonize or antagonize receptor signaling, and antibody dependence on FcγR interactions to confer therapeutic activity.

We first characterized the ability of complete blocking, partial blocking and non-blocking antibodies to modulate TNFR2 signaling. Two extremes were identified.

On the first extreme, we identified antibodies that completely blocked ligand-binding to TNFR2, which blocked TNF-α induced TNFR2 signaling, and which did not themselves induce signaling upon binding to cell-endogenously expressed TNFR2. This group of ligand-blocking, antagonistic, antibodies forms the basis for the present invention.

On the other extreme, we identified antibodies that do not block ligand-binding to TNFR2, but upon binding to TNFR2 endogenously expressing cells agonized the receptor. This second group of antibodies constitute a separate invention and are included herein for comparison.

Antibodies and categories defined by partial blocking agonistic, partial blocking non-agonistic, and complete blocking non-antagonistic, were additionally identified demonstrating the complex biology and great heterogeneity of anti-TNFR2 antibodies clearly demonstrating that the antibodies of the present invention forms a unique group.

To determine whether an antibody has agonist or antagonistic activity it is possible to use a Natural Killer (NK) cell assay as described in Example 4. Briefly, NK cells have been described to respond to IL-2 and IL-12 stimuli with secretion of IFN-γ. Soluble TNF-α is endogenously produced and present at robust but suboptimal concentrations (~20-100 pg/ml), for TNFR2 signaling, meaning that IFN-γ can be both increased and decreased through modulation of TNFR2 signaling. Consequently, exogenous addition of TNF-α at TNFR2 signaling optimal concentration enhances IFN-γ concentrations in this

assay, as does incubation with agonist anti-TNFR2 antibody (Figure 8 C). Contrarily, co-incubation with anti-TNF-α antibody or herein described ligand-blocking antagonist antibodies, decreases IFN-γ release in this assay. Thus, this assay can be used to identify agonist or antagonist activity, or lack thereof, of anti-TNFR2 antibodies. (TNFα Augments Cytokine-Induced NK Cell IFNγ Production through TNFR2. Almishri W.et al. *J Innate Immun.* 2016;8:617-629) Consequently, in this experimental set-up, an antagonistic antibody prevents TNF-α induced signaling in TNFR2-expressing cells, and does not itself stimulate the TNFR2 receptor upon binding to the same. Specifically, antagonistic antibodies in this assay do not increase IFN-γ release upon binding to above mentioned NK cells but rather inhibit IFN-γ release. As shown in figure 8, complete blocking antibodies described in this invention did not induce TNFR2 signaling, but rather reduced TNFR2 signaling in this TNF-α containing NK cell assay, resulting in lower amounts of released IFN-γ. As indicated in Figure 8 Example 4, antibodies of this invention can therefore be classified as ligand-blocking antagonistic anti-TNFR2 antibodies. Using this assay, an antagonistic antibody is defined as an antibody resulting in >30% (e.g. 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 %) reduction of IFN-γ release given that the basal TNF-α levels in the culture is at least 20 pg/ml. Since this assay uses primary cells from PBMC donors, at least 4 donors needs to be included and the mean values should be calculated from all donors. Cells from each donor to be included in calculation of means must have responded to the positive control (soluble TNF-α) treatment with >100% (>2-fold) increased IFN-γ levels relative to treatment with isotype control.

The antagonist activity can also be demonstrated using IL-2 mediated activation of memory T cells. Activation is here measured by upregulation of the T cell activation marker CD25. Using this assay, addition of non-blocking agonistic TNFR2 antibodies further upregulate CD25 expression whereas the blocking antagonistic TNFR2 antibodies according to the invention result in lower CD25 expression compared to isotype control. This is true for the human antibodies of the invention as well as murine surrogate antibodies and is shown in example 4.

In addition to binding to TNFR2, and thereby blocking TNF-α binding and signaling, the antibody molecules according to the invention also bind to Fcγ receptors. An obligate dependence on FcγR interactions of anti-TNFR2 antibodies belonging to the TNF-α-blocking antagonistic group of antibodies of the present invention for therapeutic efficacy, was demonstrated in mouse cancer experimental models using antibody variants which productively engage, or do not productively engage, FcγR binding. The obligate dependence of such ligand blocking antagonistic antibodies for in vivo therapeutic activity, and their preferential binding/engagement of activatory over inhibitory FcγRs for maximal therapeutic in vivo activity, is shown in Example 5. Data demonstrating the

FcγR-independent in vivo activity of agonist, non-blocking anti-TNFR2 antibodies, and their differential preferential engagement of inhibitory over activating FcγRs for maximal therapeutic activity is included in this example for comparative and contrasting purposes only. Collectively our data demonstrate that several types of anti-TNFR2 antibodies can be generated. Further, our data demonstrate that it is not trivial, and could not be predicted, which antibody variants would be therapeutically most efficacious, whether they would rely on blocking, agonist or antagonist (extrinsic or intrinsic) properties, and whether or not they would depend on FcγR-engagement, or be most efficacious in antibody formats associated with preferential/strong binding to activating or inhibitory Fc gamma receptors.

The relatively high homology between mouse and human FcγR systems accounts for many of the general aspects of conserved FcγR-mediated mechanisms between the species. However, mouse and human IgG subclasses differ in their affinities for their cognate FcγRs, making it important when translating FcγR-mediated observations in the mouse system into human IgG-based therapeutics to choose an antibody, antibody subclass and/or engineered subclass variant, that shows appropriate binding to human activating vs inhibitory FcγRs. The affinity and/or avidity of human antibody molecules for individual human Fcγ receptors can be determined using surface plasmon resonance (SPR). In some embodiments, the blocking TNFR2 antibody molecule binds with higher affinity to activating Fcγ receptors than to inhibitory Fcγ receptors. With higher affinity to activating Fcγ receptors than to inhibitory Fcγ receptors, we include the meaning of variants that bind with higher affinity to activating Fcγ receptors, e.g. FcγRIIA, FcγRIIIA and/or FcγRI, compared with the inhibitory Fcγ receptor.

In some embodiments, the antibody molecule is an IgG, which may bind to Fcγ receptors through normal interaction between the Fc region of the antibody molecule and the Fcγ receptor.

In some embodiments, the antagonistic, blocking TNFR2 antibody molecule is a human IgG1. It is well known that human IgG1 binds with high affinity to activating human FcγRI, and with lower and similar affinity to human activatory Fcγ receptors FcγRIIA, FcγRIIIA, and to human inhibitory FcγRIIB. This has been demonstrated e.g. using surface plasmon resonance (SPR).

In some embodiments, the antagonistic, blocking TNFR2 antibody molecule is an IgG antibody molecule showing improved binding to one or several activatory Fc receptors and/or being engineered for improved binding to one or several activatory Fcγ receptors and/or being engineered for improved relative binding to activatory over inhibitory Fcγ receptors. In some embodiments, the anti-TNFR2 antibody is an Fc-engineered human IgG1 antibody. Examples of such engineered antibody variants include afucosylated

antibodies with selective improved antibody binding to FcγRIIIA, and antibodies engineered by directed, mutational, or by other means, amino acid substitution resulting in improved binding to one or several activating Fcγ receptors compared to inhibitory FcγRIIB (Richards et al. 2008. 'Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells', *Mol Cancer Ther*, 7: 2517-27; Lazar et al. 2006. 'Engineered antibody Fc variants with enhanced effector function', *Proc Natl Acad Sci U S A*, 103: 4005-10)

In some embodiments, the human IgG antibody that is engineered for improved binding to activating Fc gamma receptors may be a human IgG antibody carrying the two mutations S239D and I332E, or the three mutations S239D, I332E and A330L, and/or G236A mutations in its Fc portion. In some embodiments, the human IgG antibody that is engineered for improved binding to activating Fc gamma receptors may be an afucosylated human IgG antibody.

The Fcγ receptor to which the Fc region of the antagonistic, blocking antibody molecules of the present invention binds can be an Fcγ receptor expressing immune effector cell as described above.

The binding of the TNFR2 specific antibody molecule to TNFR2 surface receptors on target cell, and co-engagement of FcγR on the same cell or immune effector cells in close proximity may result in depletion, or functional modulation, of the TNFR2 positive target cells to which the antibody molecule binds. By depletion of a cell, we refer herein to depletion, deletion or elimination of the cell through physical clearance of cells.

Depletion of cells may be achieved through ADCC, i.e. antibody-dependent cell-mediated cytotoxicity or antibody-dependent cellular cytotoxicity, and/or ADCP, i.e. antibody dependent cellular phagocytosis. This means that when an antibody molecule as described herein is administered to a patient, such as a human, it binds specifically to TNFR2 expressed on the surface of cells, such as Tregs, and this binding results in depletion of the cells. In general, high-expressing cells are deleted more efficaciously compared to low-expressing cells. As shown in example 5, Fig. 14, Tregs are the highest expressing cells in the tumor setting.

ADCC is an immune mechanism through which Fc receptor-bearing effector cells can recognize and kill – i.e. deplete – antibody-coated target cells expressing tumor-derived antigens, i.e. in the present case, TNFR2, on their surface. ADCP is a similar mechanism, although it results in the target cells being killed – i.e. depleted – through phagocytosis instead of cytotoxicity.

In addition, improved binding of the Fc region of the antibody molecules to the Fcγ receptor may also improve the depletion of the target cell through Fc receptor de-

pendent death via ADCC or ADCP. This is in particular relevant for antibody molecules with improved binding to activating Fcγ receptors.

That the antibody molecules have a depleting effect on TNFR2 positive cells means that upon administration to a patient, such as a human, such an antibody molecule binds specifically to TNFR2 expressed on the surface of TNFR2 positive cells, and this binding results in depletion of such target cells.

The cells that are depleted can be a number of different cells, as explained above in connection with the discussion of what the target cell is. It is in general the cells that have the highest expression of TNFR2 that will be depleted. Other cells that also express TNFR2 but not as high may also be depleted, but to a minor extent compared to the cells that have the highest TNFR2 expression.

As mentioned above, TNFR2 is highly expressed on Tregs found in tumors in various cancer patients, and in such patients the antibody molecule of the invention will preferentially bind to Tregs and thus result in depletion of Tregs. Tregs have an inhibiting effect on the proliferation, activation and cytotoxic capacity of other immune cells such as CD8 positive ($CD8^+$) cells, and therefore depletion of Tregs will, at least indirectly, result in increased proliferation, activation and possibly migration of $CD8^+$ cells and thus an increase of the number of intratumoral $CD8^+$ cells. $CD8^+$ T cells are essential for an immune mediated clearance of tumor cells (McKinney et al. Curr Opin Immunol. 2016 Dec;43:74-80; Klebanoff et al. Immunol Rev. 2006 Jun;211:214-24; Alexander-Miller. Immunol Res. 2005;31(1):13-24). Hence, an increase in proliferation, activation and cytotoxic capacity of $CD8^+$ T cells would be highly beneficial for a cancer patient and can lead to cancer eradication.

An increase in proliferation, activation and cytotoxic capacity of $CD8^+$ T cells would also be highly beneficial for treatment of an infection caused by an intracellular pathogen. Antigens from intracellular pathogens are typically presented on MHC I molecules which are instrumental in activating $CD8^+$ T cells. $CD8^+$ T cells thereby recognize infected cells and lyse them, thereby destroying the pathogen.

The binding of the TNFR2 specific antibody molecule and blocking of TNF-α signaling may also result in functional modulation of cell phenotypes, e.g. modulation of a pro-tumoral myeloid cell into a myeloid cell with tumoricidal properties.

In some embodiments, the TNFR2 positive cells, i.e. the target cells, are CD4 positive ($CD4^+$) cells, i.e. cells that express CD4.

In some embodiments, the TNFR2 positive cells are both $CD4^+$ and $FOXP3^+$, i.e. expressing both CD4 and FOXP3. These cells are Tregs. $CD8^+$ T cells also express TNFR2, but Tregs express significantly higher levels of TNFR2 than CD8 positive T cells,

as shown in figure 14 in example 5. This makes Tregs more susceptible to depletion compared to lower expressing $CD8^+$ cells.

In some situations, the TNFR2 is preferentially expressed on immune cells in the tumor microenvironment (tumor infiltrating cells, TILS).

In some embodiments, the Tregs will be the cells in a tumor microenvironment that have the highest expression of TNFR2, resulting in the antibody molecules that specifically bind to TNFR2 (or the anti-TNFR2 antibody molecules) having a Treg depleting effect. This is discussed in more detail below, e.g. in Example 5 and in connection with Figures 13 and 15.

In some embodiments, TNFR2 positive cells will be Tregs in a solid tumor. Such Tregs will have very high expression of TNFR2, and therefore administration of an antibody molecules that specifically binds to TNFR2, will preferentially result in depletion of such Tregs.

To decide whether an antibody molecule is an antibody molecule that has a depleting effect on TNFR2 positive cells as referred to herein, it is possible to use an *in vivo* test in a PBMC-NOG/SCID model. This *in vivo* test is based on the combined use of PBMC mice and NOG/SCID mice, which is herein called a PBMC-NOG/SCID model. Both NOG mice and SCID mice are known to the skilled person (Ito M et al, (2002) NOD/SCID/γc^{null} mouse: an excellent recipient mouse model for engraftment of human cells. Blood 100(9):3175-3182; Bosma GC et al; Nature. 1983 Feb 10;301(5900):527-30; A severe combined immunodeficiency mutation in the mouse) and also the PBMC-NOG model is known (Cox et al. "Antibody-mediated targeting of the Orai1 calcium channel inhibits T cell function". PLoS One. 2013 Dec 23;8(12):e82944.; and Søndergaard et al. "Human T cells depend on functional calcineurin, tumor necrosis factor-α and CD80/CD86 for expansion and activation in mice." Clin Exp Immunol. 2013 May;172(2):300-10.) The *in vivo* test in the PBMC-NOG/SCID model comprises the following consecutive nine steps:

1) Human PBMCs (peripheral blood mononuclear cells) are isolated, washed and resuspended in sterile PBS. In some embodiments, the PBMCs are resuspended in PBS at $75x10^6$ cells/ml.

2) NOG mice are injected i.v. (intravenously) with an appropriate amount, such as 200 µl, of the cell suspension from step 1). If 200 µl are injected, this corresponds to $15x10^6$ cells/mouse.

3) A suitable time, such as 2 weeks, after injection, the spleens from the NOG mice are isolated and rendered into a single cell suspension. Optionally, a small sample from the single cell suspension is taken to determine the expression of TNFR2 on human T cells by FACS, in order to confirm the TNFR2 expression.

4) The cell suspension from step 3) is resuspended in sterile PBS. In some embodiments, the cell suspension is resuspended in sterile PBS at $50x10^6$ cells/ml. If the optional TNFR2 expression determination is included in step 3, the rest of the cell suspension is then resuspended in step 4.

5) SCID mice are injected i.p. (intraperitoneally) with an appropriate amount, such as 200 µl, of the suspension from step 4. If 200 µl are injected, this corresponds to $10x10^6$ cells/mouse.

6) A suitable time, such as 1 hour, after the injection in step 5) the SCID mice are treated with an appropriate amount, such as 10 mg/kg, of either the antibody molecule to be tested, a positive control antibody (e.g. anti-CD25 antibodies known to deplete Tregs) or an isotype control monoclonal antibody.

7) The intraperitoneal fluid of the treated SCID mice is collected a suitable time, such as 24 hours, after the treatment in step 6).

8) Human T cell subsets are identified and quantified by FACS using following markers: CD45, CD4, CD8, CD25 and/or CD127. It is well established that human Tregs can be distinguished in a human PBMC population as being $CD4^+CD25^+CD127^{low/-}$.

9) The results from identification and quantification of the T cell subsets from the mice treated with the tested antibody molecule is compared to the results from identification and quantification of the T cell subsets from the mice treated with a positive control antibody and to the results from identification and quantification of the T cell subsets from the mice treated with isotype control monoclonal antibody. A lower number of Tregs in the intraperitoneal fluid from mice treated with the antibody molecule to be tested compared to the number of Tregs in the intraperitoneal fluid from mice treated with isotype control demonstrates that the antibody molecule has a depleting effect on TNFR2 positive Tregs

This assay is demonstrated in more detail below in Example 5, in combination with Figure 15.

As mentioned above, other cells can also express TNFR2, such as cancer cells. In some embodiments, the antibody molecule binds preferentially to TNFR2 expressed on cancer cells, and the binding then results in depletion of the cancer cells directly.

Antibodies are well known to those skilled in the art of immunology and molecular biology. Typically, an antibody comprises two heavy (H) chains and two light (L) chains. Herein, we sometimes refer to this complete antibody molecule as a full-size or full-length antibody. The antibody's heavy chain comprises one variable domain (VH) and three constant domains (CH1, CH2 and CH3), and the antibody's molecule light chain comprises one variable domain (VL) and one constant domain (CL). The variable domains (sometimes collectively referred to as the F_V region) bind to the antibody's target,

or antigen. Each variable domain comprises three loops, referred to as complementary determining regions (CDRs), which are responsible for target binding. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and in humans several of these are further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2.

Another part of an antibody is the Fc region (otherwise known as the fragment crystallizable domain), which comprises two of the constant domains of each of the antibody's heavy chains. As mentioned above, the Fc region is responsible for interactions between the antibody and Fc receptor.

The term antibody molecule, as used herein, encompasses full-length or full-size antibodies as well as functional fragments of full length antibodies and derivatives of such antibody molecules.

Functional fragments of a full-size antibody have the same antigen binding characteristics as the corresponding full-size antibody and include either the same variable domains (i.e. the VH and VL sequences) and/or the same CDR sequences as the corresponding full-size antibody. A functional fragment does not always contain all six CDRs of a corresponding full-size antibody. It is appreciated that molecules containing three or fewer CDR regions (in some cases, even just a single CDR or a part thereof) are capable of retaining the antigen-binding activity of the antibody from which the CDR(s) are derived. For example, in Gao *et al.*, 1994, J. Biol. Chem., 269: 32389-93 it is described that a whole VL chain (including all three CDRs) has a high affinity for its substrate.

Molecules containing two CDR regions are described, for example, by Vaughan & Sollazzo 2001, Combinatorial Chemistry & High Throughput Screening, 4: 417-430. On page 418 (right column – 3 Our Strategy for Design) a minibody including only the H1 and H2 CDR hypervariable regions interspersed within framework regions is described. The minibody is described as being capable of binding to a target. Pessi *et al.*, 1993, Nature, 362: 367-9 and Bianchi *et al.*, 1994, J. Mol. Biol., 236: 649-59 are referenced by Vaughan & Sollazzo and describe the H1 and H2 minibody and its properties in more detail. In Qiu *et al.*, 2007, Nature Biotechnology, 25:921-9 it is demonstrated that a molecule consisting of two linked CDRs are capable of binding antigen. Quiocho 1993, Nature, 362: 293-4 provides a summary of "minibody" technology. Ladner 2007, Nature Biotechnology, 25:875-7 comments that molecules containing two CDRs are capable of retaining antigen-binding activity.

Antibody molecules containing a single CDR region are described, for example, in Laune *et al.*, 1997, JBC, 272: 30937-44, in which it is demonstrated that a range of hexapeptides derived from a CDR display antigen-binding activity and it is noted that synthetic peptides of a complete, single, CDR display strong binding activity. In Monnet *et al.*, 1999, JBC, 274: 3789-96 it is shown that a range of 12-mer peptides and associated framework regions have antigen-binding activity and it is commented on that a CDR3-like peptide alone is capable of binding antigen. In Heap *et al.*, 2005, J. Gen. Virol., 86: 1791-1800 it is reported that a "micro-antibody" (a molecule containing a single CDR) is capable of binding antigen and it is shown that a cyclic peptide from an anti-HIV antibody has antigen-binding activity and function. In Nicaise *et al.*, 2004, Protein Science, 13:1882-91 it is shown that a single CDR can confer antigen-binding activity and affinity for its lysozyme antigen.

Thus, antibody molecules having five, four, three or fewer CDRs are capable of retaining the antigen binding properties of the full-length antibodies from which they are derived.

The antibody molecule may also be a derivative of a full-length antibody, or a fragment of such an antibody provided that such a derivative or fragment retains the Fcγ receptor binding capability. When a derivative is used, it should have the same antigen binding characteristics as the corresponding full-length antibody in the sense that it binds to the same epitope on the target as the full-length antibody.

Thus, by the term "antibody molecule", as used herein, we include all types of antibody molecules and functional fragments thereof and derivatives thereof, including: monoclonal antibodies, polyclonal antibodies, synthetic antibodies, recombinantly produced antibodies, multi-specific antibodies, bi-specific antibodies, human antibodies, antibodies of human origin, humanized antibodies, chimeric antibodies, single chain antibodies, antibody heavy chains, homo-dimers of antibody heavy chains, heterodimers of antibody heavy chains, and heterodimers of antibody light chains, .

Further, the term "antibody molecule", as used herein, includes all classes of antibody molecules and functional fragments, including: IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgD, and IgE, unless otherwise specified.

In some embodiments, the antibody molecule is a human antibody molecule, a humanized antibody molecule or an antibody molecule of human origin. In some such embodiments, the antibody molecule is an IgG antibody. In some embodiments, the antibody molecule is of an isotype that engages activating Fc receptors in an optimal way. In some embodiments the antibody molecule is an IgG1 antibody.

The skilled person will appreciate that the mouse IgG2a and human IgG1 engage with activatory Fcγ receptors, and share the ability to activate deletion of target cells

through activation of activatory Fcγ receptor bearing immune cells by e.g. ADCP and ADCC. In some embodiments, the anti-TNFR2 antibody is a murine or a humanized murine IgG2a antibody.

In some embodiments, the antibody molecule that specifically binds TNFR2 is human IgG2 antibody molecule.

In some embodiments, the anti-TNFR2 antibody is a murine antibody that is cross-reactive with human TNFR2.

As outlined above, different types and forms of antibody molecules are encompassed by the invention, and would be known to the person skilled in immunology. It is well known that antibodies used for therapeutic purposes are often modified with additional components which modify the properties of the antibody molecule.

Accordingly, we include that an antibody molecule described herein or an antibody molecule used as described herein (for example, a monoclonal antibody molecule, and/or polyclonal antibody molecule, and/or bi-specific antibody molecule) comprises a detectable moiety and/or a cytotoxic moiety.

By "detectable moiety", we include one or more from the group comprising of: an enzyme; a radioactive atom; a fluorescent moiety; a chemiluminescent moiety; a bioluminescent moiety. The detectable moiety allows the antibody molecule to be visualized *in vitro,* and/or *in vivo*, and/or *ex vivo*.

By "cytotoxic moiety", we include a radioactive moiety, and/or enzyme, for example wherein the enzyme is a caspase, and/or toxin, for example wherein the toxin is a bacterial toxin or a venom; wherein the cytotoxic moiety is capable of inducing cell lysis.

We further include that the antibody molecule may be in an isolated form and/or purified form, and/or may be PEGylated. PEGylation is a method by which polyethylene glycol polymers are added to a molecule such as an antibody molecule or derivative to modify its behavior, for example to extend its half-life by increasing its hydrodynamic size, preventing renal clearance.

As discussed above, the CDRs of an antibody bind to the antibody target. The assignment of amino acids to each CDR described herein is in accordance with the definitions according to Kabat EA et al. 1991, In "Sequences of Proteins of Immunological Interest" Fifth Edition, NIH Publication No. 91-3242, pp xv- xvii.

As the skilled person would be aware, other methods also exist for assigning amino acids to each CDR. For example, the International ImMunoGeneTics information system (IMGT(R)) (http://www.imgt.org/ and Lefranc and Lefranc "The Immunoglobulin FactsBook" published by Academic Press, 2001).

In some embodiments the antibody molecule that specifically binds TNFR2 is a human antibody.

In some embodiments, the antibody molecule that specifically binds TNFR2 is an antibody of human origin, i.e. an originally human antibody that has been modified as described herein.

In some embodiments, the antibody molecule that specifically binds TNFR2 is a humanized antibody, i.e. an originally non-human antibody that has been modified to increase its similarity to a human antibody. The humanized antibodies may, for example, be of murine antibodies or lama antibodies.

In some embodiments, the anti-TNFR2 antibody is a monoclonal antibody.

In some embodiments, the anti-TNFR2 antibody is a polyclonal antibody.

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VH-CDR1 sequences listed in Table 1 below.

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VH-CDR2 sequences listed in Table 1 below.

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VH-CDR3 sequences listed in Table 1 below.

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VL-CDR1 sequences listed in Table 1 below

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VL-CDR2 sequences listed in Table 1 below.

In some embodiments, the antibody molecule that specifically binds TNFR2 comprises one of the VL-CDR3 sequences listed in Table 1 below.

In some embodiments the anti-TNFR2 antibody molecule is an antibody molecule comprising the 6 CDRs having SEQ. ID. NOs: 1, 2, 3, 4, 5 and 6; or an antibody molecule comprising the 6 CDRs having SEQ. ID. NOs: 9, 10, 11, 12, 13 and 14; or an antibody molecule comprising the 6 CDRs having SEQ. ID. NOs: 17, 18, 19, 20, 21 and 22.

In some embodiments the anti-TNFR2 antibody molecule is an antibody molecule comprising the 6 CDRs having SEQ. ID. NOs: 1, 2, 3, 4, 5 and 6.

In some embodiments, the anti-TNFR2 antibody molecule is an antibody molecule selected from the group consisting of antibody molecules comprising a VH selected from the group consisting of SEQ. ID. NOs: 7, 15 and 23.

In some embodiments, the anti-TNFR2 antibody molecule is an antibody molecule selected from the group consisting of antibody molecules comprising a VL selected from the group consisting of SEQ. ID. NOs: 8, 16 and 24.

In some embodiments the anti-TNFR2 antibody molecule is an antibody molecule comprising a VH having SEQ. ID. NO: 7.

In some embodiments the anti-TNFR2 antibody molecule is an antibody molecule comprising a VL having SEQ. ID. NO: 8.

In some embodiments the anti-TNFR2 antibody molecule comprises a VH having SEQ. ID. NO: 7 and a VH having SEQ. ID. NO: 8.

In some embodiments the anti-TNFR2 antibody molecule comprises a CH having SEQ. ID. NO: 217.

In some embodiments the anti-TNFR2 antibody molecule comprises a CL having SEQ. ID. NO: 218.

In some embodiments the anti-TNFR2 antibody molecule comprises a VH having SEQ. ID. NO: 7, a VH having SEQ. ID. NO: 8, a CH having SEQ. ID. NO: 217 and a CL having SEQ. ID. NO: 218.

Table 1: Specific sequences of antagonistic TNFR2 blocking antibody molecules according to the invention (in the VH and VL sequences, the CDR sequences are marked in bold text)

Antibody clone	Region	Sequence	SEQ. ID. NO:
001-H10	VH-CDR1	FDDYGMSWVRQAPG	1
	VH-CDR2	SVIYSGGSTYYADSVKGR	2
	VH-CDR3	CARDRSSSWYRDGMDV	3
	VL-CDR1	CTGSSSNIGAGYDVH	4
	VL-CDR2	GNSNRPS	5
	VL-CDR3	CAAWDDSLSGWV	6
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FDDYGMSWVR-QAPG**KGLEWV**SVIYSGGSTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDRSSS-WYRDGMDV**WGQGTLVTVSS	7
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**GNSNRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLSGWV**FGGGTKLTVLG	8
004-H02	VH-CDR1	FDDYGMSWVRQAPG	9
	VH-CDR2	STIYSGDNAYYGASVRGR	10
	VH-CDR3	ARVYSSSWRKRAFDI	11
	VL-CDR1	CSGTSSNIESNTVN	12
	VL-CDR2	SDNQRPS	13
	VL-CDR3	CAAWDDSLSGWV	14
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FDDYGMSWVR-QAPG**KGLEWV**STIYSGDNAYYGASVRGR**FTISRD-	15

		NSKNTLYLQMNSLRAEDTAVYYC**ARVYSSSWRKRAFD-**IWGQGTLVTVSS	
	VL	QSVLTQPPSASGTPGQRVTIS**CSGTSSNI-ESNTVN**WYQQLPGTAPKLLIY**SDNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLSGWV**FGGGTKLTVLG	16
005-B08	VH-CDR1	FSDYYMSWIRQAPG	17
	VH-CDR2	AIISYDGGGKYFADPVKGR	18
	VH-CDR3	ARYYGDGGFDP	19
	VL-CDR1	CTGSSSNIGAGYVVH	20
	VL-CDR2	SNNQRPS	21
	VL-CDR3	CAAWDDSLNGPV	22
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSDYYMSWIRQAPG**KGLEWV**AIISYDGGGKY-FADPVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARYYG-DGGFDP**WGQGTLVTVSS	23
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYVVH**WYQQLPGTAPKLLIY**SNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLNGPV**FGGGTKLTVLG	24

Table 2: Specific sequences of TNFR2 blocking antibody molecules that are not antagonistic and that are used herein for comparison (in the VH and VL sequences, the CDR sequences are marked in bold text)

005-B02	VH-CDR1	FSDYYMSWIRQAPG	25
	VH-CDR2	ALIWYDGGNEYYADSVKGR	26
	VH-CDR3	VRETGNYGMDV	27
	VL-CDR1	CTGSSSNIGAGYDVH	28
	VL-CDR2	RNNQRPS	29
	VL-CDR3	CATWDDRVNGPV	30
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSDYYMSWIRQAPG**KGLEWV**ALIWYDGGNEY-YADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**VRETGNYGMDV**WGQGT LVTVSS	31

	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**RNNQRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY-**CATWDDRVNGPV**FGGGTKLTVLG	32
001-E06	VH-CDR1	FSSNYMSWVRQAPG	33
	VH-CDR2	ALIWYDGSNKYYADSVKGR	34
	VH-CDR3	AKDPLFDS	35
	VL-CDR1	CTGRSSNIGAGYDVH	36
	VL-CDR2	DNNKRPS	37
	VL-CDR3	CAAWDDSLNGPV	38
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSNYMSWVR-QAPG**KGLEWV**ALIWYDGSNKYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AKDPLFDS**WGQGTLVT VSS	39
	VL	QSVLTQPPSASGTPGQRVTIS**CTGRSSNI-GAGYDVH**WYQQLPGTAPKLLIY**DNNKRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLNGPV**FGGGTKLTVLG	40
001-G04	VH-CDR1	FNTYSMNWVRQAPG	41
	VH-CDR2	SVLYSDDDTHYADSVKGR	42
	VH-CDR3	ARDCGGDCHSGDDAFDI	43
	VL-CDR1	CSGSSSNIGSNTVN	44
	VL-CDR2	DNDKRPS	45
	VL-CDR3	CAAWHDSLNGWV	46
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FNTYSMNWVR-QAPG**KGLEWV**SVLYSDDDTHYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDCGG-DCHSGDDAFDI**WGQGTLVTVSS	47
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**DNDKRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWHDSLNGWV**LGGGTKLTVLG	48
001-G10	VH-CDR1	FSAYGMHWVRQAPG	49
	VH-CDR2	AVVSYDGREKHYADSVKGR	50
	VH-CDR3	ARSDGGYDSDSGYY	51
	VL-CDR1	CSGSTSNIGSNFVY	52
	VL-CDR2	DNNKRPS	53

	VL-CDR3	CSSYAYSDNIL	54
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSAYGMHWVR-QAPG**KGLEWV**AVVSYDGREKHYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARSDG-GYDSDSGYY**WGQGTLVTVSS	55
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSTSNIGSNFVY-**WYQQLPGTAPKLLIY**DNNKRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY**CS-SYAYSDNIL**FGGGTKLTVLG	56
001-C08	VH-CDR1	FSNAWMSWVRQAPG	57
	VH-CDR2	SGISSSGSSAYYADSVKGR	58
	VH-CDR3	ARHYYYHIAGYYYDTFDI	59
	VL-CDR1	CSGSSSNIGGNTVN	60
	VL-CDR2	GNTNRPS	61
	VL-CDR3	CAAWDDSLSGVV	62
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSNAWMSWVRQAPG**KGLEWV**SGISSSGS-SAYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARHYYYHIAGYYYDTFD-I**WGQGTLVTVSS	63
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGGNTVN**WYQQLPGTAPKLLIY**GNTNRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLSGVV**FGGGTKLTVLG	64
001-H09	VH-CDR1	FSSYAMSWVRQAPG	65
	VH-CDR2	ATISYHGSDKDYADSVKGR	66
	VH-CDR3	ARDANYHSSGYYYDVFDI	67
	VL-CDR1	CSGSSSNIGSNTVN	68
	VL-CDR2	GNSNRPS	69
	VL-CDR3	CAAWDDSLSTWV	70
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYAMSWVR-QAPG**KGLEWV**ATISYHGSDKDYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDANY-HSSGYYYDVFDI**WGQGTLVTVSS	71
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**GNSNRPS**GVPDRFSG-SKSGTSASLAISGLR-	72

		SEDEADYY**CAAWDDSLSTWV**FGGGTKLTVLG	
005-F10	VH-CDR1	FSDYYMTWIRQAPG	73
	VH-CDR2	SGISGSGGYIHYADSVKGR	74
	VH-CDR3	AREGLLPDAFD	75
	VL-CDR1	CSGSSSNIGNNYVS	76
	VL-CDR2	RNNQRPS	77
	VL-CDR3	CAAWDDSVSGWV	78
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSDYYMTWIRQAPG**KGLEWV**SGISGSGGYIHY-ADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AREGLLPDAFD-**IWGQGTLVTVSS	79
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGNNYVS**WYQQLPGTAPKLLIY**RNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSVSGWV**FGGGTKLTVLG	80
001-B11	VH-CDR1	**FSSYSMNWVRQAPG**	81
	VH-CDR2	**AVMSYDEYNTYYADSVKGR**	82
	VH-CDR3	**AKGFYGDYPLWDY**	83
	VL-CDR1	**CSGGNSNIGTNTVD**	84
	VL-CDR2	**SNNQRPS**	85
	VL-CDR3	**CAAWDDSVNGPV**	86
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYSMNWVR-QAPG**KGLEWV**AVMSYDEYNTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AKGFYGDY-PLWDY**WGQGTLVTVSS	87
	VL	QSVLTQPPSASGTPGQRVTIS**CSGGNSNIGTNTVD**WYQQL PGTAPKLLIY**SNNQRPS**GVPDRFSGSKSGTSASLAISGLR-SEDEADYY**CAAWDDSVNGPV**FGGGTKLTVLG	88
001-C07	VH-CDR1	FSSYEMNWVRQAPG	89
	VH-CDR2	STITGGGSIYDANSVQGR	90
	VH-CDR3	ARDSTYHSSGYYYDVFDI	91
	VL-CDR1	CSGSSSNIGSNTVN	92
	VL-CDR2	GNSNRPS	93
	VL-CDR3	CAAWDDSLSGHWV	94
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYEMNWVR-QAPG**KGLEWV**STITGGGSIYDANSVQGR**FTISRD-	95

		NSKNTLYLQMNSLRAEDTAVYYC**ARDSTYHSSGYYYDVFD**IWGQGTLVTVSS	
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**GNSNRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY**CAAWDDSLSGH-WV**FGGGTKLTVLG	96
001-D01	VH-CDR1	FSSYGMHWVRQAPG	97
	VH-CDR2	SAVFGSGHGNTFYADAVKGR	98
	VH-CDR3	AREQLWFGQDAFDI	99
	VL-CDR1	CSGSSSNIGSNTVN	100
	VL-CDR2	GNSNRPS	101
	VL-CDR3	CQSYDSSLSASV	102
	VH	EVQLLESGGGLVQPGGPLRLSCAASGFT**FSSYGMHWVR-QAPG**KGLEWV**SAVFGSGHGNTFYADAVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AREQLWFGQDAFD-**IWGQGTLVTVSS	103
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**GNSNRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSYDSSLSASV**FGGGTKLTVLG	104
003-F10	VH-CDR1	FSDAWMTWVRQAPG	105
	VH-CDR2	SDLSDSGGSTYYADSVKGR	106
	VH-CDR3	GRLAAGGPVDY	107
	VL-CDR1	CTGSSSNIGAGYDVH	108
	VL-CDR2	SNNQRPS	109
	VL-CDR3	CSVWDDSLNSWV	110
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSDAWMTWVR-QAPG**KGLEWV**SDLSDSGGSTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**GRLAAGGPVDY**WGQGTLVTVSS	111
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**SNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CSVWDDSLNSWV**FGGGTKLTVLG	112

In order to determine or demonstrate the features of the antibody molecules of the present invention, they were compared to antibody molecules that do not block TNF-α ligand binding to TNFR2. Such antibodies are shown in Table 3.

Table 3: Specific sequences of non-blocking TNFR2 antibody molecules mentioned herein as reference antibodies (in the VH and VL sequences, the CDR sequences are marked in bold text)

Antibody clone	Region	Sequence	SEQ. ID. NO:
001-F02	VH-CDR1	FSDYYMSWVRQAPG	113
	VH-CDR2	ANINTDGSEKYYLDSVKGR	114
	VH-CDR3	AREEYGAFDI	115
	VL-CDR1	CSGSSSNIGSNTVN	116
	VL-CDR2	DNNKRPS	117
	VL-CDR3	CQSFDRGLSGSIV	118
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSDYYMSWVR-QAPG**KGLEWV**ANINTDGSEKYYLDSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AREEYGAFD-**IWGQGTLVTVSS	119
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**DNNKRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY**CQSFDR-GLSGSIV**FGGGTKLTVLG	120
001-F06	VH-CDR1	FSSYAMHWVRQAPG	121
	VH-CDR2	SAISGGATTTYYADSVKGR	122
	VH-CDR3	AKGGTGDPYYFDY	123
	VL-CDR1	CTGSSSNIGAGYDVH	124
	VL-CDR2	RNNQRPS	125
	VL-CDR3	CAARDDGLSGPV	126
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYAMHWVR-QAPG**KGLEWV**SAISGGATTTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AKGGTGDPYYFDY**WGQ GTLVTVSS	127
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**RNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAARDDGLSGPV**FGGGTKLTVLG	128

001-A09	VH-CDR1	FSSNYMSWVRQAPG	129
	VH-CDR2	SVISGSGGSTYYADSVKGR	130
	VH-CDR3	ARDRGWFDP	131
	VL-CDR1	CSGSRSNIDNSYVS	132
	VL-CDR2	RNNQRPS	133
	VL-CDR3	CATWDDSLSGPV	134
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSNYMSWVR-QAPG**KGLEWV**SVISGSGGSTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDRGWFDP**WGQGTLV TVSS	135
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSRSNIDNSYV-S**WYQQLPGTAPKLLIY**RNNQRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY-**CATWDDSLSGPV**FGGGTKLTVLG	136
001-B05	VH-CDR1	FSNAWMSWVRQAPG	137
	VH-CDR2	SSISSASGYIYYGDSVKGR	138
	VH-CDR3	ARGTLYGDFDEF	139
	VL-CDR1	CSGSSSNIGNNAVN	140
	VL-CDR2	GNTNRPS	141
	VL-CDR3	CQSYDSSLSGYVV	142
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSNAWMSWVRQAPG**KGLEWV**SSISSASGYIY-YGDSVKGR**FTISRD-NSKNTLYLQMNNLRAEDTAVYYC**ARGTLYGDFDEF-**WGQGTLVTVSS	143
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGNNAVN**WYQQLPGTAPKLLIY**GNTNRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSYDSSLSGYVV**FGGGTKLTVLG	144
001-B09	VH-CDR1	FSRHAMNWVRQAPG	145
	VH-CDR2	SSISTGSSYIDYADSVKGR	146
	VH-CDR3	AREKGHYYYGMDV	147
	VL-CDR1	CTGSSSNIGAGYDVH	148
	VL-CDR2	GNSYRPS	149
	VL-CDR3	CQSYDTSLSAYVV	150
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSRHAMNWVR-QAPG**KGLEWV**SSISTGSSYIDYADSVKGR**FTISRD-	151

		NSKNTLYLQMNSLRAEDTAVYYC**AREKGHYYYG-MDV**WGQGTLVTVSS	
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**GNSYRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSYDTSLSAYVV**FGGGTKLTVLG	152
001-C03	VH-CDR1	FSNAWMSWVRQAPG	153
	VH-CDR2	SAISVSGINTYYADSVKGR	154
	VH-CDR3	ARDTGSLGVDY	155
	VL-CDR1	CSGSSSNIGSNTVN	156
	VL-CDR2	RNNQRPS	157
	VL-CDR3	CQSYDSSLSISV	158
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSNAWMSWVRQAPG**KGLEWV**SAISVSGINTY-YADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDTGSLGVDY**WGQGT LVTVSS	159
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**RNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSYDSSLSISV**FGGGTKLTVLG	160
001-C05	VH-CDR1	FSSNEMSWIRQAPG	161
	VH-CDR2	SVIYSGGSTYYADSVKGR	162
	VH-CDR3	ARREGWLVPFDY	163
	VL-CDR1	CSGSSSNIGSNTVN	164
	VL-CDR2	GNIIRPS	165
	VL-CDR3	CQSFDTTLSGSIV	166
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSNEMS-WIRQAPG**KGLEWV**SVIYSGGSTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARREG-WLVPFDY**WGQGTLVTVSS	167
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSS-NIGSNTVN**WYQQLPGTAPKLLIY**GNIIRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSFDTTLSGSIV**FGGGTKLTVLG	168
001-G05	VH-CDR1	FSSYAMSWVRQAPG	169
	VH-CDR2	SVISGSGGSTYYADAVKGR	170

	VH-CDR3	TTDSGSGSYL	171
	VL-CDR1	CTGSSSNIGAGYDVH	172
	VL-CDR2	SNNQRPS	173
	VL-CDR3	CAAWDDSLNGPV	174
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYAMSWVR-QAPG**KGLEWV**SVISGSGGSTYYADAVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**TT-DSGSGSYL**WGQGTLVTVSS	175
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**SNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYYCAAWDDSLNGPVFGGGTKLTVLG	176
001-A10	VH-CDR1	FSDYYMTWIRQAPG	177
	VH-CDR2	SSISGGSTYYADSRKGR	178
	VH-CDR3	AREPGYSYGFFDY	179
	VL-CDR1	CTGSSSNIGAGYDVH	180
	VL-CDR2	SNNQRPS	181
	VL-CDR3	CQSYDRSLSGSIV	182
	VH	EVQLLESGGGLVQPGGSLRLS-CAASGFT**FSDYYMTWIRQAPG**KGLEWV**SSISGGSTY-YADSRKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AREPGY-SYGFFDY**WGQGTLVTVSS	183
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYDVH**WYQQLPGTAPKLLIY**SNNQRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CQSYDRSLSGSIV**FGGGTKLTVLG	184
001-C06	VH-CDR1	SSSYWMSWVRQAPG	185
	VH-CDR2	SAISGSGGSTYYADSVKGR	186
	VH-CDR3	AREYSGYEFDF	187
	VL-CDR1	CTGSSSNIGARSDVH	188
	VL-CDR2	GNRNRPS	189
	VL-CDR3	CQSFDRGLSGSIV	190
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**SSSYWMSWVR-QAPG**KGLEWV**SAISGSGGSTYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**AR-EYSGYEFDF**WGQGTLVTVSS	191

	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GARSDVH**WYQQLPGTAPKLLIY**GNRNRPS**GVPDRFSG-SKSGTSASLAISGLRSEDEADYY**CQSFDR-GLSGSIV**FGGGTKLTVLG	192
001-H03	VH-CDR1	FSSNYMSWVRQAPG	193
	VH-CDR2	SSISSSSSYIYYADSVKGR	194
	VH-CDR3	ARDRGRTGTDY	195
	VL-CDR1	CSGTTSNIGSYAVN	196
	VL-CDR2	GNINRPS	197
	VL-CDR3	CQSYDSSLSASL	198
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSNYMSWVR-QAPG**KGLEWV**SSISSSSSYIYYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARDRGRT-GTDY**WGQGTLVTVSS	199
	VL	QSVLTQPPSASGTPGQRVTIS**CSGTTSNIGSYAVN**WYQQL PGTAPKLLIY**GNINRPS**GVPDRFSGSKSGTSASLAISGLR-SEDEADYY**CQSYDSSLSASL**FGGGTKLTVLG	200
004-E08	VH-CDR1	FSRYWMHWVRQVPG	201
	VH-CDR2	SGISDSGVVTYYADSVKGR	202
	VH-CDR3	ARAQSVAFDI	203
	VL-CDR1	CSGSSSNIGAGHDVH	204
	VL-CDR2	YDDLLPS	205
	VL-CDR3	CAAWDDSLSGWV	206
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSRY-WMHWVRQVPG**KGLEWV**SGISDSGVVTY-YADSVKGR**FTISRDNSKNTLYLQMNSLRAEDTAVYYC**AR-AQSVAFDI**WGQGTLVTVSS	207
	VL	QSVLTQPPSASGTPGQRVTIS**CSGSSSNIGAGH-DVH**WYQQLPGTAPKLLIY**YDDLLPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLSGWV**FGGGTKLTVLG	208
005-A05	VH-CDR1	FSSYAMSWVRQAPG	209
	VH-CDR2	STIIGSGANTWYADSVKGR	210
	VH-CDR3	ARHEGYYYYGMDV	211
	VL-CDR1	CTGSSSNIGAGYVVH	212
	VL-CDR2	GNSNRPS	213
	VL-CDR3	CAAWDDSLNGRV	214

	VH	EVQLLESGGGLVQPGGSLRLSCAASGFT**FSSYAMSWVR-QAPG**KGLEWV**STIIGSGANTWYADSVKGR**FTISRD-NSKNTLYLQMNSLRAEDTAVYYC**ARHEGYYYYG-MDV**WGQGTLVTVSS	215
	VL	QSVLTQPPSASGTPGQRVTIS**CTGSSSNI-GAGYVVH**WYQQLPGTAPKLLIY**GNSNRPS**GVPDRFSG-SKSGTSASLAISGLR-SEDEADYY**CAAWDDSLNGRV**FGGGTKLTVLG	216

The sequences in Tables 1, 2 and 3 above are all of human origin and derived from the n-CoDeR® library, as explained in detail in Example 1.

In some embodiments, the antibody molecules that specifically bind TNFR2 described herein may also comprise one or both of the constant regions (CH and/or CL) listed in Table 4 below.

Table 4:

Region	**Sequence**	**SEQ. ID. NO:**
CH	ASTKGPSVFPLAPSSKSTSGG-TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS-LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE-PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS-RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK-TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA-PIEK-TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI-AVEWESNGQPENNYKTTPPVLDSDGS-FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL-SPGK	217
CL	QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW-KADSSPVKAGVETTTPSKQSNNKYAASSYLSLT-PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	218
CH	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLT-WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSIT-CNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIK-DVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQ-TQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTI-	219

	SKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTN- NGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERN- SYSCSVVHEGLHNHHTTKSFSRTPGK	
CH	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLT- WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSIT- CNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIK- DVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQ- TQTHREDY**A**STLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTI- SKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTN- NGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERN- SYSCSVVHEGLHNHHTTKSFSRTPGK	220
CL	QPKSSPSVTLFPPSSEELETNKA- TLVCTITDFYPGVVTVDWKVDGTPVTQGMETTQPSKQSNNKY- MASSYLTLTARAWERHSSYSCQVTHEGHTVEKSLSRADCS	221

The first CH (SEQ. ID. NO: 217) and the fist CL (SEQ. ID. NO: 218) sequences in Table 4 above are of human origin. The second CH (SEQ. ID. NO: 219) and the third CH (SEQ. ID. NO: 220) in Table 4 are both from murine IgG2a, with that difference that the third CH sequence (SEQ. ID. NO: 220) contains an N297A mutation. The second CL sequence (SEQ. ID. NO: 221) is from murine lambda light chain constant region. These murine sequences are used in the examples for the surrogate antibodies.

In some embodiments, the antibody molecules bind human TNFR2 (hTNFR2).

In some embodiments, it is advantageous that the antibody molecule binds both to hTNFR2 and to cynomologus monkey TNFR2 (cmTNFR2 or cynoTNFR2). Cross-reactivity with TNFR2 expressed on cells in cynomolgus monkey, also called crab-eating macaque or Macaca fascicularis, may be advantageous since this enables animal testing of the antibody molecule without having to use a surrogate antibody, with particular focus on tolerability.

In some embodiments, it is necessary to use a surrogate antibody to test an antibody molecule's functional activity in relevant in vivo models in mice. To ensure the comparability between the antibody molecule's effect in humans and the *in vivo* results for the surrogate antibody in mice, it is essential to select a functionally equivalent surrogate antibody having the same *in vitro* characteristics as the human antibody molecule.

In some embodiments, the antibody molecule does not bind specifically to an epitope of TNFR2 comprising or consisting of the sequence KCSPG.

In some embodiments, the antibody molecule of the present invention or used according to the invention is an antibody molecule that is capable of competing with the

specific antibodies provided herein, for example capable of competing with antibody molecules comprising a VH selected from the group consisting of SEQ. ID. NOs: 7, 15 and 23; and/or a VL selected from the group consisting of SEQ. ID. NOs: 8, 16 and 24, for binding to TNFR2.

By "capable of competing for" we mean that the competing antibody is capable of inhibiting or otherwise interfering, at least in part, with the binding of an antibody molecule as defined herein to the specific target TNFR2.

For example, such a competing antibody molecule may be capable of inhibiting the binding of an antagonistic, blocking antibody molecule described herein by at least about 10%; for example at least about 20%, or at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, about 100% and/or inhibiting the ability of the antibody described herein to prevent or reduce binding of TNFR2 to the specific target ligand TNF-α by at least about 10%; for example at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100%.

Competitive binding may be determined by methods well known to those skilled in the art, such as Enzyme-linked immunosorbent assay (ELISA).

ELISA assays can be used to evaluate epitope-modifying or blocking antibodies. Additional methods suitable for identifying competing antibodies are disclosed in *Antibodies: A Laboratory Manual*, Harlow & Lane, which is incorporated herein by reference (for example, see pages 567 to 569, 574 to 576, 583 and 590 to 612, 1988, CSHL, NY, ISBN 0-87969-314-2).

In some embodiments, it is of interest to use not the antibody molecule itself but a nucleotide sequence encoding such an antibody molecule. The present invention thus encompasses nucleotide sequences encoding the above antagonistic blocking TNFR-2 antibody molecules.

The above described antagonistic blocking antibody molecules and nucleotide sequences can be used in medicine, and then such an antibody molecule and/or nucleotide sequence can be included in a pharmaceutical composition, as discussed further below.

The above described antagonistic blocking antibody molecules, nucleotide sequences and/or pharmaceutical compositions can be used in the treatment of cancer, as discussed further below.

The above described antagonistic blocking antibody molecules, nucleotide sequences and/or pharmaceutical compositions can be used in the treatment of an infection caused by an intracellular pathogen, as discussed further below.

The above described antagonistic blocking antibody molecules and/or nucleotide sequences can be used in the manufacture of a pharmaceutical composition for use in the treatment of cancer.

The above described antagonistic blocking antibody molecules and/or nucleotide sequences can be used in the manufacture of a pharmaceutical composition for use in the treatment of an infection caused by an intracellular pathogen.

The above described antagonistic blocking antibody molecules and/or pharmaceutical compositions can be used in a method for treatment of cancer in a patient, wherein a therapeutically effective amount of an antibody molecule or pharmaceutical composition is administered to the patient.

The above described antagonistic blocking antibody molecules and/or pharmaceutical compositions can be used in a method for treatment of an infection caused by an intracellular pathogen in a patient, wherein a therapeutically effective amount of an antibody molecule or pharmaceutical composition is administered to the patient.

In some embodiments relating to treatment of cancer, the cancer is a solid or leukemias cancer. A solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign (not cancer), or malignant (cancer). Malignant solid tumors are herein denoted solid cancer. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors or cancers are sarcomas, carcinomas, and lymphomas.

More specific examples of solid cancers are lung cancer, breast cancer, colorectal cancer, prostate cancer, bladder cancer, ovarian cancer, endometrial cancer, kidney cancer, liver cancer, pancreatic cancer, thyroid cancer, brain cancer, central nervous system cancer, melanoma, neuroblastoma, Wilms tumor, rhabdomyosarcoma, retinoblastoma, head and neck cancer, gastric cancer, lymphoma and bone cancer.

More specific examples of leukemic cancers are acute lymphocytic leukemia, Chronic myeloproliferative disease, acute non-lymphocytic leukemia, B cell acute lymphocytic leukemia, chronic lymphocytic leukemia, T cell acute lymphocytic leukemia, non-Hodgkin lymphomas and chronic lymphoproliferative diseases.

In some embodiments relating to treatment of an infection caused by an intracellular pathogen, such as virus or bacteria., Specific examples of intracellular pathogens are *Legionella pneumophila*, *R. rickettsia*, *Mycobacterium tuberculosis, Listeria monocytogenes, Salmonella* spp, invasive *Escherichia coli*, *Neisseria spp*, Brucella spp, *Shigella spp,* Influenza virus, Herpes virus, Hepatitis virus, Coxsackievirus, Epstein-Barr virus or Rhinovirus.

In some embodiments relating to treatment of cancer, the above described antagonistic blocking antibody molecules can be used in combination with an antibody mol-

ecule that specifically binds to a check-point inhibitor. Alternatively, the above discussed nucleotide sequences encoding a blocking TNFR2 antibody molecule can be used in combination with antibody molecule that specifically binds to a check-point inhibitor or a co-stimulatory agonistic antibody. Antibodies to check-point inhibitors include antibodies targeting CTLA4, PD1, PD-L1, VISTA, TIGIT, CD200, CD200R, BTLA, LAG3, TIM3, B7-H3, B7-H4, B7-H7, . Examples of co-stimulatory agonistic antibodies are antibodies targeting OX40, 41BB, OX40L, 41BBL, GITR, ICOS, DR3, DR4, DR5, CD40, CD27, RANK, HVEM, LIGHT and B7-H6. Alternatively, the above discussed antagonistic blocking TNFR2 antibody molecules can be used in combination with a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor or a costimulatory agonist. Alternatively, the above discussed nucleotide sequences encoding a blocking TNFR2 antibody molecule can be used in combination with a nucleotide sequence that encodes an antibody molecule that specifically binds to a check-point inhibitor or a costimulatory agonist. In some such embodiments, the antibody molecule that specifically binds to a check-point inhibitor is an anti-PD-1 antibody. PD-1 (or PD1) antibodies are thought to block the inhibitory signal mediated through PD-L1 primarily in $CD8^+$ T cells; and thereby allowing an increased T cell mediated anti-tumor response. An antagonistic TNFR2 antibody depleting Tregs would work through a separate mechanism to increase the anti-tumor response. Hence these treatments could synergize with each other. The same is true for other check-point inhibitors and agonistic costimulatory antibodies.

Additionally, the above discussed antagonistic blocking TNFR2 antibody molecules can be used in combination with other anti-cancer treatments such as chemotherapy (e.g. but not limited to doxorubicin, paraplatin, cyclophosphamide, paclitaxel, gemcitabine, 5-fluorouracil, docetaxel, vincristine, Mitoxantrone, mutamycin, epirubicin and methotrexate), small molecule tyrosin kinase or serine/threonine kinase inhibitors (e.g. but not limited to ibrutinib, imatinib, suntinib, regorafenib, sorafenib, dasatinib, erlotinib, vandetanib, midostaurin, vemurafenib, dabrafenib, palbociclib, ribociclib, Trametinib or alectinib), inhibitors targeting growth factor receptors (e.g. but not limited to drugs targeting EGFR/HER1/ErbB1, EGFR2/HER2/ErbB2, EGFR3/HER3/ErbB3, VEGFR, PDGFR HGFR, RET, insulin-like growth factor receptor IGFR, FGFR), anti-angiogenic agents (e.g. but not limited to Bevacizumab, Everolimus, Lenalidomide, Thalidomide, Ziv-aflibercept) or irradiation. Typically, the above mentioned anti-cancer drugs all cause cancer cell death which will lead to exposure of neo-antigens and inflammation. At a time where neo-antigens are exposed, and there is an influx of inflammatory cells in the tumor, there can occur synergistic effects of the anti-cancer drug, and the addition of an

antagonistic ligand blocking TNFR2 antibody, which can deplete Tregs and thereby enhance the immune system even further.

It would be known to the person skilled in medicine, that medicines can be modified with different additives, for example to change the rate in which the medicine is absorbed by the body; and can be modified in different forms, for example to allow for a particular administration route to the body.

Accordingly, we include that the antagonistic blocking antibody molecules, nucleotide sequences, plasmids, viruses and/or cells described herein may be combined with a pharmaceutically acceptable excipient, carrier, diluent, vehicle and/or adjuvant into a pharmaceutical composition. In this context, the term pharmaceutical composition can be used interchangeably with the terms pharmaceutical preparation, pharmaceutical formulation, therapeutic composition, therapeutic preparation, therapeutic formulation and therapeutic entity.

The pharmaceutical compositions described herein may comprise, or in some embodiments consist of, antibody molecules, nucleotide sequences, plasmids, viruses or cells.

The pharmaceutical compositions described herein may in some embodiments consist of or comprise plasmids comprising nucleotide sequences encoding the above described antibody molecules or comprising the above described nucleotide sequences.

In some embodiments, the pharmaceutical compositions may comprise nucleotide sequences encoding parts of or a complete antibody molecule described herein integrated in a cell or viral genome or in a viriome. The pharmaceutical composition may then comprise a cell or a virus as a delivery vehicle for an antibody of the invention (or a delivery vehicle for a nucleotide sequence encoding an antibody of the invention). For example, in an embodiment, the virus may be in the form of a therapeutic oncolytic virus comprising nucleotide sequences encoding at least one of the antibody molecules described herein. In some embodiments, such an oncolytic virus comprises nucleotide sequences encoding a full-length human IgG antibody.

In some embodiments the invention relates to a virus comprising a nucleotide sequence of the invention or a plasmid of the invention. Preferably, the virus is an oncolytic virus, such as a therapeutic oncolytic virus. Such viruses are known to those skilled in the arts of medicine and virology.

In some embodiments, such an oncolytic virus comprises nucleotide sequences encoding amino acid sequence having at least 80% identity with a sequence set out in table 1 above. In some embodiments, such an oncolytic virus comprises an amino acid sequence having at least 85% identity with a sequence set out in table 1 above. In some embodiments, such an oncolytic virus comprises an amino acid sequence having at least

90% identity with a sequence set out in table 1 above. In some embodiments, such an oncolytic virus comprises an amino acid sequence having at least 95% identity with a sequence set out in table 1 above.

As an example, a nucleotide sequence encoding the antibody 001-H10 could be as presented in Table 5..

Table 5: Example of nucleotide sequences encoding the antibody 001-H10 – the parts of the sequences that are underlined in the table encodes the VH and VL sequences, respectively, of 001-H10

Encoding	Sequence	SEQ. ID. NO:
001-H10 VH	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGG- TACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT- TCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAA- GCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG- TTATTTATAGCGGTGGTAGTACATATTAC- GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAA- GAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACAC- TGCCGTGTATTACTGTGCGAGAGATCGAAGCAGCAGCTGGTAC- CGCGATGGTATGGACGTCTGGGGCCAAGGTACACTGGTCAC- CGTGAGCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG- CACCCTCCTCCAAGAGCACCTCTGGGGG- CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC- CGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG- CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAG- CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGAC- CTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTG- GACAAGAAAGTTGAGCCCAAATCTT- GTGACAAAACTCACACATGCCCACCGTGCCCAGCAC- CTGAACTCCTGGGGGGACCGTCAG- TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCG- GACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA- GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG- CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAC- GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG- GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA-	222

	GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG- CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG- GATGAGCTGACCAAGAACCAGGTCAGCCTGAC- CTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG- GAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC- GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAA- GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC- GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC- GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA	
001-H10 VL	CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGG- CAGAGGGTCACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGG- CAGGTTATGATGTACACTGGTATCAGCAGCTCCCAGGAAC- GGCCCCCAAACTCCTCATCTATGGTAACAG- CAATCGGCCCTCAGGGGTCCCTGACCGAT- TCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAG- TGGGCTCCGGTCCGAGGATGAGGCTGATTATTACTGTGCAGCATGG- GATGACAGCCTGAGTGGTTGGGTGTTCGGCGGAGGAACCAA- GCTGACGGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCAC- TCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACAC- TGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAG- TGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA- GACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAG- CAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGC- TACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAG- TGGCCCCTACAGAATGTTCATGA	

Some oncolytic viruses have capacity to host large enough DNA insertions to accommodate integration of full-length human antibody sequences. Attenuated Vaccinia viruses and Herpes Simplex Viruses are examples of therapeutic oncolytic viruses whose genome is sufficiently large to permit integration of full-length IgG antibody sequences (Chan et al. 2014. 'Oncolytic Poxviruses', *Annu Rev Virol*, 1: 119-41; Bommareddy. et al 2018. 'Integrating oncolytic viruses in combination cancer immunotherapy', *Nat Rev Immunol*, 18: 498-513).Full-length IgG antibodies have successfully been integrated into oncolytic *Vaccinia* virus, resulting in expression and extracellular release (production) of full-length IgG antibodies upon infection of virus-susceptible host cells e.g. cancer cells (Kleinpeter et al. 2016. 'Vectorization in an oncolytic vaccinia virus of an antibody, a Fab and a scFv against programmed cell death -1 (PD-1) allows their

intratumoral delivery and an improved tumor-growth inhibition', *Oncoimmunology*, 5: e1220467). Adenoviruses can also be engineered to encode full-length IgG antibodies that are functionally produced and secreted upon cellular infection (Marino et al. 2017. 'Development of a versatile oncolytic virus platform for local intra-tumoural expression of therapeutic transgenes', *PLoS One*, 12: e0177810).

The invention also encompasses pharmaceutical compositions comprising a virus, such as an oncolytic virus, as discussed above, and a pharmaceutically acceptable diluent, vehicle and/or an adjuvant.

The invention also comprises other therapeutic modalities, or "shapes" of drugs, such as antibody drug conjugates, fusion proteins etc., and pharmaceutical composition comprising such therapeutic modalities.

The antibody molecules, nucleotide sequences, plasmids, viruses, cells and/or pharmaceutical compositions described herein may be suitable for parenteral administration including aqueous and/or non-aqueous sterile injection solutions which may contain anti-oxidants, and/or buffers, and/or bacteriostats, and/or solutes which render the formulation isotonic with the blood of the intended recipient; and/or aqueous and/or non-aqueous sterile suspensions which may include suspending agents and/or thickening agents. The antibody molecules, nucleotide sequences, plasmids, cells and/or pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (*i.e.* lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, and/or granules, and/or tablets of the kind previously described.

For parenteral administration to human patients, the daily dosage level of the anti-TNFR2 antibody molecule will usually be from 1 mg/kg bodyweight of the patient to 20 mg/kg, or in some cases even up to 100 mg/kg administered in single or divided doses. Lower doses may be used in special circumstances, for example in combination with prolonged administration. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

Typically, a pharmaceutical composition (or medicament) described herein comprising an antibody molecule will contain the anti-TNFR2 antibody molecule at a concentration of between approximately 2 mg/ml and 150 mg/ml or between approximately 2 mg/ml and 200 mg/ml.

Generally, in humans, oral or parenteral administration of the antibody molecules, nucleotide sequences, plasmids, viruses, cells and/or pharmaceutical compositions described herein is the preferred route, being the most convenient. For veterinary use, the antibody molecules, nucleotide sequences, plasmids, viruses, cells and/or pharmaceutical compositions described herein are administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal. Thus, the present invention provides a pharmaceutical formulation comprising an amount of an antibody molecule, nucleotide sequence, plasmid, virus and/or cell of the invention effective to treat various conditions (as described above and further below). Preferably, the antibody molecules, nucleotide sequences, plasmids, viruses, cells and/or pharmaceutical compositions described herein is adapted for delivery by a route selected from the group comprising: intravenous (IV or i.v.); intramuscular (IM or i.m.); subcutaneous (SC or s.c.) or intratumoral.

The present invention also includes antibody molecules, nucleotide sequences, plasmids, viruses, cells and/or pharmaceutical compositions described herein comprising pharmaceutically acceptable acid or base addition salts of the target binding molecules or parts of the present invention. The acids which are used to prepare the pharmaceutically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulphate, bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, p- toluenesulphonate and pamoate [*i.e.* 1 ,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others. Pharmaceutically acceptable base addition salts may also be used to produce pharmaceutically acceptable salt forms of the agents according to the present invention. The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of the present agents that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmacologically acceptable cations such as alkali metal cations (*e.g.* potassium and sodium) and alkaline earth metal cations (*e.g.* calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines, among others. The antibody molecules, nucleotide sequences, plasmids, viruses and/or cells described herein may be lyophilized for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophi-

lization method (*e.g.* spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (*e.g.* with conventional immunoglobulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted upward to compensate. In one embodiment, the lyophilized (freeze dried) polypeptide binding moiety loses no more than about 20%, or no more than about 25%, or no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilization) when re-hydrated.

The anti-TNFR2 antibody molecules, nucleotide sequences and pharmaceutical compositions described herein can be used use in the treatment of cancer in a subject or patient. Herein, the terms subject and patient are used interchangeably

"Patient" (or subject) as the term is used herein refers to an animal, including human, that has been diagnosed as having a specific disease.

In some embodiments, the patient (or subject) is an animal, including human, that has been diagnosed as having cancer and/or that exhibits symptoms of cancer.

In some embodiments, the patient (or subject) is an animal, including human, that has been diagnosed as having an infection caused by an intracellular pathogen and/or that exhibits symptoms of an infection caused by an intracellular pathogen.

In some embodiments, the patient (or subject) is a patient having high TNFR2 expression in diseased tissue. In this context, high expression means a higher level of TNFR2 expression compared to corresponding healthy tissue. Normally the healthy tissue used for such a comparison is a reference tissue (or standard reference) collected from healthy tissue from one or several healthy individuals. The level of expression can be measured by standard techniques such as immunohistochemistry (IHC), fluorescence-activated cell sorting (FACS) or mRNA expression measurements.

We include that the patient could be mammalian or non-mammalian. Preferably, the mammalian patient is a human, a horse, a cow, a sheep, a pig, a camel, a dog or a cat. Most preferably, the mammalian patient is a human.

By exhibit symptoms of cancer, we include that the patient displays a cancer symptom and/or a cancer diagnostic marker, and/or the cancer symptom and/or a cancer diagnostic marker can be measured, and/or assessed, and/or quantified.

It would be readily apparent to the person skilled in medicine what the cancer symptoms and cancer diagnostic markers would be and how to measure and/or assess and/or quantify whether there is a reduction or increase in the severity of the cancer symptoms, or a reduction or increase in the cancer diagnostic markers; as well as how

those cancer symptoms and/or cancer diagnostic markers could be used to form a prognosis for the cancer.

Cancer treatments are often administered as a course of treatment, which is to say that the therapeutic agent is administered over a period of time. The length of time of the course of treatment will depend on a number of factors, which could include the type of therapeutic agent being administered, the type of cancer being treated, the severity of the cancer being treated, and the age and health of the patient, amongst others reasons.

By "during the treatment", we include that the patient is currently receiving a course of treatment, and/or receiving a therapeutic agent, and/or receiving a course of a therapeutic agent.

In some embodiments the cancer to be treated in accordance with the present invention is a solid tumor.

Each one of the above described cancers is well-known, and the symptoms and cancer diagnostic markers are well described, as are the therapeutic agents used to treat those cancers. Accordingly, the symptoms, cancer diagnostic markers, and therapeutic agents used to treat the above mentioned cancer types would be known to those skilled in medicine.

Clinical definitions of the diagnosis, prognosis and progression of a large number of cancers rely on certain classifications known as staging. Those staging systems act to collate a number of different cancer diagnostic markers and cancer symptoms to provide a summary of the diagnosis, and/or prognosis, and/or progression of the cancer. It would be known to the person skilled in oncology how to assess the diagnosis, and/or prognosis, and/or progression of the cancer using a staging system, and which cancer diagnostic markers and cancer symptoms should be used to do so.

By "cancer staging", we include the Rai staging, which includes stage 0, stage I, stage II, stage III and stage IV, and/or the Binet staging, which includes stage A, stage B and stage C, and/or the Ann Arbour staging, which includes stage I, stage II, stage III and stage IV.

It is known that cancer can cause abnormalities in the morphology of cells. These abnormalities often reproducibly occur in certain cancers, which means that examining these changes in morphology (otherwise known as histological examination) can be used in the diagnosis or prognosis of cancer. Techniques for visualizing samples to examine the morphology of cells, and preparing samples for visualization, are well known in the art; for example, light microscopy or confocal microscopy.

By "histological examination", we include the presence of small, mature lymphocyte, and/or the presence of small, mature lymphocytes with a narrow border of cytoplasm, the presence of small, mature lymphocytes with a dense nucleus lacking discern-

ible nucleoli, and/or the presence of small, mature lymphocytes with a narrow border of cytoplasm, and with a dense nucleus lacking discernible nucleoli, and/or the presence of atypical cells, and/or cleaved cells, and/or prolymphocytes.

It is well known that cancer is a result of mutations in the DNA of the cell, which can lead to the cell avoiding cell death or uncontrollably proliferating. Therefore, examining these mutations (also known as cytogenetic examination) can be a useful tool for assessing the diagnosis and/or prognosis of a cancer. An example of this is the deletion of the chromosomal location 13q14.1 which is characteristic of chronic lymphocytic leukemia. Techniques for examining mutations in cells are well known in the art; for example, fluorescence in situ hybridization (FISH).

By "cytogenetic examination", we include the examination of the DNA in a cell, and, in particular the chromosomes. Cytogenetic examination can be used to identify changes in DNA which may be associated with the presence of a refractory cancer and/or relapsed cancer. Such may include: deletions in the long arm of chromosome 13, and/or the deletion of chromosomal location 13q14.1, and/or trisomy of chromosome 12, and/or deletions in the long arm of chromosome 12, and/or deletions in the long arm of chromosome 11, and/or the deletion of 11q, and/or deletions in the long arm of chromosome 6, and/or the deletion of 6q, and/or deletions in the short arm of chromosome 17, and/or the deletion of 17p, and/or the t(11:14) translocation, and/or the (q13:q32) translocation, and/or antigen gene receptor rearrangements, and/or BCL2 rearrangements, and/or BCL6 rearrangements, and/or t(14:18) translocations, and/or t(11:14) translocations, and/or (q13:q32) translocations, and/or (3:v) translocations, and/or (8:14) translocations, and/or (8:v) translocations, and/or t(11:14) and (q13:q32) translocations.

It is known that patients with cancer exhibit certain physical symptoms, which are often as a result of the burden of the cancer on the body. Those symptoms often reoccur in the same cancer, and so can be characteristic of the diagnosis, and/or prognosis, and/or progression of the disease. A person skilled in medicine would understand which physical symptoms are associated with which cancers, and how assessing those physical systems can correlate to the diagnosis, and/or prognosis, and/or progression of the disease. By "physical symptoms", we include hepatomegaly, and/or splenomegaly.

BRIEF DESCRIPTION OF THE DRAWINGS

In the examples below, reference is made to the following figures:

Figure 1 demonstrates that antibodies of the invention bind TNFR2. Fig. 1 A-D: Human antibodies were shown by ELISA to bind to human TNFR2 protein in a dose-dependent manner generating different EC50 values. Fig. 1 E: The murine antibodies 3-F10 and 5-A05 bind to mTNFR2 with a similar affinity.

Figure 2 shows binding of TNFR2 specific n-CoDeR® antibodies to *in vitro* activated $CD4^+$ T cells. Human blood derived $CD4^+$ T cells (Fig. 2 A-D) and mouse splenic $CD4^+$ T cells (Fig. 2 E) were activated with IL-2 and CD3/CD28 Dynabeads®. The affinity of TNFR2 specific n-CoDeR® antibodies to activated cells were analyzed by FACS at concentrations ranging from 0.002-267 nM (human) and 0.00003-133 nM (mouse). The curves show mean fluorescence intensity (MFI) after subtraction of isotype control background (Fig. 2 A (complete and partial-blockers), Fig. 2 B (partial-blockers), Fig. C and D (non-blockers), Fig. 2 E (mouse complete-blocker (3-F10) and non-blocker (5-A05)).

While human TNFR2 antibodies bind with different affinities to *in vitro* activated CD4s (ranging EC50 values from 0.59 to 53nM) the mouse TNFR2 antibodies bind with similar affinity (EC50 values ranging from 0.072 to 0.11nM).

Figure 3 shows that TNFR2 n-CoDeR® antibodies specifically bind to TNFR2. Human blood derived $CD4^+$ T cells (Fig. 3 A) and mouse splenic $CD4^+$ T cells (Fig. 3 B) were activated 3 days with recombinant IL-2 and CD3/CD28 activation beads. *In vitro* activated cells were blocked with a polyclonal TNFR2 antibody (grey line) or left in PBS (black line) for 30 min before stained with suboptimal concentration of the different TNFR2 n-CoDeR® antibodies or isotype control (dashed line) for 15 min. Cells were then washed and incubated with an APC conjugated secondary antibody for 30 min before analyzed by flow cytometry.

All antibodies could be blocked by the polyclonal TNFR2 antibody, hence show that the TNFR2 n-CoDeR® antibodies (human and mouse) are specific to TNFR2.

Figure 4 shows cross-reactivity of human TNFR2 specific n-CoDeR® antibodies to Cynomolgus. $CD4^+$ T cells were isolated from cynomolgus blood and stimulated with PMA and Ionomycin. After 2 days the cells were labelled with 0.1, 1 or 10µg/ml TNFR2 specific n-CoDeR® antibodies or isotype control followed by incubation with an APC conjugated secondary a-human antibody. Cells were analyzed by flow cytometry. The figure shows percentages of $TNFR2^+$ T cells for the individual antibodies over the isotype control. The results are the mean value and SD from 2-3 individual experiments.

Most TNFR2 antibodies show cross-reactive binding to Cynomolgus cells.

Figure 5 show that all herein described TNFR2 specific n-CoDeR® antibodies bind other epitopes on the TNFR2 protein than the TNFR2 clone MR2-1. Human blood derived $CD4^+$ T cells were stimulated with rhIL-2 and CD3/CD28 activation beads 2-3 days. Activated cells were either blocked by 40 µg/ml MR2-1 antibody (Fig. 5 A, black bars) or left with PBS (Fig. 5 A, grey bars) 30 min, then TNFR2 specific n-CoDeR® antibodies/polyclonal TNFR2 (pTNFR2) were added and cells were incubated 15 min. Percent bound TNFR2 n-CoDeR® antibodies were analyzed by FACS after incubation with APC conjugated secondary antibodies. In Fig. 5 B, activated $CD4^+$ T cells were blocked

with 40µg/ml TNFR2 specific n-CoDeR® antibodies/pTNFR2 (black bars) or left with PBS (grey bar) and then incubated 15 min with PE conjugated MR2-1 antibody. Cells were then analyzed by FACS.

MR2-1 antibody did not interfere with the binding of the TNFR2 specific n-CoDeR® antibodies and the n-CoDeR® antibodies did not affect the binding of MR2-1 to activated cells showing that the n-CoDeR® antibodies all bind other domains of the TNFR2 protein than the MR2-1 antibody.

Figure 6 shows ligand blocking activity of anti-human TNFR2 antibodies. Blocking ELISAs were performed with n-CoDeR® mAbs specific for hTNFR2 to evaluate ligand blocking characteristics. Fig. 6 A: All antibodies were incubated at 10 µg/ml. Subsequently, all antibodies reducing the signal achieved with the isotype control by more than 50% (indicated by the dotted line) were dosed to further explore the ligand blocking potential. Fig. 6 B shows complete blocking mAbs, Figs. 6 C and D show partially blocking mAbs and Fig. 6 E shows weak blocking mAbs. All other mAbs are considered non-blocking mAbs.

Figure 7 shows ligand blocking activity of anti-mouseTNFR2 antibodies. Blocking ELISAs were performed with n-CoDeR® mAbs specific for mTNFR2 to evaluate ligand blocking characteristics. Fig. 7 A: All antibodies were incubated at 10 µg/ml. Subsequently, all antibodies reducing the signal achieved with the isotype control by more than 50% (indicated by the dotted line) were dosed to further explore the ligand blocking potential. Fig. 7 B shows complete blocking mAbs, Figs. 7 C and D show partially blocking mAbs and Fig. 7 E shows weak blocking mAbs. All other mAbs are considered non-blocking mAbs. Based on this, antibodies 3-F10 and 5-A05 were chosen to represent complete blocking antibodies and non-blocking antibodies respectively.

Figure 8 Categorization of TNFR2 specific n-CoDeR® antibodies according to their ability to agonize/antagonize TNFR2 signaling and ability to block TNF-α binding to TNFR2. The ability of TNFR2 specific n-CoDeR® antibodies to enhance or reduce IFN-γ production was monitored using IL-2 and IL-12 stimulated NK cells, and was plotted as a function of antibodies ability to block TNF-α ligand binding to TNFR2 as described above. Fig. 8 A: Human blood derived NK cells were stimulated with 20 ng/ml rhIL-2 and 20 ng/ml rhIL-12 with the addition of 10 µg/ml TNFR2 specific n-CoDeR® antibodies, isotype control or 100 ng/ml rhTNF-α for 24h. The amount of IFN-γ in the culture supernatants was measured using MSD. The quantity of IFN-γ are normalized to isotype control (IFN-γ values of isotype control = 1 in figure) and shown in Fig. 8 A. Human antibodies that had a higher EC50 value than 25 nM to *in vitro* activated $CD4^+$ T cells were not included in the analysis. Fig. 8 B: Human NK cells also produce TNF-α in these cultures (data shown mean TNF-α levels of two donors. The cell cultures supernatants were col-

lected and the amounts of IFN-γ produced were analyzed using MSD. The results are normalized against an isotype control. IFN-γ results are the mean value of 3 donors in 2 independent experiments. The results reveal two extreme groups characterized by 1) antibodies with complete blocking and antagonist properties, and 2) antibodies with agonist non-blocking properties, respectively. Agonistic non-blocking antibodies are agonistic and enhance the IFN-γ production from cytokine stimulated NK cells while blocking antibodies are antagonistic and inhibit IFN- γ release. Figure 8 C shows that the IFN- γ release is TNF-α dependent as neutralization of soluble TNF-α decreases the IFN- γ, while adding exogenous TNF-α increases IFN- γ. Figure 8 D show that addition of a blocking anti-TNF-α antibody results in a dose-dependent neutralization of soluble TNF-α. At a dose of 1 µg/ml, no soluble TNF-α can be detected in the supernatant.

Figure 9 shows that non-blocking agonistic but not blocking antagonistic TNFR2 specific n-CoDeR® antibodies enhance the proportion of $CD25^+$ cells within the memory $CD4^+$ T cells population. Human blood derived $CD4^+$ T cells (Fig. 9 A) and mouse splenic $CD4^+$ T cells (Fig. 9 B) were activated with recombinant IL-2 and TNFR2 specific n-CoDeR® antibodies, isotype control or recombinant TNF-α. After 3 days of culture the cells were stained for CD25 and CD45RO (human)/CD44 and CD62L (mouse) and analyzed by flow cytometry. The results show the percentage of CD25 expressing cells on the memory ($CD45RO^+$ cells (human)/$CD44^+CD62L^-$ (mouse)) population over the percentage of $CD25^+$ cells recovered in cultures with isotype control. The results are the mean value and SEM of 7 donors (Fig. 9 A, human) and 3 mice (in 2 independent experiments, Fig. 9 B). In both human and mouse cultures non-blocking TNFR2 antibodies induced the percentage of $CD25^+$ memory cells, while blocking antibodies had no such effect on the memory population. For both human cultures (Fig. 9 A) and murine (Fig. 9 B), the addition of exogenous TNF-α increase the $CD25^+$ memory T cell population*=$p<0,05$ as calculated by one-way ANOVA.

Figure 10 A shows that ligand blocking antagonistic antibodies have most pronounced anti-tumor effect as mIgG2a, an isotype preferentially engaging activatory Fc receptors. Balb/c mice were injected subcutaneously with $1x10^6$ CT26 cells. After 8 days, at a mean tumor size of 3x3 mm mice were treated twice weekly with 10 mg/kg antibody i.p. as indicated in figures. Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated. The upper figure shows the tumor growth in isotype control treated mice, then the two figures below, in the left panel, show the antagonistic ligand-blocker antibody (middle figure) and the agonistic non-ligand blocking antibody (lower figure) in FcγR defective Ig format. The middle panel show the same antibodies in murine IgG2a format, engaging primarily activatory FcγRs and the right panel the antibodies in murine IgG1 format engaging primarily the inhibitory

FcγRIIb. For Fig. 10 B surviving mice were followed for 70 days. As seen in the figures, the blocking antagonistic antibody is most efficacious as tumor treatment in an IgG2a format engaging primarily activatory FcγRs and has no effect in a format with defective FcγR binding. On the other hand, the non-blocking agonistic antibody is most efficacious as tumor treatment in an IgG1 format preferentially engaging the inhibitory FcγR. In addition, the agonistic antibody has intrinsic, FcγR-independent, anti-tumor effects as seen using the N297A antibody format. ***=p<0,001 compared to isotype control as calculated by Log-rank Mantel Cox test

Figure 11 shows that ligand blocking antagonistic antibodies are effective as anti-tumor treatment in combination with anti-PD1. C57/BL6 mice were injected subcutaneously with $1x10^6$ MC38 cells. At a mean tumor size of 3x3 mm mice were treated twice weekly with 10 mg/kg antibody i.p. as indicated in figures. The figure shows tumor growth curves of individual mice. Fig. 11 A: isotype control, Fig. 11 B: PD-1 targeting antibody, Fig. 11 C: 3-F10 antibody (surrogate antibody, ligand blocker, antagonist), Fig. 11 D: combination of 3-F10 and PD1. Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated. Fig. 11 E shows survival curves of the four different treatment groups, **=p<0,01, ***=p<0,001 compared to isotype control as calculated by Log-rank Mantel Cox test.

Figure 12 shows that blocking antagonistic antibodies are effective as anti-tumor treatment in combination with anti-PD-L1. C57/BL6 mice were injected subcutaneously with $1x10^6$ MC38 cells. At a mean tumor size of 5x5 mm, mice were treated twice with isotype control antibody or 3F10 (day 1 and 4), or four consecutive days with anti-PD-L1 followed by a fifth injection two days later (in total five injections day 1,2,3,4 and 7), or a combination of both. All antibodies were administered at 10 mg/kg i.p. Figure show mean tumor growth +/- SEM, n=10/group. *=p<0,05, ***=p<0,001 as calculated using one-way ANOVA test.

Figure 13: C57/BL6 mice were injected subcutaneously with $1x10^6$ B16.F10 cells. After 3 days mice were treated twice weekly with 10 mg/kg antibody i.p. as indicated in figures. Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated. Fig. 13 A: isotype control, Fig. 13 B: 3-F10 antibody (surrogate antibody, ligand blocker, antagonist). Fig. 13 C shows survival curves of the two different treatment groups. *=p<0,05 compared to isotype control as calculated by Log-rank Mantel Cox test

Figure 14 shows that ligand blocking antagonistic surrogate antibody 3F10 alters immune cell composition in tumors. Mice were inoculated with CT26 tumor cells as described and injected with antibodies as indicated once the tumors reached a size of approximately 7x7 mm. after 3 injections, at day 8 after treatment start, mice were sacri-

ficed and tumors harvested. Tumor single cell suspensions were analyzed for immune cell content by FACS. Fig. 14 A: Ligand blocking antagonistic surrogate antibody 3F10 causes Treg depletion and Fig. 14 B: $CD8^+$ T cell influx or expansion. This causes a shift in $CD8^+$/Treg T cell ratio as depicted in Fig. 14 C. Figure 14 D shows that not only T cells numbers but also the number of myeloid cells, here tumor associated macrophages (TAMs, defined as being $CD11b^+F4/80^+MHCII^+$ but negative for both Ly6G and Ly6C) are very significantly reduced. The ligand non-blocking agonistic surrogate antibody 5A05 also modulates TAM numbers but is still significantly different from the ligand blocking antibody 3F10.

Figure 15 shows that T cells in human tumors express levels of TNFR2 similar to T cells retrieved from a PBMC reconstituted NOG mice. Briefly, NOG mice were injected i.v. with 15-20x10^6 PBMC cells. After 10-12 days, the spleens were removed from mice, single cell suspension prepared and TNFR2 expression was assessed by FACS. Previously, TNFR2 expression had been assessed on T cells retrieved from blood and tumor samples from 3 or 9 cancer patients respectively. As shown in the figure, the TNFR2 expression on Tregs and $CD8^+$ T cells are very comparable between the human T cells grown and activated in vivo in the NOG mice, and T cells from human tumors.

Figure 16 shows that the ligand blocking antagonistic antibody 1-H10 depletes Tregs *in vivo* in an FcγR dependent manner. NOG mice were injected i.v. with 15-20x10^6 PBMC cells. After 10-12 days, the spleens was removed from mice, single cell suspension prepared and then injected i.p. into SCID mice. (10-15x10^6/mice). After 1 h, mice were treated with 10 mg/kg antibody i.p. and 24 hours after antibody injection, i.p. fluid was collected from mice and the cells in the fluid were analyzed using FACS. Fig. 16 A shows the mean percentage of stained Tregs (defined as $CD45^+CD3^+CD4^+CD25^+CD127^{low/neg}$) out of the human $CD45^+$ population and show that Tregs are significantly depleted by the blocking antibody 1-H10. Fig. 16 B shows the mean percentage of CD8+ T out of the human $CD45^+$ and shows that 1-H10 significantly increases the $CD8^+$ T cell population. Fig. 16 C shows that the ratio of $CD8^+$ T cells against the Tregs is significantly increased by 1-H10. Data in Fig. 16 A-C is presented as mean of four different experiments where each dot represents one mouse. Yervoy and a commercially available anti-CD25 antibody were used as positive controls. All data is normalized to isotype control so that in Fig. 16 A and B isotype control is set to 100% and in Fig. 16 C to 1. Fig. 16 D shows a separate experiment where FcγR binding defective 1-H10 IgG1N297Q antibody was used (designated 1-H10NQ in the figure) to assess the dependency of FcγR binding for Treg depletion. As shown in the figure, the depletion is most efficacious in the wild type IgG1 format (1-H10) compared to the Fc defective (1-H10NQ).

Figure 17 shows that antagonistic ligand non-blocking TNFR2 antibodies do not induce cytokine release in vitro. IFN-γ release induced by various TNFR2 specific antibodies was measured in three different in vitro systems. As positive controls, and anti-CD3 antibody = OKT3, an anti-CD52 antibody = Alemtuzumab and an anti-CD28 antibody were used. Isotype control was used as a negative control. Each dot represents PBMC from one human donor. Fig. 17 A shows results from high density cell cultures were PBMCs were cultured at $1x10^7$ cells/ml. After 48 h, 10 µg/ml antibody was added and incubated for 24 h. As seen in the figure, both Alemtuzumab and OKT3 induced significant IFN-γ release but not any of the TNFR2 specific antibodies. Fig. 17 B shows Solid Phase in vitro cultures performed by coating wells of a 96-well plate with antibodies before adding PBMCs. Again, both Alemtuzumab and OKT3 induced significant IFN-γ release along with some of the TNFR2 specific antibodies. However, the complete blocking antibody 1-H10 did not induce cytokine release above isotype control antibody. Fig. 17 C shows stimulation of whole blood with antibody and here Alemtuzumab induced significant IFN-γ release but not any of the TNFR2 specific antibodies.

Figure 18 shows that antagonistic ligand non-blocking TNFR2 antibodies do not induce cytokine release in vivo. NOG mice were injected i.v. with $25-x10^6$ PBMC cells. After 14 days, when the blood of the mice was shown to consist of approximately 40% human T-cells, mice were treated with 10 µg antibody. Body temperature was measured 1h post injection (Fig. 18 A). The experiment was terminated 5 h post injection and blood was analyzed for IFN-γ (Fig. 18 B) or TNF-α (Fig. 18 C) content. ****=p<0,0001 and **=p<0,01 as calculated with one-way ANOVA.

Figure 19 shows binding to TNFR2 variants lacking individual domains. Antibody binding to TNFR2 variants expressed on HEK cells were tested in a flow cytometry approach. Lack of domain 1 and 2 does not significantly affect binding (Fig. 19 A and B), while 3 and partially 4 completely abrogates interaction between the antibody and TNFR2 (Fig. 19 C and D). Similarly lack of domain 1+3 prevents binding of all antibodies (except 1F06) completely (Fig. 19 E), while the lack of domain 2+4 abrogate binding completely for the agonistic antibodies (1F02, 1F06, 4E08) and significantly reduces binding also for the antagonists (1H10, 4H02, 5B08) (Fig. 19 F). Dark grey indicates positive control and white negative control antibody.

Figure 20 shows a comparison of amino acid sequence of human (H-D3) and mouse (M-D3) domain 3 of TNFR2. Similar amino acids are marked in white, while differences are marked in grey. The five sequences below represent the 5 different constructs the antibodies are tested against. Exchanges of human-to-mouse sequence are underlined, while the non-marked sequence is completely human. Domains 1, 2 and 4 are human and do not contain any substitutions or mutations.

Figure 21 shows binding to wild type human and mouse TNFR2 (left panels). Mutated hTNFR2 constructs (m1, m2, m3 and m4) were used to narrow down the binding site for different anti hTNFR2 antibodies. Flow cytometry analysis revealed that mutations in aa 119-132 do not affect antibody binding, while mutations in aa 151-160 completely abrogate binding of all antibodies. Mutations in 134-144 disrupt binding for blocking and antagonistic antibodies only but does not affect the agonistic antibodies significantly. Dark grey bars indicate positive control and white negative control antibody. Dashed line is the level of the negative control antibody.

EXAMPLES

Specific, non-limiting examples which embody certain aspects of the invention will now be described.

In many of the examples, in particular the *in vivo* examples, the antibody 3-F10 has been used. This is a mouse antibody, which is a surrogate antibody to the human antibodies disclosed herein. It has been selected based on its ability to bind murine TNFR2, its blocking of murine TNF-α ligand binding to TNFR2, and based on its antagonistic activity in a murine T cell activation assay as described in example 4. In some examples, the 3-F10 antibody was tested and compared in different antibody formats associated with strong and preferential binding to activatory over inhibitory Fc gamma receptors (mIgG2a), strong and preferential binding to mouse inhibitory FcγR (mIgG1) or defective binding to mouse FcγR (mIgG2a N297A).

In some examples, the antibody 5-A05, has been used. This is a mouse surrogate antibody for the human anti-TNFR2, non-blocking, agonistic antibodies included herein for reference and for comparative reasons. 5-A05 was selected as surrogate based on its ability to bind murine TNFR2, lack of blocking effects on murine TNF-α ligand binding to TNFR2, and on its agonistic activity in a murine T cell activation assay as described in example 4. In some examples, the 5-A05 antibody was tested and compared in different antibody formats associated with strong and preferential binding to activating over inhibitory Fc gamma receptors (mIgG2a), strong and preferential binding to mouse inhibitory FcγR over activatory FcγR (mIgG1) or defective binding to mouse Fcγ (N297A).

In some of the examples and figures a slightly different naming of the antibody clones is used, for example, clone 001-H10 is sometimes shortened to 1-H10 or 1H10, 005-B08 is sometimes shortened to 5-B08 or 5B08 etc.

Example 1 – Generation of TNFR2 specific antibodies

(See also figure 1 and the above description of this figure.)

Isolation of scFv antibody fragments

The n-CoDeR® scFv library (BioInvent, Söderlind E, et al *Nat Biotechnol.* 2000;18(8):852-6) was used to isolate scFv antibody fragments recognizing human or mouse TNFR2.

The phage library was used in three consecutive pannings against recombinant human or mouse protein (Sino Biological). After phage incubation, the cells were washed to remove unbound phages. Binding phages were eluted with trypsin and amplified in *E.coli.* The resulting phage stock was converted to scFv format. *E.coli* was transformed with scFv bearing plasmids and individual scFv clones were expressed.

Identification of unique TNFR2 binding scFv

Converted scFv from the third panning were assayed using a homogeneous FMAT analysis (Applied Biosystems, Carlsbad, CA, USA) for binding to 293 FT cells transfected to express human or mouse TNFR2 or a non-related protein.

Briefly, transfected cells were added to clear-bottom plates, together with the scFv-containing supernatant from expression plates (diluted 1:7), mouse anti-His Tag antibody (0. 4 µg/ml; R&D Systems) and an APC-conjugated goat anti-mouse antibody (0.2 µg/ml; cat.no. 115-136-146, Jackson Immunoresearch). FMAT plates were incubated at room temperature for 9 h prior to reading. Bacterial clones binding TNFR2 transfected cells but non cells transfected with a non-related protein were classified as actives and cherry picked into 96-well plate.

IgG binding to TNFR2 in ELISA

96-well plates (Lumitrac 600 LIA plate, Greiner) were coated overnight at 4°C with recombinant human or mouse TNFR2-Fc protein (Sino Biological) at 1 pmol/well. After washing, titrated doses of anti-TNFR2 mAbs from 20 µg/ml to 0.1 ng/ml (133 nM to 1 pM) were allowed to bind for 1 hour. Plates were then washed again, and bound antibodies were detected with an anti-human-F(ab)-HRP secondary antibody (Jackson ImmunoResearch) diluted in 50 ng/ml. Super Signal ELISA Pico (Thermo Scientific) was used as substrate and the plates were analyzed using Tecan Ultra Microplate reader.

The data, which are shown in Table 6 and in Fig. 1 A-D, show that the human anti-TNFR2 antibodies all bind to human TNFR2 protein. The EC50 values are ranging from 0.082 nM for 1-C08 to 4.4 nM for 1-A09.

In addition, the mouse antibody surrogate clones 3-F10 and 5-A05 also bind to mTNFR2 protein. These two clones bind with a very similar affinity (Table 6 and Figure 1E).

Table 6 EC50 values of antibodies binding to TNFR2 protein (human protein except for clone 3F10 and 5A05)

Clone	EC_{50} (nM)

1-C08	0.082
1-E06	0.20
1-G10	0.29
1-H10	0.29
4-H02	0.20
5-B02	0.15
5-B08	0.17
1-G04	1.7
1-H09	0.30
1-D01	0.37
5-F10	0.22
1-B11	0.25
1-C07	0.26
1-B05	0.23
1-F02	0.31
1-F06	0.15
4-E08	0.38
1-G05	0.54
1-A09	4.4
1-B09	0.18
1-C03	0.75
1-C05	0.38
3-F10 (mouse)	0.97
5-A05 (mouse)	1.4

Example 2 – Specificity of antibodies

(See also figures 2-5 and the above description of these figures.)

Isolation of CD4$^+$ T cells

PBMCs from human buffy coats and Cynomolgus macaques (M. fascicularis) whole blood were isolated using Ficoll-Paque PLUS (GE Healthcare) gradients. CD4$^+$ T cells were isolated from PBMCs by magnetic cell sorting using CD4$^+$ T cell isolation kit (human) or CD4 MicroBeads, non-human primate (Cynomolgus) both from Miltenyi. Mouse CD4$^+$ T cells were isolated from spleen using CD4$^+$ T cell isolation kit (mouse) from Miltenyi.

Titration of TNFR2 specific n-CoDeR® antibodies

The ability and affinity of TNFR2 n-CoDeR® antibodies to bind TNFR2 expressed on cells were obtained using *in vitro* activated CD4$^+$ T cells. Human CD4$^+$ T cells were stimulated with 50ng/ml rhIL-2 (R&D systems) and Dynabeads® T-Activator CD3/CD28 for T-Cell Expansion and Activation (Gibco) 2-3 days at 37°C. *In vitro* activated cells were labelled with increasing amount of n-CoDeR® antibodies specific for TNFR2 or isotype control, ranging from 0,002-267nM. Cells were then incubated with an APC conjugated a-human IgG secondary ab (Jackson) followed by analysis by flow cytometry (FACSVerse, BD). The resulting titration curves are shown in Figure 2 A-D. Mouse CD4$^+$ T cells were stimulated with 135 U/ml rmIL-2 (R&D systems) and Dynabeads® T-

Activator CD3/CD28 for T-Cell Expansion and Activation (Gibco) 2-3 days at 37°C. *In vitro* activated cells were labelled with increasing amount of n-CoDeR® antibodies specific for TNFR2 or isotype control, ranging from 0.00003-133 nM. Cells were then incubated with an APC conjugated a-mouse IgG secondary ab (Jackson) followed by analysis by flow cytometry (FACSVerse, BD). The titration curves are shown in Figure 2E. The EC50 values for the titration curves were calculated in Microsoft Excel and are shown in Table 7. For the human antibodies the EC50 values differed from 0.6 nM (4-H02) to 52.7 nM (1-C03). The mouse antibodies bound to *in vitro* activated cells with similar affinity (0.072 nM (3-F10) and 0.11 nM (5-A05)).

Specificity of TNFR2 n-CoDeR® antibodies

The specificity of TNFR2 antibodies to TNFR2 were obtained in FACS blocking experiments with a commercial polyclonal TNFR2 antibody (R&D systems). $CD4^+$ T cells (mouse and human) stimulated 2-3 days with 50ng/ml rhIL-2 (R&D systems) (human)/ 135U/ml rm IL-2 (R&D systems) (mouse) and Dynabeads® T-Activator CD3/CD28 for T-Cell Expansion and Activation (Gibco) were blocked with 40µg/ml polyclonal TNFR2 antibody (R&D systems) for 30 min, immediately followed by 15 min incubation with TNFR2 n-CoDeR® antibodies or isotype control. The concentration of n-CoDeR® antibodies used was based on the titration curves for the individual TNFR2 n-CoDeR® antibodies and a suboptimal concentration for each antibody was chosen. Cells were then washed and incubated 30 min with an APC conjugated secondary antibody (Jackson). Cells were analyzed by flow cytometry (FACSVerse, BD). All binding of TNFR2 specific n-CoDeR® antibodies (both human and mouse) could be blocked by a polyclonal TNFR2 antibody as shown in Figure 3. These results verifying that TNFR2 n-CoDeR® antibodies specifically bind TNFR2 on *in vitro* activated $CD4^+$ T cells.

Epitope mapping of TNFR2 specific n-CoDeR® antibodies against the TNFR2 antibody clone MR2-1

The TNFR2 antibody clone MR2-1 (Invitrogen) binds a specific domain of the TNFR2 protein. If the TNFR2 specific n-CoDeR® antibodies bound to the same domain as MR2-1 was tested by FACS blocking experiments.

Human $CD4^+$ T cells were stimulated 2-3 days with 50 ng/ml rhIL-2 (R&D systems) and Dynabeads® T-Activator CD3/CD28 for T-Cell Expansion and Activation (Gibco). Activated cells were blocked with 40 µg/ml MR2-1 (black bars in Figure 5 A), or PBS (grey bars in Figure 5). After 30 min incubation, cells were immediately stained for TNFR2 specific n-CoDeR® antibody or polyclonal TNFR2 (pTNFR2) for 15 min. After incubation with an APC conjugated secondary anti-human IgG reagent (Jackson), cells were analyzed by flow cytometry (FACSVerse, BD). In figure 5 B, activated $CD4^+$ T cells were blocked with 40µg/ml TNFR2 specific n-CoDeR® antibodies or pTNFR2 (black

bars) or left with PBS (grey bar) and then incubated 15 min with PE conjugated MR2-1 antibody. Again, cells were then analyzed by FACS. Since the percentage of MR2-1+ cells were the same for n-CoDeR® blocked as non-blocked cells (Figure 5 B) and the binding of n-CoDeR® antibodies were the same with or without MR2-1 block (Figure 5 A) these data show that the n-CoDeR® antibodies bind other epitopes of the TNFR2 protein than the MR2-1 antibody.

Binding of TNFR2 n-CoDeR® antibodies to Cynomolgus

To validate the cross-reactivity of TNFR2 antibodies to Cynomolgus, Cynomolgus $CD4^+$ T cells were stimulated 2 days with 50ng/ml PMA (Sigma) and 100 ng/ml Ionomycin (Sigma) to cause upregulation of TNFR2. Cells were incubated with TNFR2 specific n-CoDeR® antibodies at 3 different concentrations (0.1, 1 and 10 µg/ml) and then incubated with an APC conjugated secondary a-human IgG reagent (Jackson). Cells were analyzed by flow cytometry (FACSVerse, BD) and the result show that most of the human TNFR2 specific n-CoDeR® antibodies could bind Cynomolgus TNFR2, the results for the individual antibodies are presented in Figure 4.

In summary, the data in example 2 show that the human antibodies specifically bind to TNFR2 endogenously expressed on human immune cells. Furthermore, the data show that this binding can be blocked by adding a polyclonal commercially available antibody against TNFR2, which indicates very high specificity for TNFR2. The same is true for the surrogate clones 3F10 and 5A05 regarding murine cells expressing murine TNFR2. Also, the binding of the human clones is unaffected by MR2-1 antibodies showing a different epitope specificity compared to MR2-1.

Table 7. EC_{50} values calculated on the titration of TNFR2 specific antibodies to *in vitro* activated $CD4^+$ T cells.

Clone	EC_{50} (nM)
1-C08	2.6
1-E06	4.1
1-G10	3.3
1-H10	1.1
4-H02	0.59
5-B02	0.80
5-B08	1.2
1-G04	18
1-H09	16
1-D01	3.9

5-F10	32
1-B11	27
1-C07	36
1-B05	1.5
1-F02	0.79
1-F06	2.5
4-E08	2.3
1-G05	0.66
1-A09	48
1-B09	29
1-C03	53
1-C05	12
3-F10 (mouse)	0.072
5-A05 (mouse)	0.11

Example 3 – test of ligand blocking characteristics

(See also figures 6-7 and the above description of these figures.)

ELISA method

96-well plates were coated with hTNFR2 (Sinobioologicals Cat. No. 10414-H08H) or mTNFR2 (Sinobioologicals Cat. No. 50128 M08H) at 2.5 pmol/well in ELISA coating buffer (0.1 M sodium carbonate pH 9.5) and incubated overnight at 4°C. After washing in ELISA wash buffer (PBS with 0.05% Tween20), the plates were incubated under slow agitation for 1 h in room temperature with n-CoDeR® mAbs at 10 µg/ml (one-dose ELISA) or 33 nM and subsequent 1:2 dilutions (titration ELISA) in block buffer containing 0.45% fish gelatin. Subsequently, recombinant hTNF-α-bio (R&D Cat. No. BT210) or mTNF-α (Gibco Cat. No. PMC3014) were added at a final concentration of 5 nM and 2 nM respectively and allowed to incubate for another 15 min. Thereafter, plates were washed. For the human ELISAs, Streptavidin-HRP (Jackson Cat. No. 016-030-084) diluted 1:2000 in block buffer were added and again incubated for 1 h at room temperature followed by washes first in ELISA buffer and then in Tris buffer (pH 9.8). The substrate (Super Signal ELISA Pico from Thermo Scientific Cat. No. 37069) were thereafter diluted according to the manufacturers instruction, added to the wells and incubated in darkness for 10 min before reading in a Tecan Ultra. For the mouse ELISAs, rabbit anti-mTNF-α (Sinobiologicals Cat. No. 50349-RP02) diluted to 1 µg/ml in was added and allowed to incubate for 1 h in room temperature. After washing, anti-Rabbit-HRP diluted 1:10 000 in block buffer was added and again incubated for 1 h at room temperature. Substrate adding and reading were performed as above.

The data is presented in Tables 8 and 9 below, and in Figures 6 and 7.

Table 8. EC50 values of ligand blocking human antibodies: Antibodies were titrated and EC 50 values were calculated.

Clone	EC50 (nM)	Block
001-H10	0.9	Complete
004-H02	0.4	Complete
005-B08	0.3	Complete
005-B02	0.2	Complete
001-E06	0.3	Partial
001-G10	1.6	Partial
001-C08	1.1	Partial
001-H09	1.4	Partial
005-F10	0.03	Partial
001-G04	3.2	Partial
001-B11	1.0	Weak
001-C07	0.8	Weak
001-D01	1.4	Weak

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Table 9. EC50 values of ligand blocking murine antibodies: Antibodies were titrated and EC 50 values were calculated.

Clone	EC50 (nM)	Block
3-F10	1.9	Complete
4-C01	2.7	Complete
4-A06	2.0	Partial
4-A07	>500	Partial
4-F06	6.2	Partial
5-C09	8.6	Partial
2-D09	4.4	Partial
4-B12	>500	Partial
3-G06	13	Partial
2-H01	25	Weak
4-C02	>500	Weak
4-G09	2.6	Weak
4-C03	8.3	Weak

Blocking definitions

- Complete blockers are defined as reducing the TNF-α binding with more than 98%
- Partial blockers are defined as reducing the TNF-α binding with 60-98%
- Weak blockers are defined as reducing the TNF-α binding with less than 60%
- Non-blocking antibodies are defined as not reaching more than 50% block in high-dose, one-point ELISA as shown in figures 6A and 7A

The data shown in this example show that various antibodies have been generated ranging from antibodies which completely inhibit the ligand TNF-α from binding to antibodies that do not inhibit ligand blocking at all. This is true for both human antibodies and murine surrogates.

Example 4 – *In vitro* functionality of antibodies

(See also figures 8-9 and the above description of these figures.)

TNFR2 antibodies ability to regulate cytokine stimulated NK cells IFN-γ production

The agonistic/antagonistic characteristics of TNFR2 specific antibodies were evaluated using a NK cell assay described by Almishri *et al.* (TNFα Augments Cytokine-Induced NK Cell IFNγ Production through TNFR2. Almishri W.et al. *J Innate Immun.* 2016;8:617-629).

In brief, human NK cells were isolated from human PBMCs by MACS using "NK isolation kit" (Miltenyi). 100 µl NK cells ($1x10^6$ cells/ml) were cultured with 20 ng/ml rhIL-2 (R&D systems) and 20 ng/ml rhIL-12 (R&D systems) together with 10 µg/ml TNFR2 specific antibodies, 10 µg/ml isotype control or 100 ng/ml TNF-α (R&D systems) in U-bottom plates (Corning® 96 Well TC-Treated Microplates, Sigma-Aldrich). Supernatants were collected after 24h and the amount of IFN-γ produced was assessed by MSD.

As control, anti-TNF-α antibody neutralizing TNF-α (Cat. No. AF-210-NA, R&D systems) was included. As seen in figure 8 D, a dose of 1 µg/ml completely neutralized soluble TNF-α and this dose also lowered the IFN-γ release.

Human non-blocking TNFR2 antibodies distinctly enhanced the IL-2 and IL-12 stimulated NK cells IFN-γ production (2-3 times more IFN-γ than isotype control) while antagonistic antibodies (here shown by complete blockers) showed antagonistic effects on NK cells and decreased IFN-γ production (Figure 8 A).

This test was considered non-representative to perform with the mouse surrogate antibodies due to lack of endogenously produced TNF-α in the murine cultures, as well as expression of inhibitory FcγR on murine NK cells while only activating FcγR was expressed on the human counterpart. Instead, the memory T cell activation assay (induc-

tion of CD25) as described below was used to address the agonist or antagonistic properties of the murine surrogate antibodies.

Induction of CD25 expressing memory CD4$^+$ T cells by TNFR2 antibodies

To further understand the agonistic/antagonistic characteristics of the TNFR2 antibodies, their ability of enhancing the proportion of CD25 expressing memory CD4$^+$ T cells were evaluated.

Briefly, human CD4$^+$ T cells were isolated from PBMCs by MACS using the "CD4$^+$ T cell isolation kit" from Miltenyi. CD4s were cultured with 10 ng/ml rhIL-2 (R&D systems) and 10 µg/ml TNFR2 specific antibody or indicated amount of rhTNF-α (R&D systems). After 3 days the expression of CD25 on memory cells (CD45RO$^+$ cells) were analyzed by FACS (Figure 9A).

Similarly, mouse CD4$^+$ T cells were isolated from spleen by MACS using the "CD4$^+$ T cell isolation kit" (Miltenyi) and cultured with 10 ng/ml rmIL-2 (R&D systems) and 10 µg/ml TNFR2 specific antibody or indicated amount of rmTNF-α (R&D systems). The expression of CD25 on memory cells (CD44$^+$CD62L$^-$ cells) were analyzed by FACS after 3 days (Figure 9B).

The percentage of CD25 expressing cells was enhanced in memory cell cultures stimulated with non-blocking TNFR2, in both human and mouse. However, stimulation with blocking antibodies did not increase CD25 expression in these cultures but rather decreased this.

In summary, the data in example 4 show that ligand blocking antibodies are antagonistic as measured by several methods in vitro: inhibition of NK cell mediated IFN-γ release and activation of CD4$^+$ memory cells as measured by CD25 expression. The antagonistic ligand blocking antibodies are shown according to the invention while the agonistic ligand non-blocking antibodies are included for comparison.

Example 5 – Surrogate ligand blocking, antagonistic anti-mouse TNFR2 mAb has *In vivo* anti-tumor effect

(See also figures 10-16 and the above description of these figures.)

Therapeutic effect in different tumor models

To assess the in vivo anti-tumor effect of ligand blocking, antagonistic anti-TNFR2 mAbs a mouse surrogate, called 3F10$_1$, was investigated in vivo in different tumor models, using different isotype formats, and alone or in combination with anti-PD-1 as described below.

Mice were bred and maintained in local facilities in accordance with home office guidelines. Six to eight weeks-old female BalbC and C57/BL6 mice were supplied by

Taconic (Bomholt, Denmark) and maintained in local animal facilities. CT26, MC38 and B16.F10 cells (ATCC) were grown in glutamax buffered RPMI, supplemented with 10% FCS. When cells were semi confluent they were detached with trypsin and resuspended in sterile PBS at $10x10^6$ cells/ml. Mice were s.c. injected with 100 µl cell suspension corresponding to $1x10^6$ cells/mouse. 3-8 days after injection dependent on model, mice were treated twice weekly with 10 mg/kg antibody i.p (isotype control, 3-F10 or 5-A05) and as indicated in figures. Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated

The ligand blocking, ant agonistic anti-mouse TNFR2 mAb 3-F10 show therapeutic anti-tumor effect in three different tumor models (Fig. 10-13), with curative effect in more treatment sensitive CT26 (Fig. 10) and tumor growth inhibiting effect in more treatment resistant MC38 and B16 (Fig. 11-13).

The anti-tumor effect of ligand blocking, antagonistic anti-mouse TNFR2 mAb is Fc:FcγR-dependent

To assess the importance of Fc-FcγR interaction on the in vivo anti-tumor effect of the ligand blocking, antagonistic anti-TNFR2 mouse surrogate mAb different Fc formats of this antibody was investigated in vivo in the CT26 tumor model as described below.

Mice were bred and maintained as described above. CT26 cells (ATCC) were grown and injected as described above. When tumors reached 3x3 mm, mice were treated twice weekly with 10 mg/kg antibody i.p (isotype control, 3-F10 IgG1, 3-F10 IgG2a or 3-F10-N297A (Fc defective). Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated.

The Fc-defective 3-F10-N297A shows minimal or no therapeutic activity compared to isotype control indicating that Fc-engagement is crucial to the therapeutic efficacy of this ligand blocking, antagonistic anti-mouse TNFR2 mAb (Fig. 10 A and B). Both IgG1 and IgG2a formats show significant therapeutic efficacy. However, the IgG2a format preferentially binding to activating Fcγ-receptors shows superior therapeutic effect indicative of depletion/phagocytosis of Tregs being one important mechanism of action of this ligand blocking, antagonistic anti-mouse TNFR2 mAb (Fig. 10 A-B). This is in contrast to a non-blocking, agonistic surrogate antibody 5A05 which shows some activity in the Fc defective format and shows best activity in the murine IgG1 format, known to preferentially bind the inhibitory FcγR. The ligand-blocking antagonistic antibody (3F10) is in accordance with the invention and the ligand non-blocking agonistic antibody (5A05) is included for reference.

Combinational effect with anti-PD-1 mAb

To assess the combinational in vivo anti-tumor effect of ligand blocking, antagonistic anti-TNFR2 mAbs a mouse surrogate (3-F10) with anti-PD-1, the treatment combination was investigated in vivo in the MC38 tumor model as described below.

Mice were bred and maintained as described above. MC38 cells (ATCC) were grown and injected as described above. Eight days after injection, mice were treated twice weekly with 10 mg/kg antibody i.p (isotype control, anti-mouse PD-1, 3-F10 or a combination of anti-mouse P-D-1 and 3-F10) and as indicated in figure 11 A-E. Tumors were measured two times/week until they reached a diameter of 15 mm, where after the mice were terminated.

The anti-mouse PD-1 and the ligand blocking, antagonistic anti-mouse TNFR2 mAb 3-F10 both show tumor growth inhibiting therapeutic effect in effect in the MC38 model (Fig. 11 A-E). When the anti-PD1 and the antagonistic anti-mouse TNFR2 mAb 3-F10 are combined, tumors are cured in the treatment resistant MC38 (Fig. 11 D-E).

Combinational effect with anti-PD-L1 mAb

To assess the combinational in vivo anti-tumor effect of ligand blocking antagonistic anti-TNFR2 mAbs, we further combined the mouse surrogate (3F10) with anti-PD-L1 for treatment in the MC38 tumor model as described below.

Mice were bred and maintained as described above. MC38 cells (obtained from Dr M. Cragg, Southampton University) were grown and injected as described above. Six days after injection, mice were treated twice with isotype control antibody or 3F10 (day 1 and 4), or four consecutive days with anti-PD-L1 (clone 10F.9G2, Bioxcell) followed by a fifth injection two days later (in total five injections day 1,2,3,4 and 7), or a combination of both. All antibodies were administered at 10 mg/kg i.p. Tumors were measured with calipers twice weekly until they reached a volume of 2000 mm3, where after the mice were terminated.

The anti-mouse PD-L1 and the ligand blocking, antagonistic anti-mouse TNFR2 mAb 3-F10 both show tumor growth inhibiting therapeutic effect in effect in the MC38 model (Fig. 12). When the anti-PD-L1 and the antagonistic anti-mouse TNFR2 mAb 3-F10 are combined, the anti-tumor effect is even further enhanced (Fig. 12).

Immune cell modulation in vivo

To investigate the effects in immune cell in the tumor in vivo, BalbC mice were inoculated with CT26 cells as described above. After the tumors reached approximately 7x7 mm, the mice were treated with 10 mg/kg antibodies administered i.p. as indicated in figures. Mice were treated at day 1, 4 and 7 and terminated at day 8. Tumors were dis-

sected out, mechanically divided into small pieces and digested using a mixture of Collagenase 100 µg/ml liberase and 100 µg/ml Dnase in 37°C for 2x5 min with Vortex in between. After filtration through a 70 µm filter, the cell suspension was washed (400 g for 10 min) with PBS containing 10% FBS. Thereafter, the cells were resuspended in MACS buffer and stained with an antibody panel staining CD45, CD3, CD8, CD4 and CD25 or an antibody panel staining MHCII, F4/80, Ly6C, CD11b and Ly6G. Before staining, the cells were blocked for unspecific binding using 100 µg/ml IVIG (purified intravenous immunoglobulins). Cells were analyzed in a FACS Verse. Mouse Tregs were quantified as being $CD45^+CD3^+CD4^+CD25^+$ and TAMs as being $CD11b^+Ly6G^-Ly6C^-F4/80^+MHCII^+$. The results are shown in Fig. 14.

As seen in figure 14, treatment with the ligand blocking/antagonistic TNFR2 antibody results in an T reg depletion in the tumor. A weaker tendency towards increase in $CD8^+$T cell influx is also seen. Together, this results in a much improved CD8+ T cell to T reg ratio (Fig. 14 C). In addition, the antagonistic antibody modulates the myeloid compartment by reducing the number of tumor associated macrophages (Fig. 14 D).

PBMC-NOG/SCID model

To confirm the in vivo findings on the depleting activity of the ligand blocking, antagonistic anti-mouse TNFR2 surrogate mAb, we analyzed the depleting capacity of the ligand blocking, antagonistic anti-human TNFR2 mAb 1-H10 in the PBMC-NOG/SCID model *in vivo* as described below.

Mice were bred and maintained in local facilities in accordance with home office guidelines. Eight weeks-old female SCID and NOG mice were supplied by Taconic (Bomholt, Denmark) and maintained in local animal facilities. For the PBMC-NOG/SCID (primary human xenograft) model, human PBMCs were isolated using Ficoll Paque PLUS and after washing the cells were resuspended in sterile PBS at $75x10^6$ cells/ml. NOG mice were i.v. injected with 200 µl cell suspension corresponding to $15x10^6$ cells/mouse. 2 weeks after injection, the spleens were isolated and rendered into a single cell suspension. Thereafter, a small sample was taken to determine the expression of TNFR2 on human T cells by FACS (Fig. 15). This FACS showed that the TNFR2 expression on Tregs and CD8+ T cells are very comparable between the human T cells grown and activated in vivo in the NOG mice, and T cells from human tumors. The majority of the cells was resuspended in sterile PBS at $50x10^6$ cells/ml. SCID mice were injected i.p. with 200 µl of the suspension corresponding to $10x10^6$ cells/mouse. 1h later, mice were treated with 10 mg/kg of either Yervoy, anti-CD25, 1-H10, 1-H10-N297Q (Fc defective version of 1-H10) or isotype control mAb. The intraperitoneal fluid of the mice was col-

lected after 24 h. Human T cell subsets were identified and quantified by FACS using following markers: CD45, CD3, CD4, CD8, CD25, CD127 (all from BD Biosciences).

1-H10 Treg depleting activity was superior to Yervoy and 1-H10N297Q (Fig. 16), confirming that ligand blocking, antagonistic anti-TNFR2 mAb 1-H10 depletes Tregs and that Fc interaction is involved in this depletion (Fig. 16 D).

In summary, example 5 shows that:

1. Antagonistic ligand blocking antibodies can have strong anti-tumor effects across several tumor models
2. This effect can be increased by combining with anti-PD1 antibodies
3. The effect is dependent on engagement of activating FcγRs
4. Treatment with the antagonistic ligand blocking surrogate antibody significantly alters the T cell composition in tumors, with an increased $CD8^+$T cell/ Treg ratio, and reduction in tumor associated macrophages.
5. In human tumors, the highest TNFR2 expressing cells are Tregs
6. In a human xenograft model where tumor TNFR2 expression is mimicked on T cells, human Tregs are delated and $CD8^+$T cell levels increased.
7. This Treg deletion is most pronounced if the antibody can engage activating FcγRs.

Example 6 – Antagonistic ligand-blocking antibodies do not induce large amounts of proinflammatory cytokines

(See also figures 17-18 and the above description of these figures.)

Release of large amounts of pro-inflammatory cytokines is one possible side effect of immune modulatory antibodies used for treatment of patients. Hence, we here measured cytokine release induced by antagonistic, ligand blocking antibodies using two different methods. The first is based on antibody stimulation in in vitro cultures, and the second is based on xenografting human immune cells to immune deficient mice. For in vitro, the set-up of the culture has been shown to largely impact the release of cytokines (Vessillier et al., J Immunol Methods. 2015 Sep; 424: 43–52). To account for differences in methodologies, three different in vitro culture set-ups were used in accordance with recent publications.

For the High Density Cell Culture (HDC) Cytokine Release Assays (CRA), PBMCs were cultured at $1x10^7$ cells/ml in serum-free CTL-Test medium (Cell Technology Limited) supplemented with 2mM glutamine, 1mM pyruvate, 100 IU/ml of penicillin and streptomycin. 2 ml of cell culture was plated in a 12-well plate. After 48 h, 10 µg/ml anti-

body was added to $1x10^5$ pre-incubated PBMCs in a 96-well flat-bottom plate and incubated for 24 h.

The PBMC Solid Phase (SP) CRA was performed by coating wells of a 96-well plate with 1 µg/ml antibody for 1 h. After washing of the plate with PBS, $1x10^5$ PBMCs in 200µl complete medium were added per well and incubated for 48 h.

The cytokine release was also measured after stimulation of 200 µl of whole blood with 5 µg/ml of antibody for 48 h.

At the end of the incubation period, plates were centrifuged and the culture supernatant was taken and stored at -20°C. Concentrations of IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-8 and TNF-α were measured using custom made MSD plates, according to the manufacturer's instructions (Meso Scale Discovery, USA).

In summary, the blocking antagonistic antibody did not induce any significant cytokine release in any of the in vitro settings. The positive control antibodies, Alemtuzumab and OKT3 did induce cytokines, most pronounced of all was IFN-γ, but as seen in figure 17, the 1H10 antibody did not induce IFN-γ beyond an isotype control antibody. No other cytokine was elevated by 1H10 (data not shown).

PBMC-NOG tolerability model

To investigate the tolerability of the ligand blocking, antagonistic anti-human TNFR2 mAb 1-H10, we analyzed the *in vivo* cytokine release in the PBMC-NOG model as described below.

Mice were bred and maintained in local facilities in accordance with home office guidelines. Eight weeks-old female NOG mice were supplied by Taconic (Bomholt, Denmark) and maintained in local animal facilities. For the PBMC-NOG (primary human xenograft) model, human PBMCs were isolated using Ficoll Paque PLUS and after washing the cells were resuspended in sterile PBS at $125x10^6$ cells/ml. NOG mice were i.v. injected with 200 µl cell suspension corresponding to $25x10^6$ cells/mouse. 2 weeks after injection, blood samples were taken to analyze the level of "humanization" meaning the amount of human cells in the blood of the NOG mice. The blood was composed of approx. 40% human T-cells and the mice were considered humanized. The mice were then treated with 10 µg of either Yervoy, anti-CD3 (OKT-3), 1-H10, or isotype control mAb. Body temperature was measured prior to antibody injection and at 1h post injection Figure 18 A. As seen in Figure 18 A, the positive control antibody OKT3 induced dramatic lowering of body temperature as previously published, and in accordance with the toxicity seen in the clinic with this antibody. In contrast, 1-H10 did not show any effect on body temperature. Five hours post injection of antibodies the experiments were terminated and blood was collected for analysis of cytokine release (MSD). The cytokines measured

were human IFN-γ, TNF-α, IL-6 and IL1β. Of these, IFN- γ and TNF-α were quantified in high enough levels to be reliable. As seen in Figure 18 B and C, the positive control antibody OKT3 induced both significant IFN-γ and TNF-α release (in accordance with the toxicity seen in the clinic with this antibody) whereas 1H10 treated mice had no significant IFN-γ release. However, there was a tendency towards increase in TNF-α release, although not significant and as dramatic as for OKT3.

In summary, example 6 shows that the TNFR2 ligand blocking antibodies, here exemplified with the antibody called 1-H10, do not induce substantial levels of cytokine release as measured by several previously published methods. Since cytokine release is a limiting factor for clinical development of several immunomodulatory antibodies, this indicates an acceptable safety profile in this regard.

Example 7 – Epitopes of generated TNFR2 targeting antibodies

Domain construct knock-outs

In a first set of experiments, DNA constructs encoding different variants of TNFR2, missing one or more of the 4 extracellular domains, described in table 10, were used. In a second set of experiments, DNA constructs encoding variants of TNFR2 where different parts of domain 3 were exchanged with the corresponding murine part, as described in table 11, were used. The latter is possible since none of the antibodies is cross-reactive to murine TNFR. In both cases, the constructs were purchased from GeneArt (ThermoFisher). The constructs were cloned into an expression vector, containing the CMV-promotor and the OriP origin of plasmid replication, and transiently expressed in suspension adapted HEK293-EBNA cells.

Table 10. TNFR2 constructs used for transfection where one or several domains have been deleted.

Construct	Description
hTNFR2	wild type, full length human TNFR2 (uniprot #P20333)
hTNFR2-Δ1	hTNFR2 with domain TNFR-Cys 1 (aa 39-76) deleted
hTNFR2-Δ2	hTNFR2 with domain TNFR-Cys 2 (aa 77-118) deleted
hTNFR2-Δ3	hTNFR2 with domain TNFR-Cys 3 (aa 119-162) deleted
hTNFR2-Δ4	hTNFR2 with domain TNFR-Cys 4 (aa 163-201) deleted
hTNFR2-Δ1+3	hTNFR2 with domain TNFR-Cys 1 and 3 (aa 39-76 and 119-162) deleted
hTNFR2-Δ2+4	hTNFR2 with domain TNFR-Cys 2 and 4 (aa 77-118 and 163-

	201) deleted

Table 11. TNFR2 constructs used for transfection various parts of domain 3 have been exchanged for the corresponding murine sequence.

Construct	Description
hTNFR2	wild type, full length human TNFR2 (uniprot #P20333)
mTNFR2	wild type, full length murine TNFR2 (uniprot #P25119)
hTNFR2-m1	hTNFR2 with aa 119-132 replaced by aa 120-133 from mTNFR2
hTNFR2-m2	hTNFR2 with aa 134-144 replaced by aa 135-146 from mTNFR2
hTNFR2-m3	hTNFR2 with aa 151-160 replaced by aa 153-162 from mTNFR2
hTNFR2-m4	hTNFR2 with aa 130-144 replaced by aa 131-146 from mTNFR2

Flow cytometry based binding analysis

HEK-293-E cells were transfected with the respective cDNA plasmids of TNFR2 variants using Lipofectamin 2000. 48h after transfection, cells were harvested and stained with the indicated antibodies for 30 minutes. After 2 washing steps with PBS, surface bound antibodies were stained with a secondary anti-IgG coupled to APC. Prior to flow cytometry analysis on BD-Verse flow cytometer, cells were washed and stained for live/dead.

Flow cytometry based binding experiments of transfected HEK 293 cells clearly showed, that domain 1 and domain 2 either does not affect the binding (domain 1), or only marginally affects binding (domain 2), of any of the antibodies to these cells. As a positive control a polyclonal anti-human TNFR2 antibody was used. The positive control antibody showed high binding to all tested constructs, whereas the negative antibody showed no binding (Fig. 19). All tested antibodies showed a complete loss of binding to TNFR2 lacking domain 3. Similarly, most antibodies could not bind to TNFR2 if domain 4 was missing. All antagonistic antibodies (1H10, 4H02 and 5B08) showed drastically reduced binding to TNFR2 Δ4 of more than 50% compared to binding to TNFR2 Δ1 and TNFR2 Δ2. Similarly, removing two domains from TNFR2 clearly showed that the lack of domain 3 or 4 severely abrogated binding of all tested antibodies to TNFR2, with the possible exception of agonistic antibody 1F06, while the lack of domain 4 abolished binding of agonistic antibodies and reduced the binding of the antagonistic antibodies significantly. (Fig. 19 E and F).

Binding to mouse-human chimeric TNFR2

To further narrow down the binding site and define the epitopes, parts of the human TNFR2 domain 3 were replaced by the corresponding mouse sequence. Since all antibodies shows very little cross-reactivity to mouse TNFR2, a loss of binding to certain constructs would allow refining the binding epitope. Fig. 20 displays the different mouse-human chimeric TNFR2 constructs. Four different replacements were made, exchanging either 14 (m1), 12 (m2), 10 (m3) or 16 (m4) amino acids from the human sequence with the corresponding mouse sequence. The other three domains (1, 2, 4) contain exclusively human sequences.

These constructs (TNFR2 domains 1-4 with mutations in 3) were then transfected into HEK293 cells and antibodies were tested for binding using a flow cytometry approach. As positive controls, polyclonal antibodies against mouse TNFR2 as well as against human TNFR2 were used. As expected, due to sequence similarity, both polyclonal control antibodies showed significant cross-reactivity and recognized both, human and mouse TNFR2. Obviously, best signals were achieved when matching the antibodies to its intended target.

Our monoclonal antibodies showed strong binding to human TNFR2, but no or only very little binding to mouse TNFR2 (Fig. 21 left panels). Similar binding with very little reduction was observed for all clones to the hTNFR2 m1 construct with mutations in aa 119-132, indicating, that none of the antibodies bind to an epitope within that region. However, mutations in aa 134-144 (hTNFR2 m2 construct) abrogated binding completely for half of the tested antibodies, corresponding to the antagonistic blocking antibodies 1-H10, 4-H02 and 5-B08, indicating that the antibodies bind at least partially within this region. The 1-G10 is a partial blocker also strongly affected by this replacement. Noteworthy, the agonistic antibodies (1-F02, 1-F06 and 4-E08) retained binding using construct 2, strongly suggesting a different epitope compared to the antagonistic antibodies. Interestingly, all antibodies lost binding to the hTNFR2 m3 construct with mutations in aa 151-160. This indicates, that all antibodies, both agonists and antagonists, have at least a partial epitope within that a sequence. Testing a slightly larger construct hTNFR2 m4 with mutations in aa 130-144 showed similar binding as with construct hTNFR2 m2.

Conclusions binding epitopes

Grouping the antibodies into their functionally role, the agonistic antibodies (1-F02, 1-F06 and 4-E08), seems to bind a very distal C-terminal part of domain 3 encompassing aa 151-160 and likely extend to a larger part of domain 4, whereas the epitope for the antagonists (1-H10, 5-B08 and 4-H02) are shifted more towards the center of do-

main 3, encompassing aa 134-160 and probably covers a smaller part of domain 4. However, despite this, their epitopes seem to overlap to some extent.

None of the antibodies bind to the N-terminal part of domain 3, aa 119-134. Binding sites to domain 4 is quite likely for all antibodies, but has not been identified completely.

CLAIMS

1. An antagonistic antibody molecule that specifically binds to TNFR2 on a target cell and thereby blocks TNF-α binding to TNFR2 and blocks TNFR2 signaling, and wherein the antibody molecule also binds to an Fcγ receptor via its Fc region.

2. An antibody molecule according to claim 1, wherein the antibody binds to with higher affinity to activating Fcγ receptors than to inhibitory Fcγ receptors.

3. An antibody molecule according to claim 1 or 2, wherein the binding of the antibody molecule to TNFR2 results in change in numbers and/or frequency of TNFR2 expressing cells in diseased tissue.

4. An antibody molecule according to any one of the claims 1-3, wherein the binding of the antibody molecule to TNFR2 results in infiltration of T-cells and/or myeloid cells into diseased tissue and/or a change in composition of T-cells and/or myeloid cells in diseased tissue.

5. An antibody molecule according to any one of the claims 1-4 wherein the antibody molecule is selected from the group consisting of: a full-size antibody, a chimeric antibody, a single chain antibody, and an antigen-binding fragment thereof retaining the ability to bind an Fc receptor via its Fc region.

6. An antibody molecule according to any one of the claims 1-5, which binds to human TNFR2 (hTNFR2) and/or to cynomolgous monkey TNFR2 (cmTNFR2).

7. An antibody molecule according to any one of the claims 1-6, wherein the antibody molecule is selected from the group consisting of a human IgG antibody molecule, a humanized IgG antibody molecule, and an IgG antibody molecule of human origin.

8. An antibody molecule according to claim 7, wherein the antibody molecule is a human IgG1 antibody.

9. An antibody molecule according to claim 7 or 8, wherein the antibody molecule has been engineered for improved binding to activating Fc gamma receptors.

10. An antibody molecule according to any one of the claims 1-9, wherein the antibody molecule is a monoclonal antibody.

11. An antibody molecule according to any one of the claims 1-10, wherein the antibody molecule does not bind specifically to an epitope comprising or consisting of the sequence KCSPG.

12. An antibody molecule according to any one of the claims 1-11, wherein the antibody molecule is selected from the group consisting of antibody molecules comprising 1-6 of the CDRs VH-CDR1, VH-CDR2, VH-CDR3, VL-CDR1, VL-CDR2 and VL-CDR3,

wherein VH-CDR1, if present, is selected from the group consisting of SEQ. ID. NOs: 1, 9 and 17;

wherein VH-CDR2, if present, is selected from the group consisting of SEQ. ID. NOs: 2, 10 and 18;

wherein VH-CDR3, if present, is selected from the group consisting of SEQ. ID. NOs: 3, 11 and 19;

wherein VL-CDR1, if present, is selected from the group consisting of SEQ. ID. NOs: 4, 12 and 20;

wherein VL-CDR2, if present, is selected from the group consisting of SEQ. ID. NOs: 5, 13 and 21; and

wherein VL-CDR3, if present, is selected from the group consisting of SEQ. ID. NOs: 6, 14 and 22.

13. An antibody molecule according to any one of the claims 1-12, wherein the antibody molecule comprises a variable heavy chain (VH) comprising the following CDRs

(i) SEQ. ID. NO: 1, SEQ. ID. NO: 2 and SEQ. ID. NO: 3; or

(ii) SEQ. ID. NO: 9, SEQ. ID. NO: 10 and SEQ. ID. NO: 11; or

(iii) SEQ. ID. NO: 17, SEQ. ID. NO: 18 and SEQ. ID. NO: 19; and/or wherein the antibody molecule comprises a variable light chain (VL) comprising the following CDRs:

(i) SEQ. ID. NO: 4, SEQ. ID. NO: 5 and SEQ. ID. NO: 6; or

(ii) SEQ. ID. NO: 12, SEQ. ID. NO: 13 and SEQ. ID. NO: 14; or

(iii) SEQ. ID. NO: 20, SEQ. ID. NO: 21 and SEQ. ID. NO: 22.

14. An antibody molecule according to any one of the claims 1-13, wherein the antibody molecule comprises a variable heavy chain (VH) amino acid sequence selected from the group consisting of SEQ. ID. NOs 7, 15 and 23; and/or wherein the antibody molecule comprises a variable light chain (VL) amino acid sequence selected from the group consisting of SEQ. ID. NOs: 8, 16 and 24.

15. An antibody molecule according to any one of the claims 1-11 wherein the antibody molecule is an antibody molecule that is capable of competing for binding to TNFR2 with an antibody molecule as defined in any one of claims 12-14.

16. An isolated nucleotide sequence encoding an antibody molecule as defined in any one of the claims 1-14.

17. A plasmid comprising a nucleotide sequence as defined in claim 16.

18. A virus comprising a nucleotide sequence as defined in claim 16 or a plasmid as defined in claim 17.

19. A virus according to claim 18, further comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor.

20. A cell comprising a nucleotide sequence as defined in claim 16, a plasmid as defined in claim 16, or a virus as defined in claim 18 or 19.

21. An antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 17, a virus according to claim 18 or 19 and/or a cell according to claim 20 for use in medicine.

22. An antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 17, a virus according to claim 18 or 19 and/or a cell according to claim 20 for use in the treatment of cancer or an infection caused by an intracellular pathogen.

23. An antibody molecule, a nucleotide sequence, a plasmid, a virus and/or a cell for use according to claim 22, wherein the patient to be treated is a patient having high TNFR2 expression in diseased tissue.

24. An antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 17, a virus according to claim 19 and/or a cell according to claim 20 for use in the treatment of cancer, in combination with:

an antibody molecule that specifically binds to a check-point inhibitor;

a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor;

a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor; and/or

a cell comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor, a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor or a virus comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor.

25. Use of an antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 17, a virus according to claim 18 and/or a cell according to claim 20 for the manufacture of a pharmaceutical composition for use in the treatment of cancer or of an infection caused by an intracellular pathogen.

26. Use according to claim 25, wherein the pharmaceutical composition is for use in the treatment of cancer or of an infection in a patient having high TNFR2 expression in diseased tissue.

27. Use according to claim 25 or 26, wherein the pharmaceutical composition is for use in the treatment of cancer and is to be administered in combination with:

an antibody molecule that specifically binds to a check-point inhibitor;

a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor;

a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor; and/or

a cell comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor, a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor or a virus comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor.

28. A pharmaceutical composition comprising or consisting of an antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 17, a virus according to claim 18 or 19 and/or a cell according to claim 20, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient.

29. A pharmaceutical composition according to claim 28, for use in the treatment of cancer or of an infection caused by an intracellular pathogen.

30. A pharmaceutical composition according to claim 29, for use in the treatment of cancer in combination with a pharmaceutical composition comprising:

an antibody molecule that specifically binds to a check-point inhibitor;

a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor;

a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor; and/or

a cell comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor, a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor or a virus comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor

31. A method for treatment of cancer or an infection caused by an intracellular pathogen in a patient comprising administering to the patient a therapeutically effective amount of an antibody molecule as defined in any one of the claims 1-15, a nucleotide sequence according to claim 16, a plasmid according to claim 20, a virus according to claim 21 or 22, a cell according to claim 23, or a pharmaceutical composition according to claim 29.

32. A method according to claim 31, wherein the patient is a patient having high TNFR2 expression in diseased tissue.

33. A method for treatment of cancer according to claim 31 or 32, wherein also a therapeutically effective amount of:

an antibody molecule that specifically binds to a check-point inhibitor;

a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor;

a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor; and/or

a cell comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor, a plasmid comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor or a virus comprising a nucleotide sequence encoding an antibody molecule that specifically binds to a check-point inhibitor

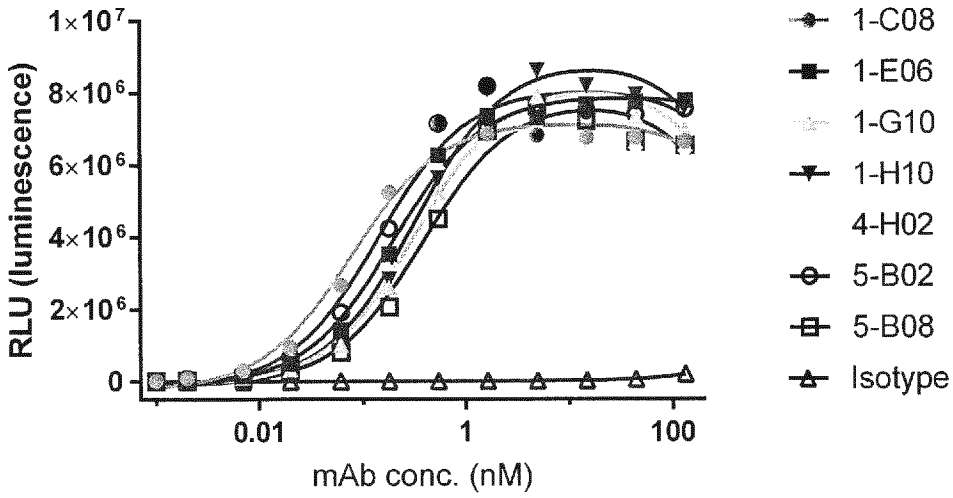
is administered to the patient.

34. An antibody molecule for use according to claim 24, a nucleotide sequence for use according to claim 24, a plasmid for use according to claim 24, a virus according to claim 19, a virus for use according to claim 24, a cell for use according to claim 24, a use according to claim 27, a pharmaceutical composition according to claim 30, or a method according to claim 33, wherein the check-point inhibitor is PD-1.

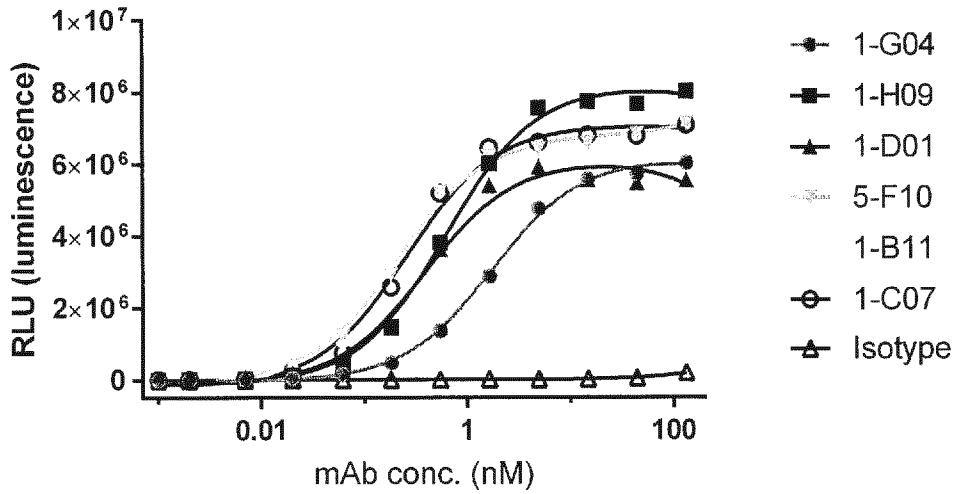
35. An antibody molecule for use according to claim 24, a nucleotide sequence for use according to claim 24, a plasmid for use according to claim 24, a virus according to claim 19, a virus for use according to claim 24, a cell for use according to claim 24, a use according to claim 27, a pharmaceutical composition according to claim 30, or a method according to claim 33, wherein the check-point inhibitor is PD-L1.

36. An antibody molecule for use according to claim 22, 23, 24, 34 or 35, a nucleotide sequence for use according to claim 22, 23, 24, 34 or 35, a plasmid for use according to claim 25, 26, 34 or 35, a virus for use according to claim 25, 26, 34 or 35, a cell for use according to claim 22, 23, 24, 34 or 35, a use according to claim 25, 26, 27, 34 or 35, a pharmaceutical composition according to claim 29, 30, 34 or 35, or a method according to claim 31, 32, 33, 34 or 35, wherein the cancer is a solid cancer.

A


RLU (luminescence)
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
0.01
1
100
mAb conc. (nM)
1-C08
1-E06
1-G10
1-H10
4-H02
5-B02
5-B08
Isotype

B


RLU (luminescence)
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
0.01
1
100
mAb conc. (nM)
1-G04
1-H09
1-D01
5-F10
1-B11
1-C07
Isotype

C

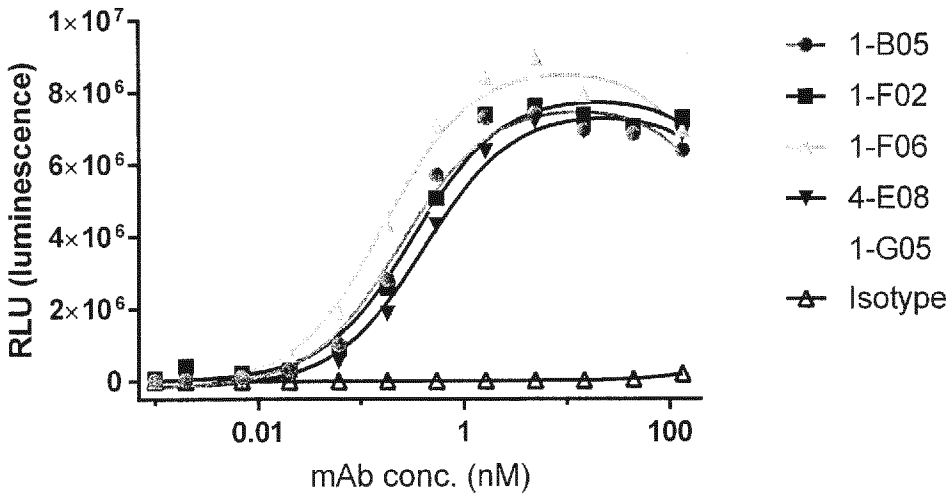
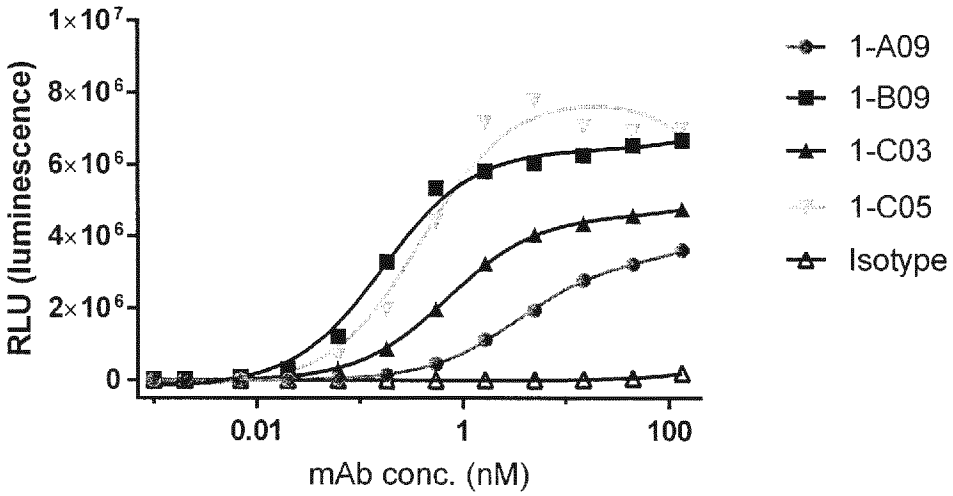

RLU (luminescence)
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
0.01
1
100
mAb conc. (nM)
1-B05
1-F02
1-F06
4-E08
1-G05
Isotype

Fig. 1

D


1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
RLU (luminescence)
0.01
1
100
mAb conc. (nM)
1-A09
1-B09
1-C03
1-C05
Isotype

E

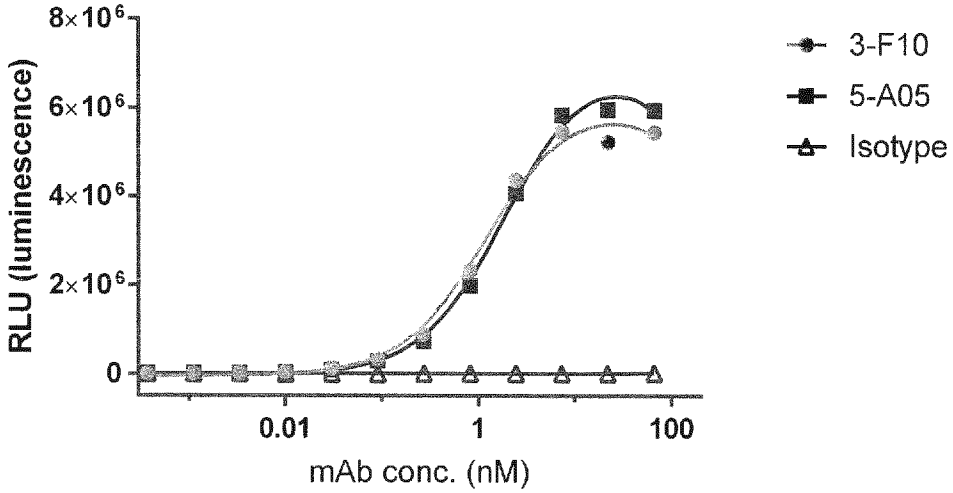

8×10^6
6×10^6
4×10^6
2×10^6
0
RLU (luminescence)
0.01
1
100
mAb conc. (nM)
3-F10
5-A05
Isotype

Fig. 1, cont.

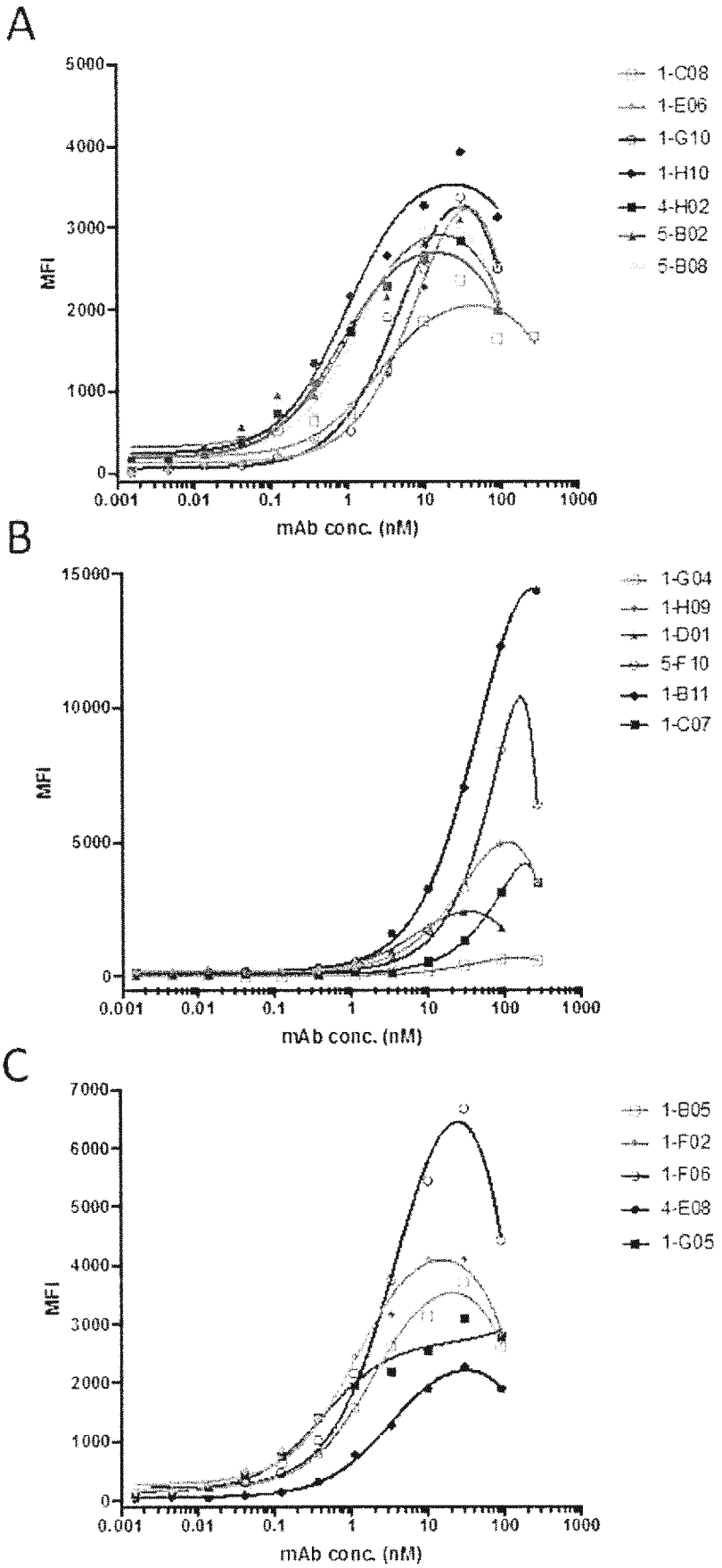
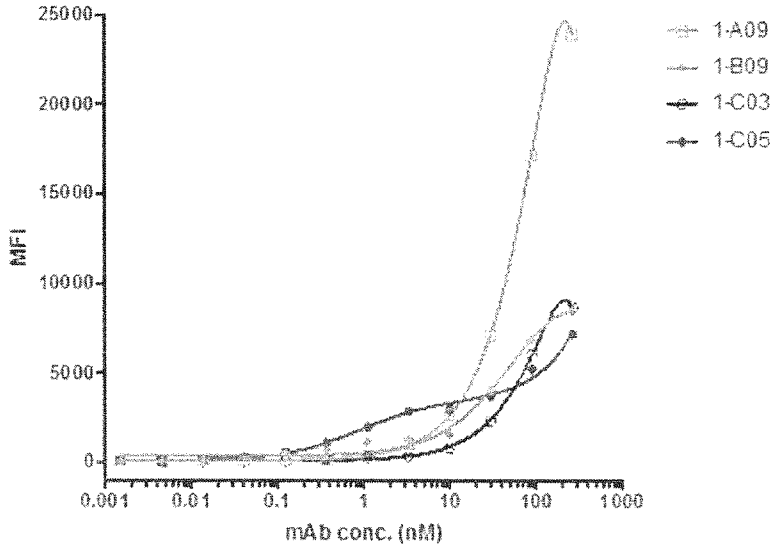

A
5000
4000
3000
2000
1000
0
MFI
0.001
0.01
0.1
1
10
100
1000
mAb conc. (nM)
1-C08
1-E06
1-G10
1-H10
4-H02
5-B02
5-B08
B
15000
10000
5000
0
MFI
0.001
0.01
0.1
1
10
100
1000
mAb conc. (nM)
1-G04
1-H09
1-D01
5-F10
1-B11
1-C07
C
7000
6000
5000
4000
3000
2000
1000
0
MFI
0.001
0.01
0.1
1
10
100
1000
mAb conc. (nM)
1-B05
1-F02
1-F06
4-E08
1-G05

Fig. 2

D


25000
20000
15000
10000
5000
0
MFI
0.001
0.01
0.1
1
10
100
1000
mAb conc. (nM)
1-A09
1-B09
1-C03
1-C05

E

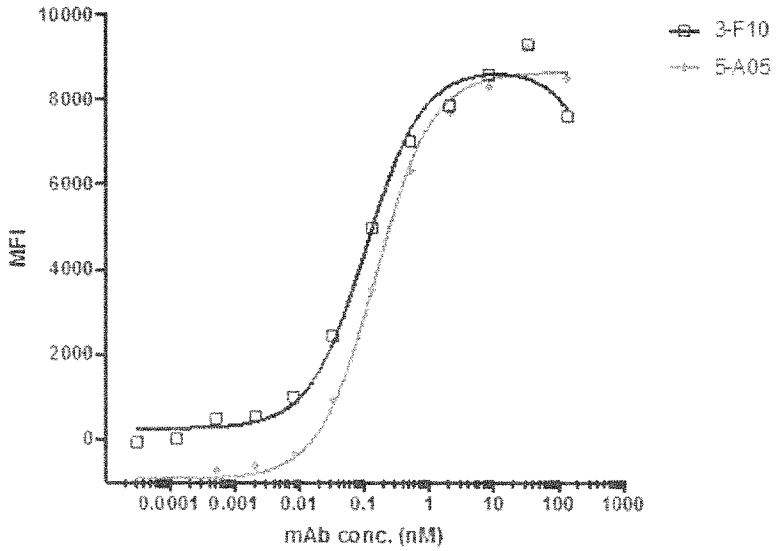

10000
8000
6000
4000
2000
0
MFI
0.0001
0.001
0.01
0.1
1
10
100
1000
mAb conc. (nM)
3-F10
5-A05

Fig. 2, cont.

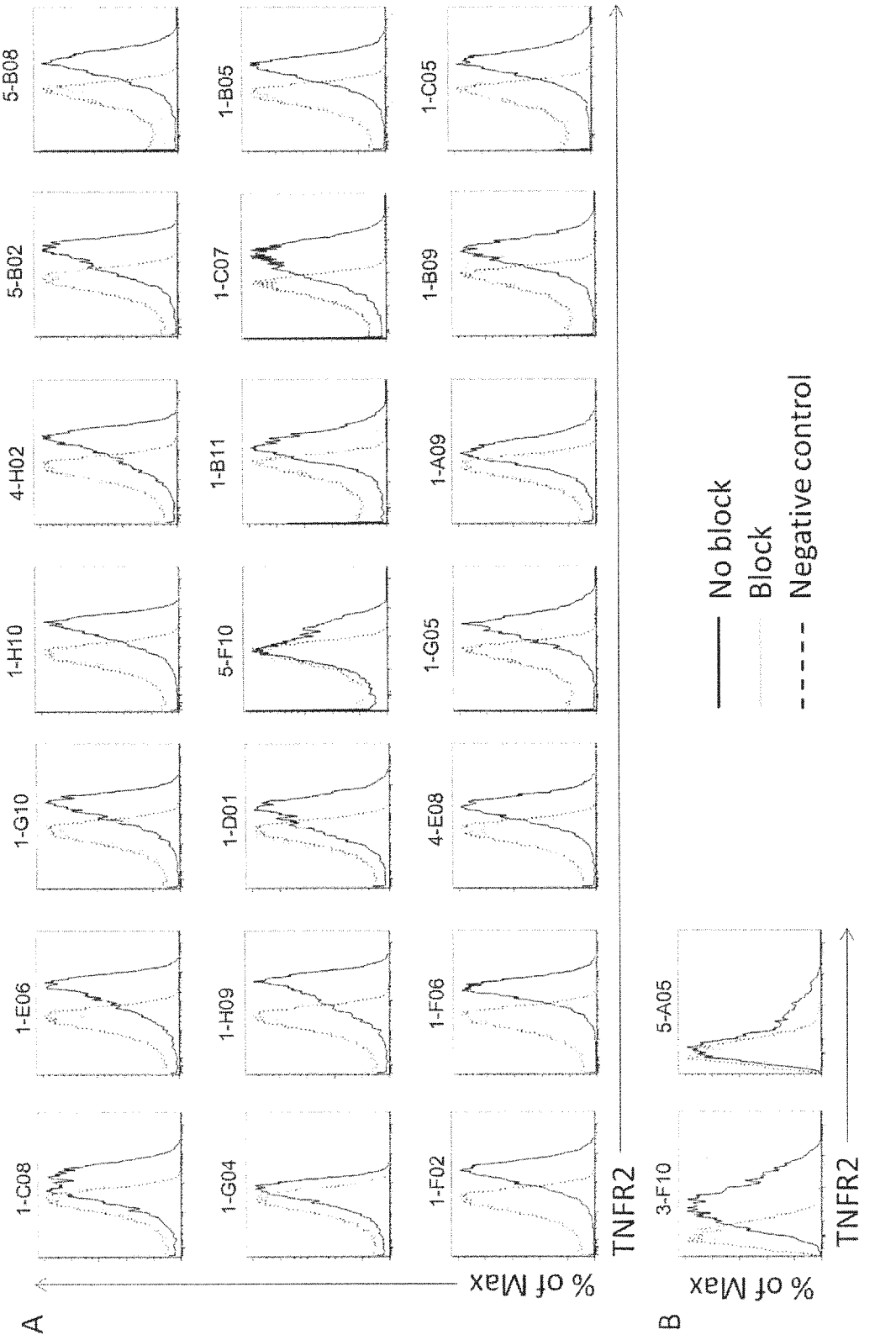

A
% of Max
TNFR2
1-C08
1-E06
1-G10
1-H10
4-H02
5-B02
5-B08
1-G04
1-H09
1-D01
5-F10
1-B11
1-C07
1-B05
1-F02
1-F06
4-E08
1-G05
1-A09
1-B09
1-C05
B
% of Max
TNFR2
3-F10
5-A05
No block
Block
Negative control

Fig. 3

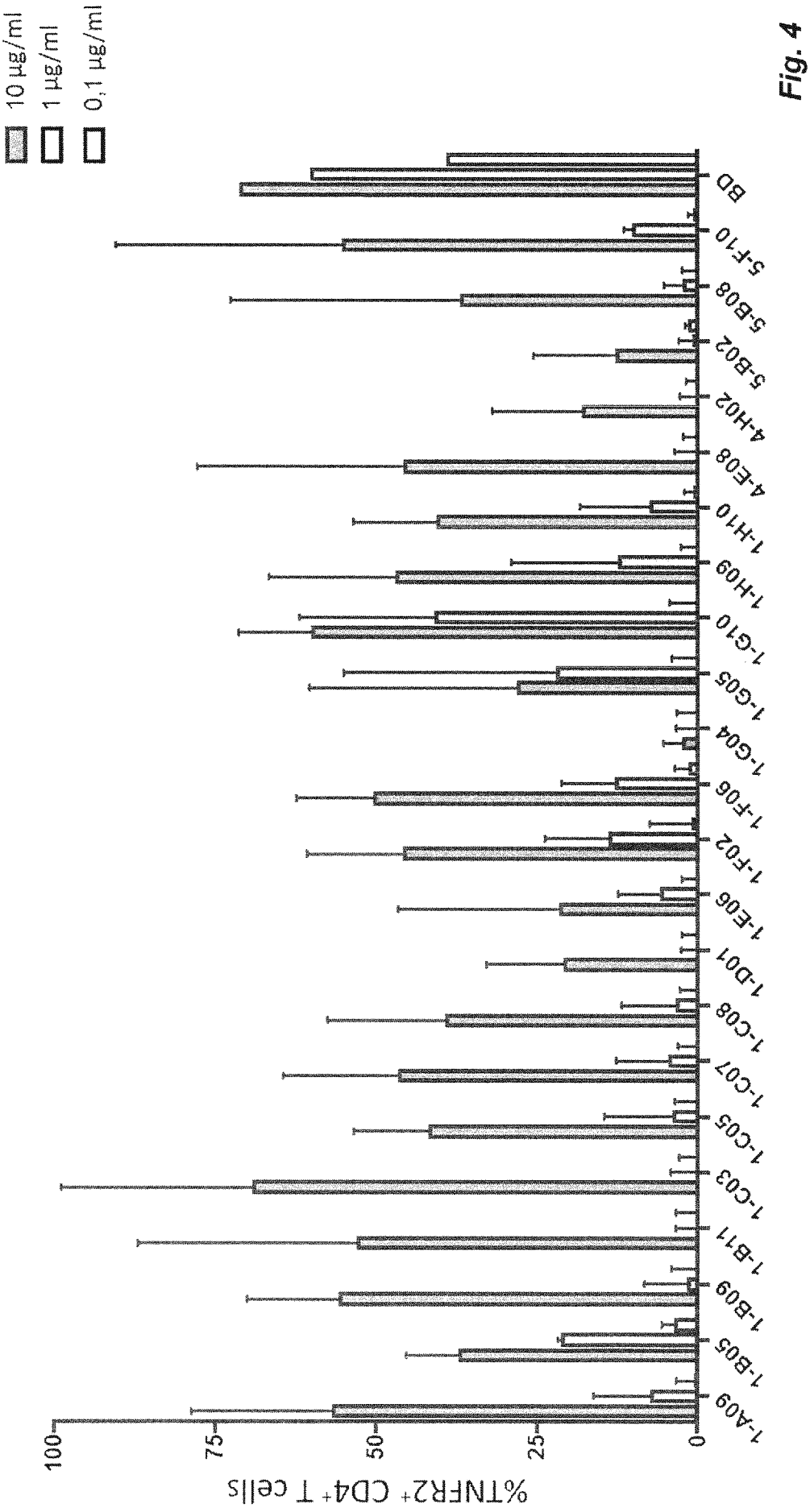

10 µg/ml
1 µg/ml
0,1 µg/ml
%TNFR2+ CD4+ T cells
100
75
50
25
0
1-A09
1-B05
1-B09
1-B11
1-C03
1-C05
1-C07
1-C08
1-D01
1-E06
1-F02
1-F06
1-G04
1-G05
1-G10
1-H09
1-H10
4-E08
4-H02
5-B02
5-B08
5-F10
BD

Fig. 4

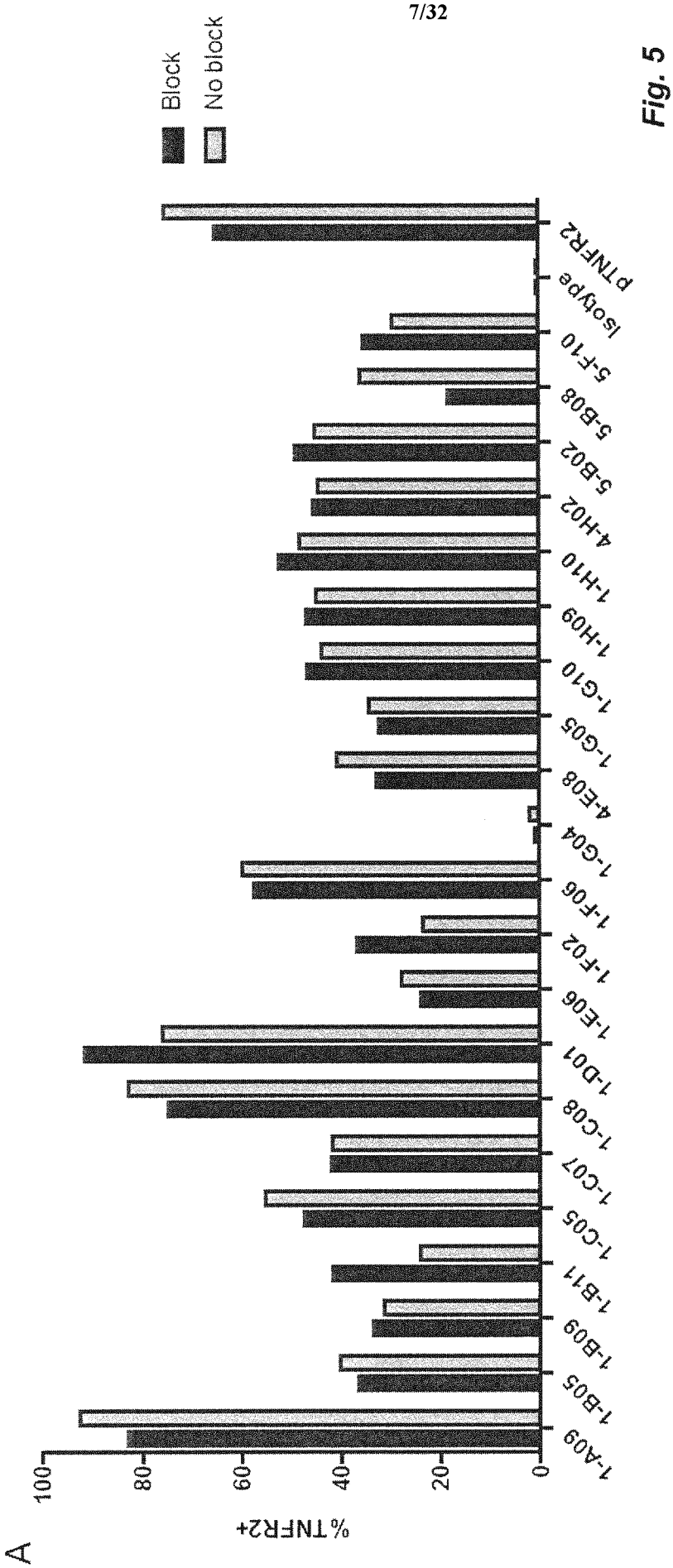

A
%TNFR2+
100
80
60
40
20
0
Block
No block
1-A09
1-B05
1-B09
1-B11
1-C05
1-C07
1-C08
1-D01
1-E06
1-F02
1-F06
1-G04
4-E08
1-G05
1-G10
1-H09
1-H10
4-H02
5-B02
5-B08
5-F10
Isotype
pTNFR2

Fig. 5

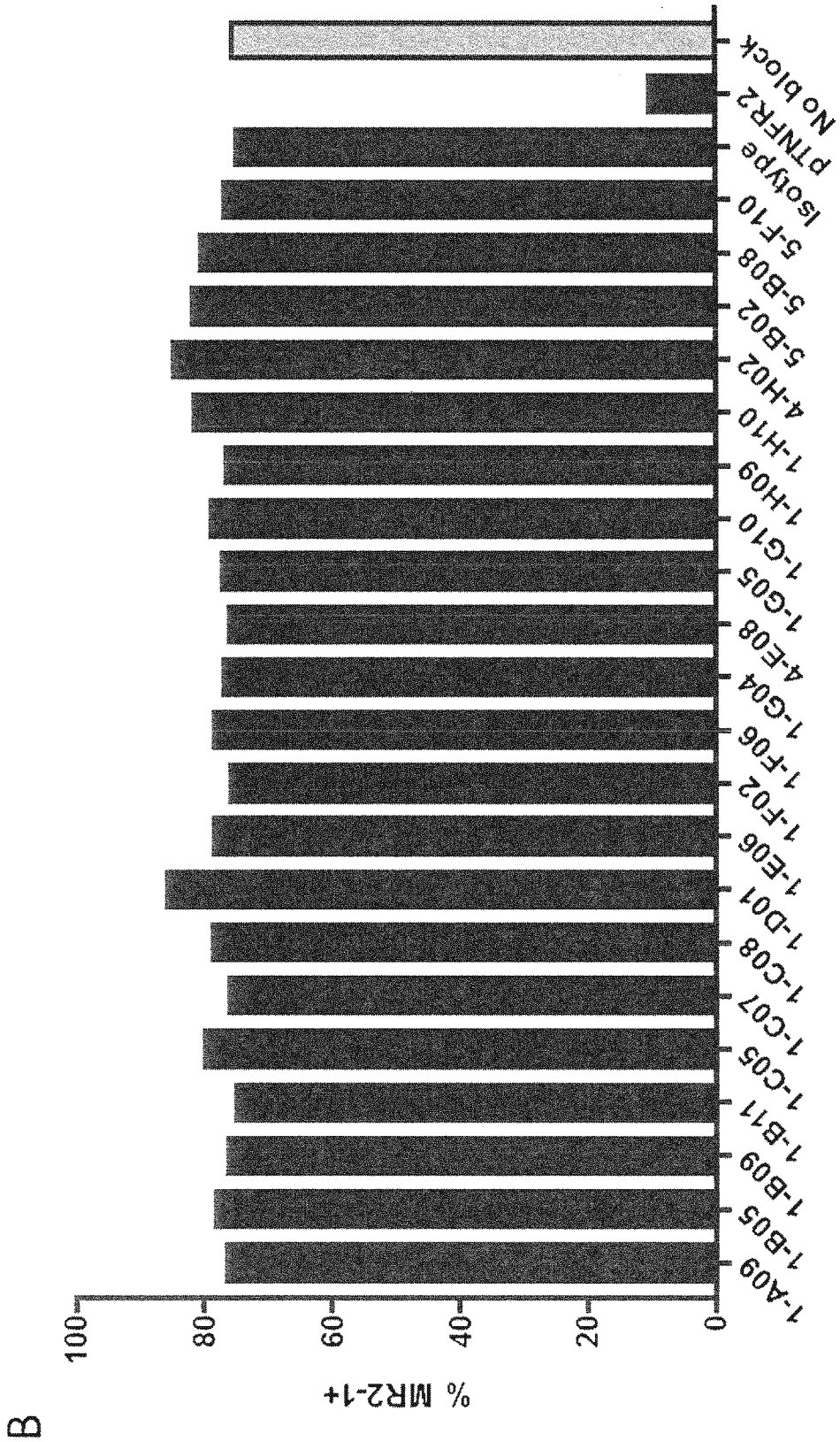
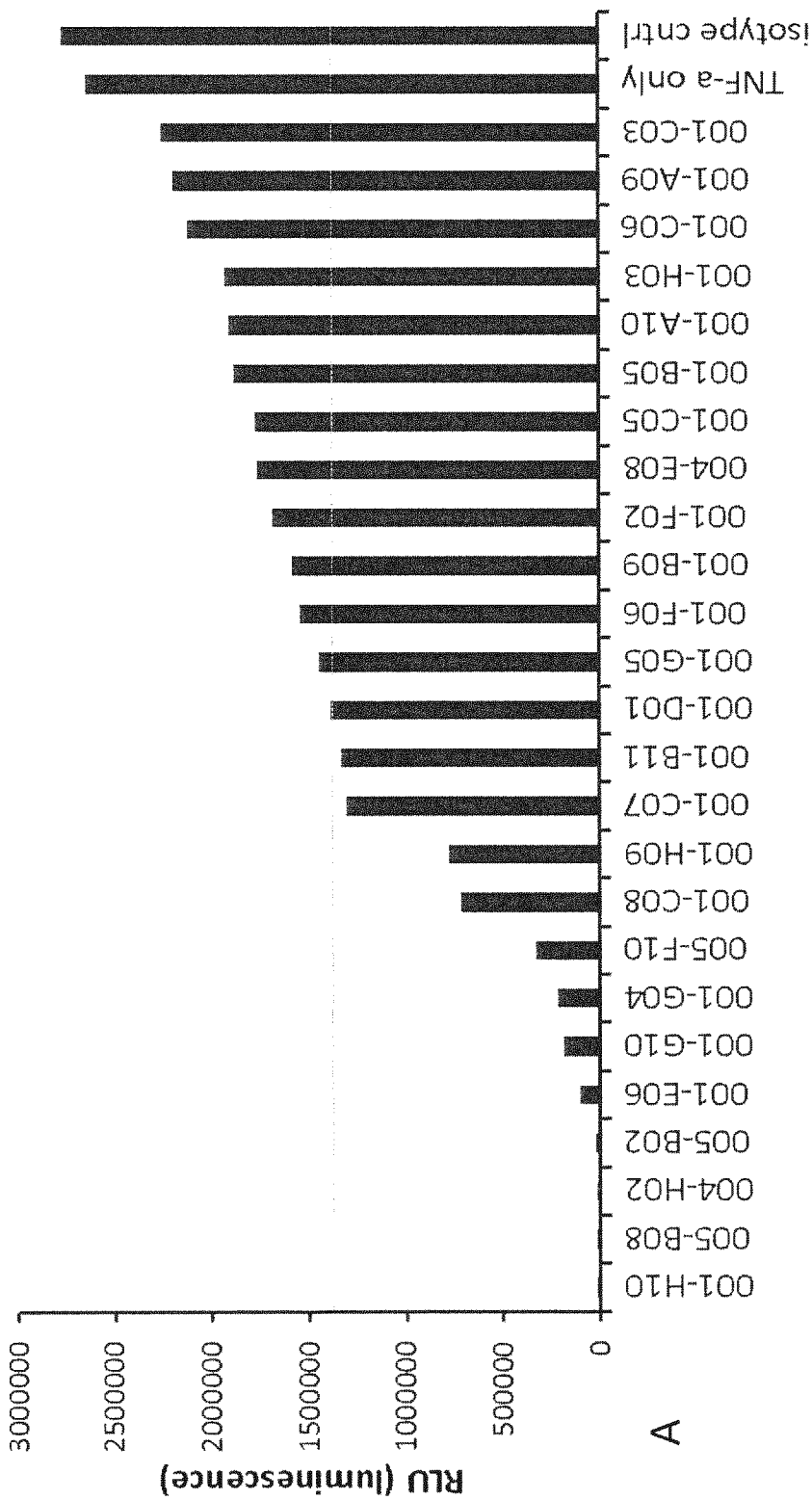

B
% MR2-1+
0
20
40
60
80
100
1-A09
1-B05
1-B09
1-B11
1-C05
1-C07
1-C08
1-D01
1-E06
1-F02
1-F06
1-G04
4-E08
1-G05
1-G10
1-H09
1-H10
4-H02
5-B02
5-B08
5-F10
Isotype
pTNFR2
No block

Fig. 5 cont.

Fig. 6


A
RLU (luminescence)
3000000
2500000
2000000
1500000
1000000
500000
0
001-H10
005-B08
004-H02
005-B02
001-E06
001-G10
001-G04
005-F10
001-C08
001-H09
001-C07
001-B11
001-D01
001-G05
001-F06
001-B09
001-F02
004-E08
001-C05
001-B05
001-A10
001-H03
001-C06
001-A09
001-C03
TNF-a only
isotype cntrl

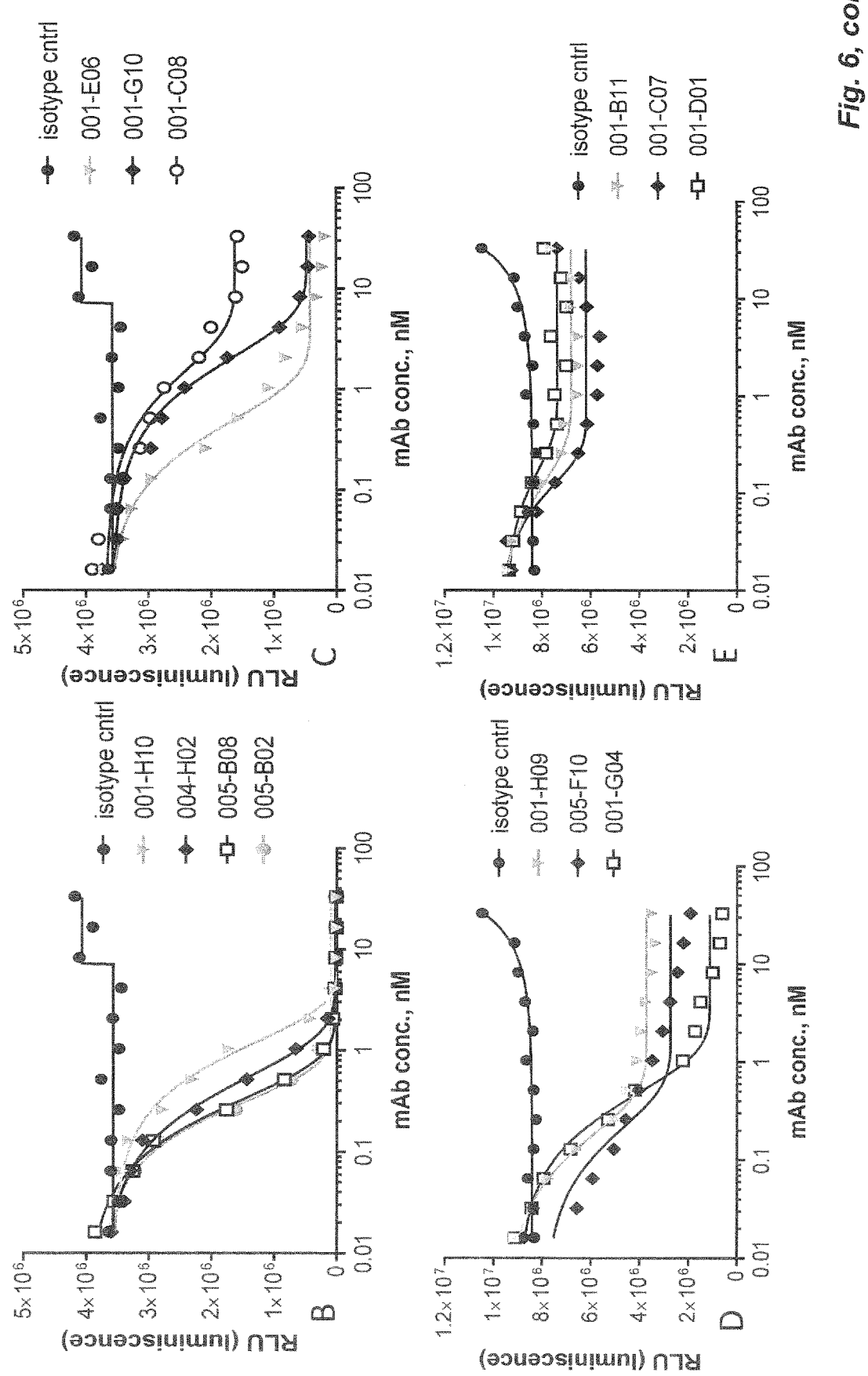
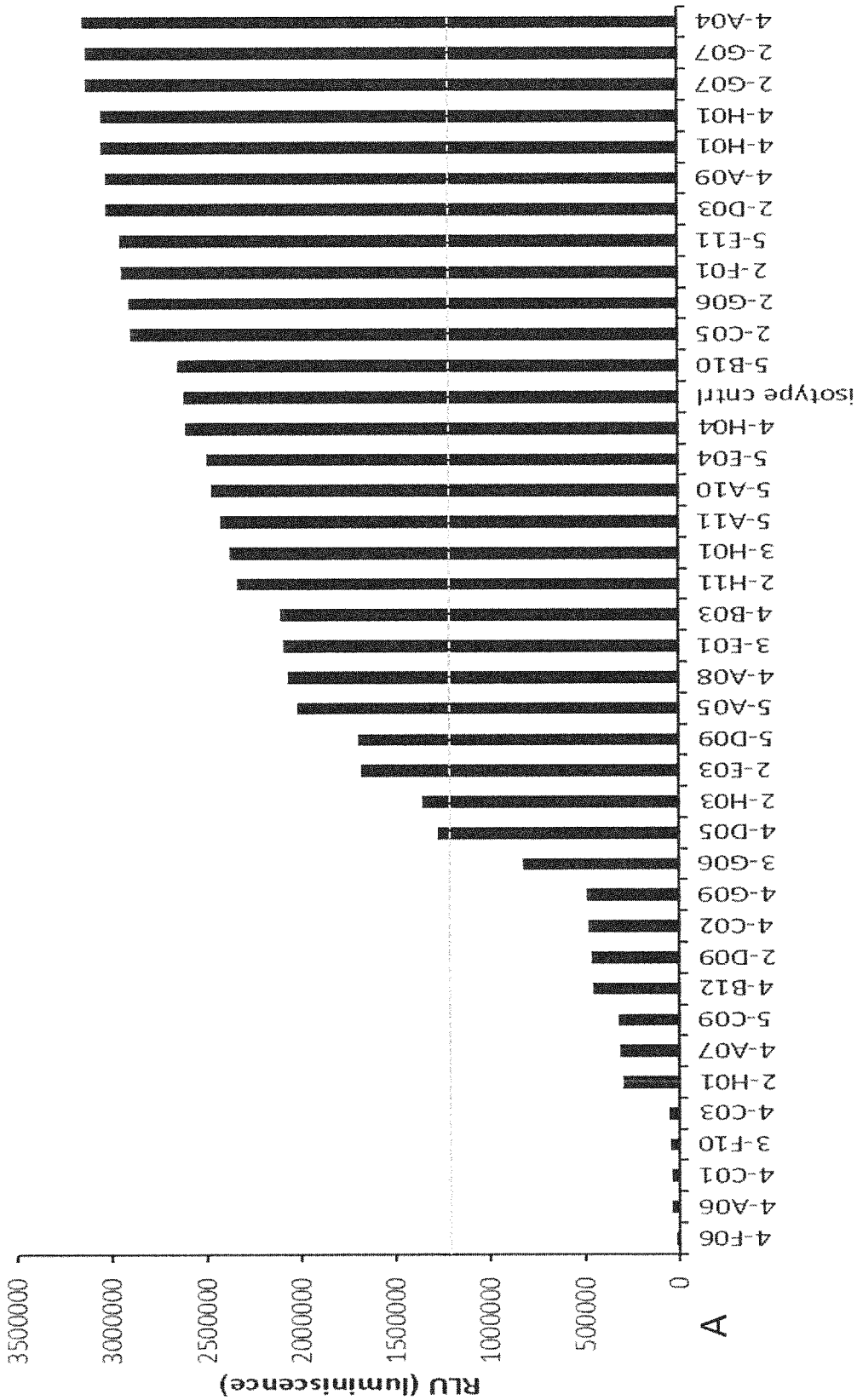

B
C
D
E
RLU (luminiscence)
mAb conc., nM
isotype cntrl
001-H10
004-H02
005-B08
005-B02
001-E06
001-G10
001-C08
001-H09
005-F10
001-G04
001-B11
001-C07
001-D01

Fig. 6, cont.

Fig. 7


RLU (luminiscence)
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
A
4-F06
4-A06
4-C01
3-F10
4-C03
2-H01
4-A07
5-C09
4-B12
2-D09
4-C02
4-G09
3-G06
4-D05
2-H03
2-E03
5-D09
5-A05
4-A08
3-E01
4-B03
2-H11
3-H01
5-A11
5-A10
5-E04
4-H04
isotype cntrl
5-B10
2-C05
2-G06
2-F01
5-E11
2-D03
4-A09
4-H01
4-H01
2-G07
2-G07
4-A04

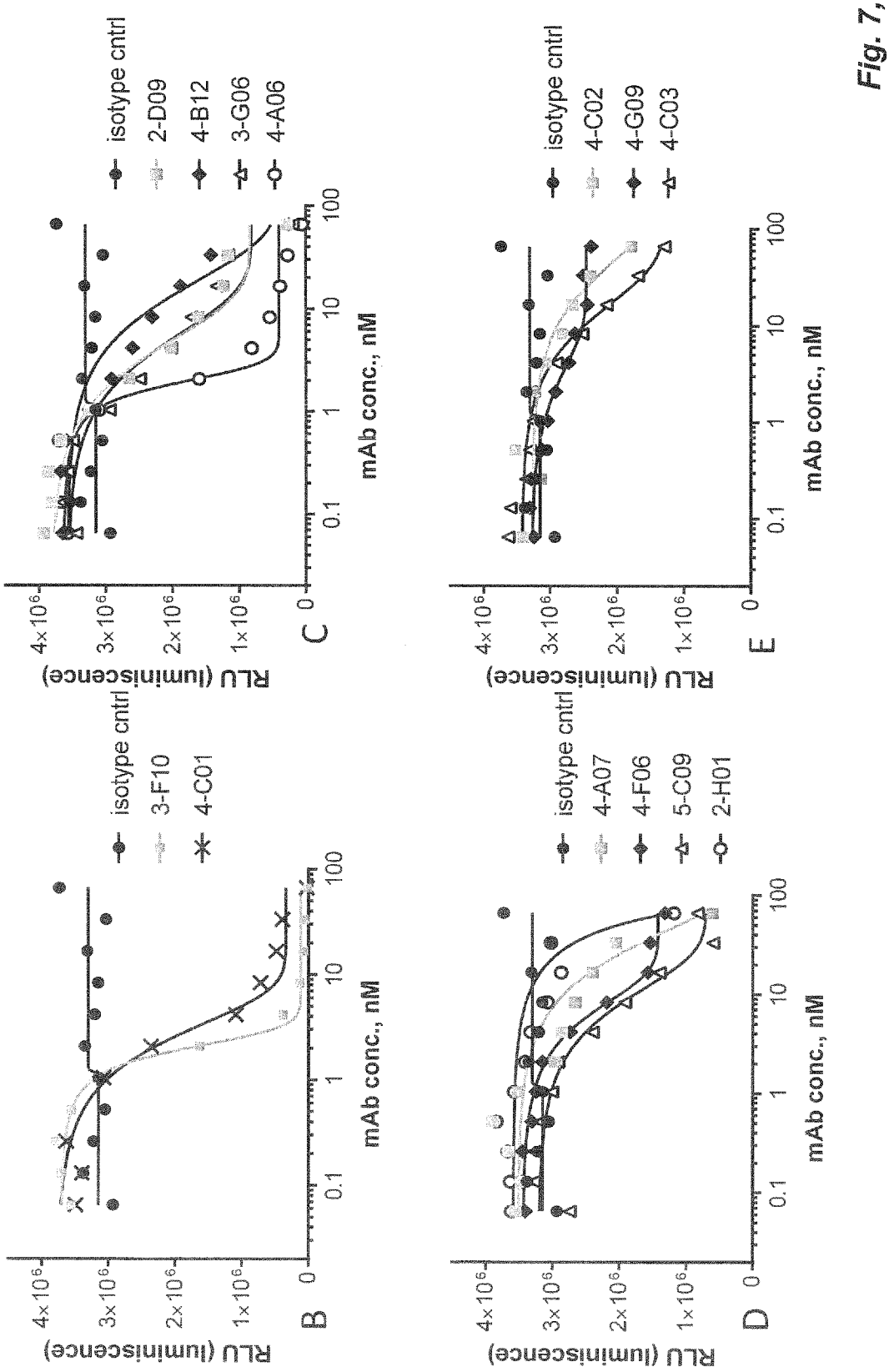

RLU (luminiscence)
4×10^6
3×10^6
2×10^6
1×10^6
0
0.1
1
10
100
mAb conc., nM
B
isotype cntrl
3-F10
4-C01
C
isotype cntrl
2-D09
4-B12
3-G06
4-A06
D
isotype cntrl
4-A07
4-F06
5-C09
2-H01
E
isotype cntrl
4-C02
4-G09
4-C03

Fig. 7, cont.

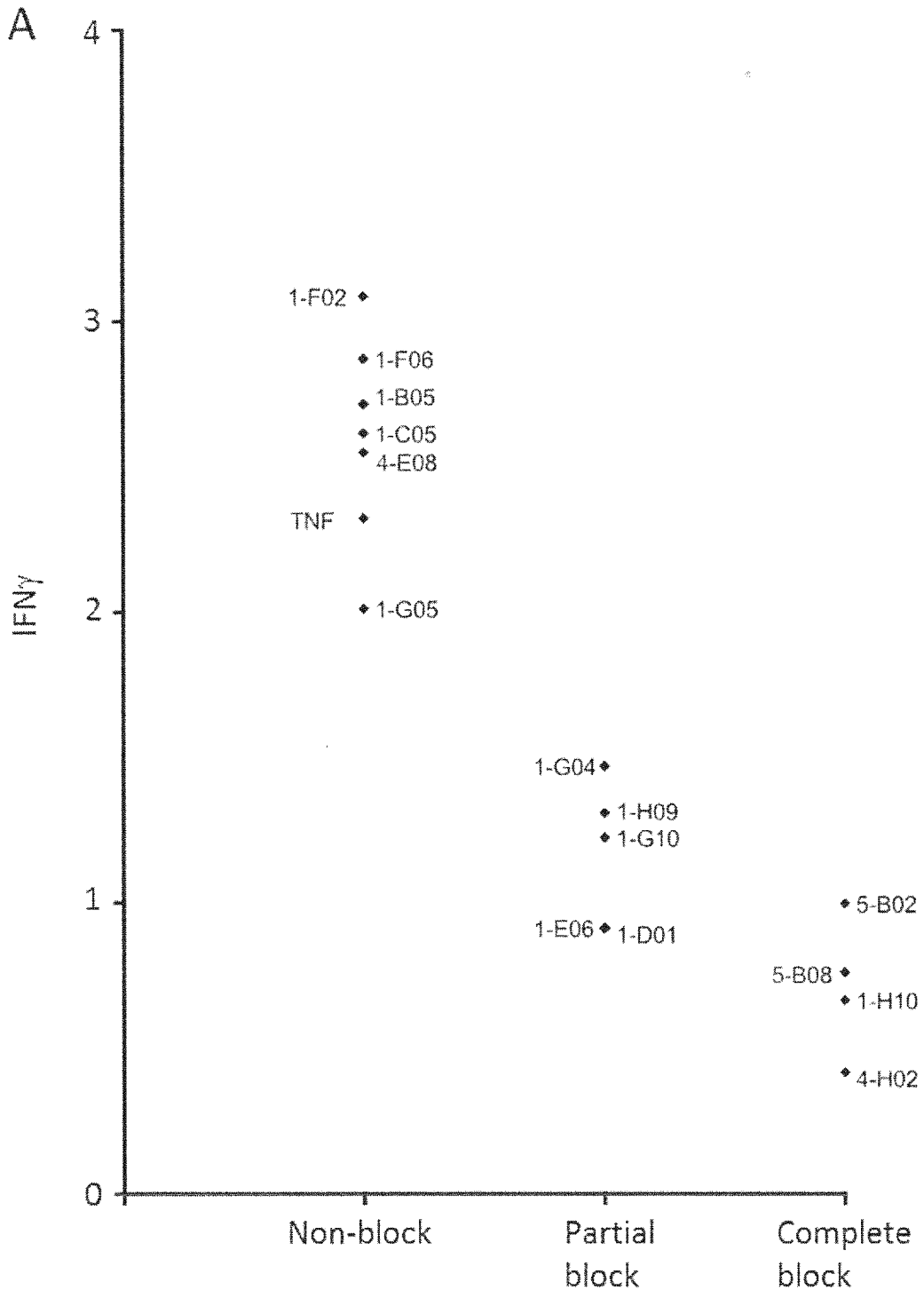

A
4
3
2
1
0
IFNγ
1-F02
1-F06
1-B05
1-C05
4-E08
TNF
1-G05
1-G04
1-H09
1-G10
1-E06
1-D01
5-B02
5-B08
1-H10
4-H02
Non-block
Partial block
Complete block

Fig. 8

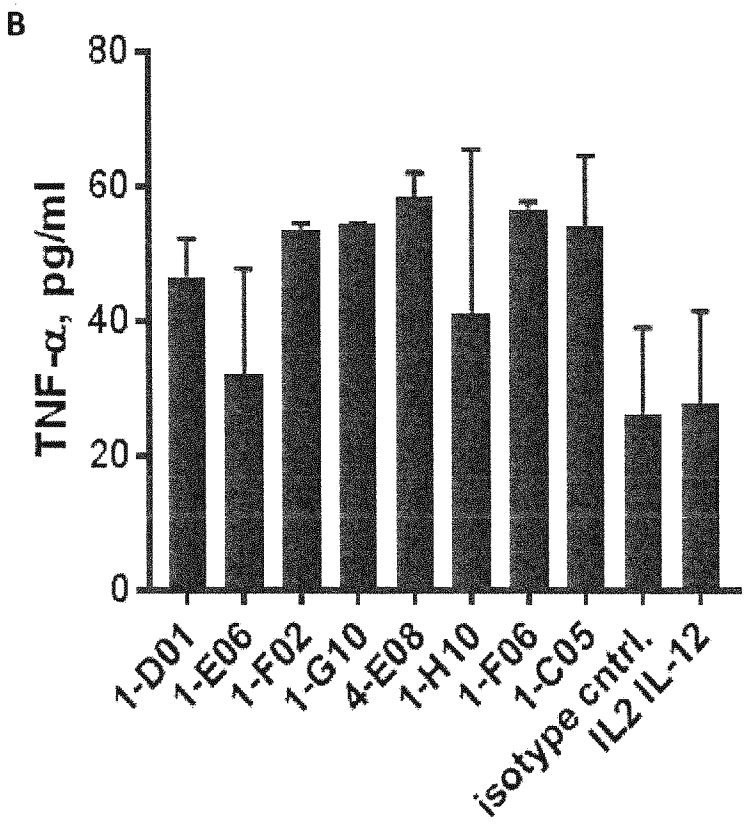
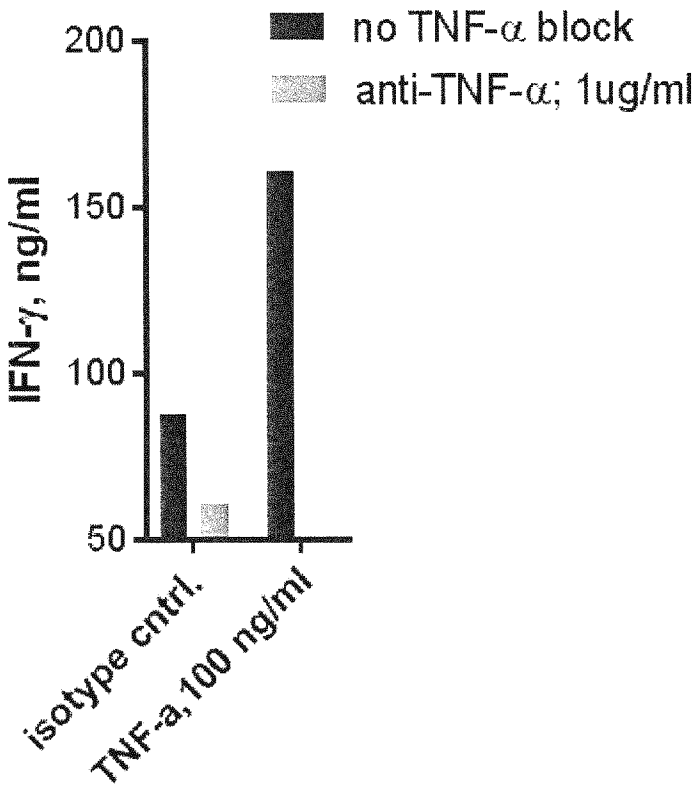

B
TNF-α, pg/ml
80
60
40
20
0
1-D01
1-E06
1-F02
1-G10
4-E08
1-H10
1-F06
1-C05
isotype cntrl.
IL2 IL-12

Fig.8, cont.

C


no TNF-α block
anti-TNF-α; 1ug/ml
IFN-γ, ng/ml
200
150
100
50
isotype cntrl.
TNF-a,100 ng/ml

D

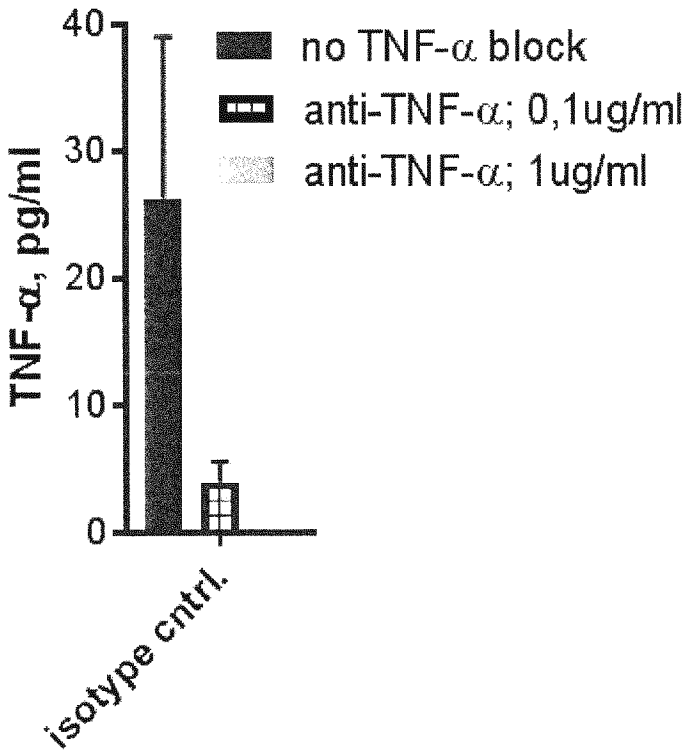

no TNF-α block
anti-TNF-α; 0,1ug/ml
anti-TNF-α; 1ug/ml
TNF-α, pg/ml
40
30
20
10
0
isotype cntrl.

Fig.8, cont.

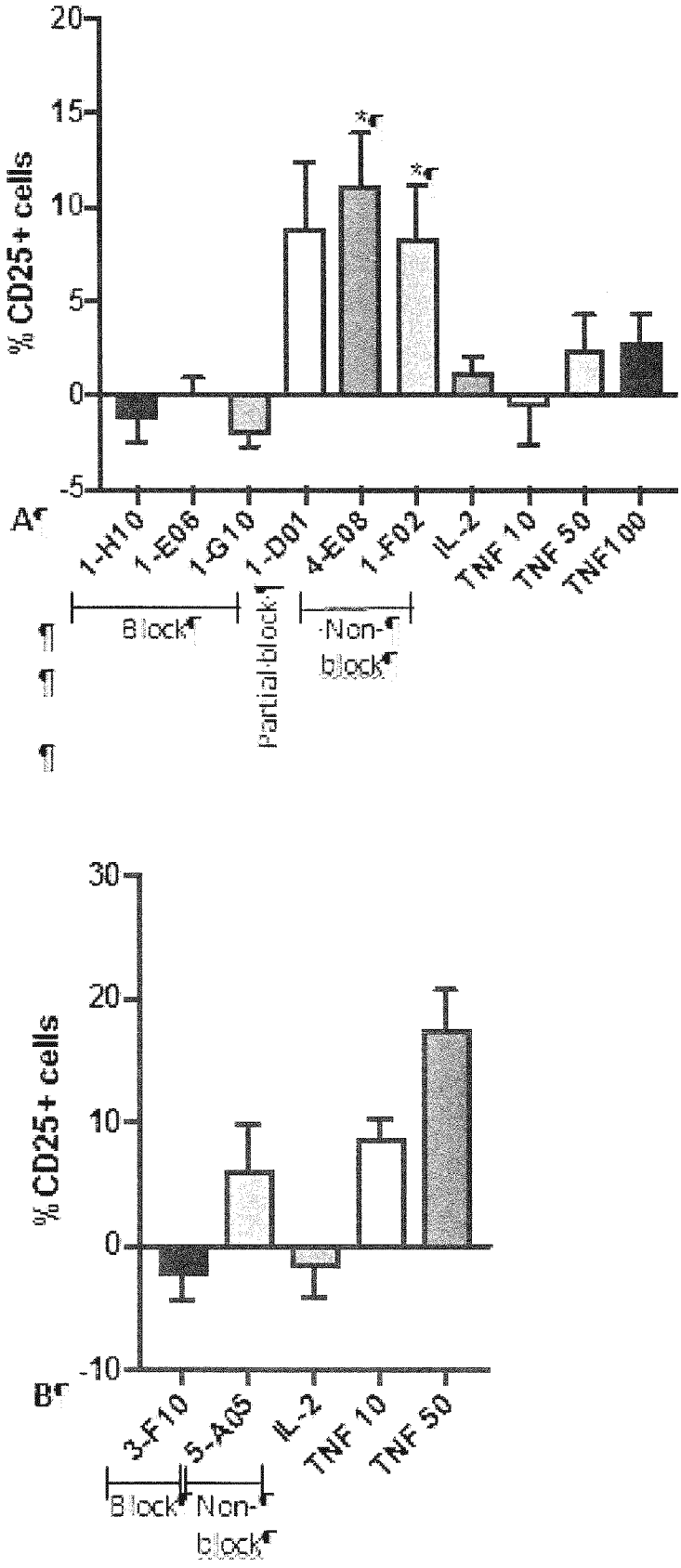

20
15
10
5
0
-5
% CD25+ cells
*
*
A
1-H10
1-E06
1-G10
1-D01
4-E08
1-F02
IL-2
TNF 10
TNF 50
TNF100
Block
Partial-block
Non-block
30
20
10
0
-10
% CD25+ cells
B
3-F10
5-A05
IL-2
TNF 10
TNF 50
Block
Non-block

Fig. 9

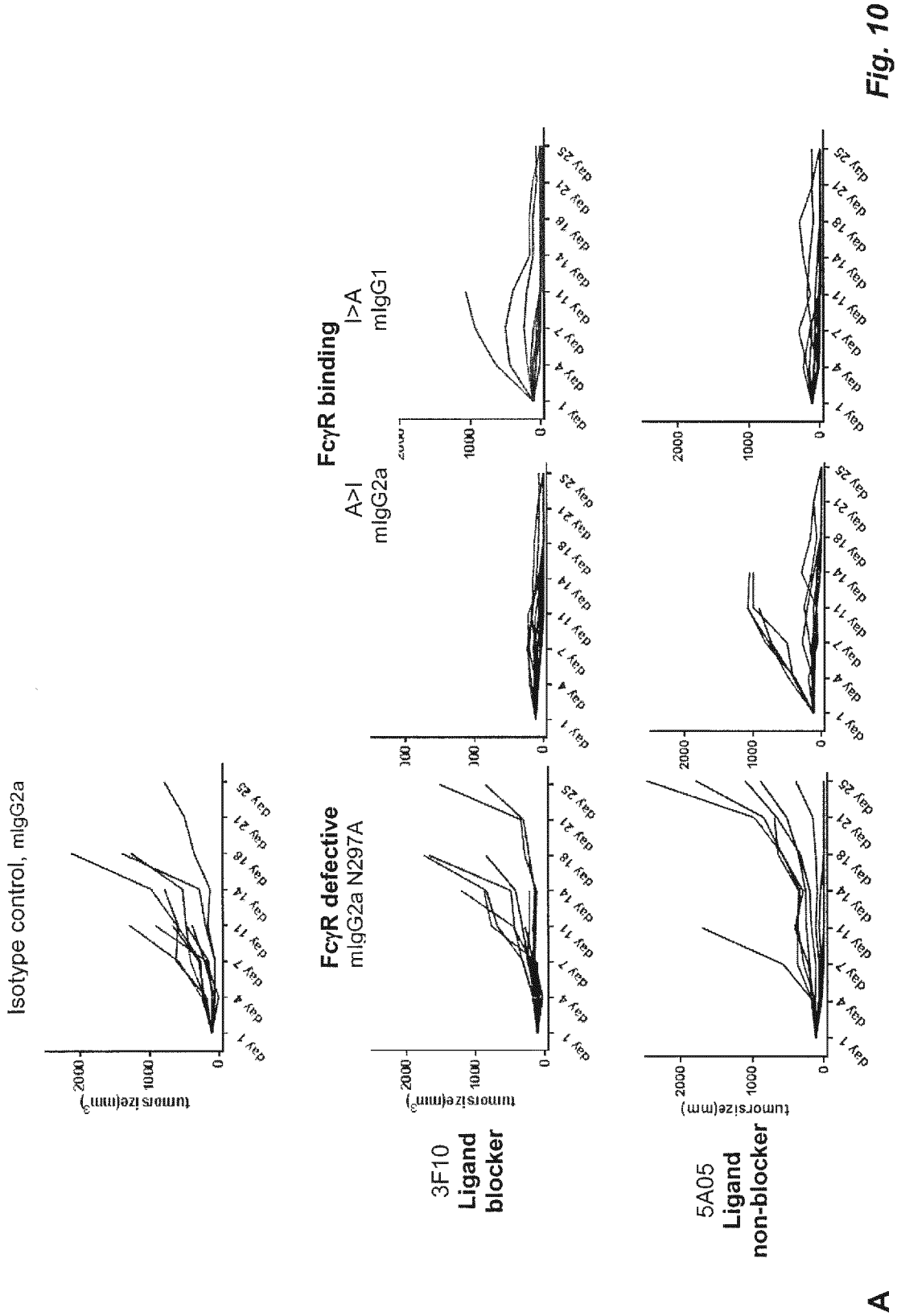
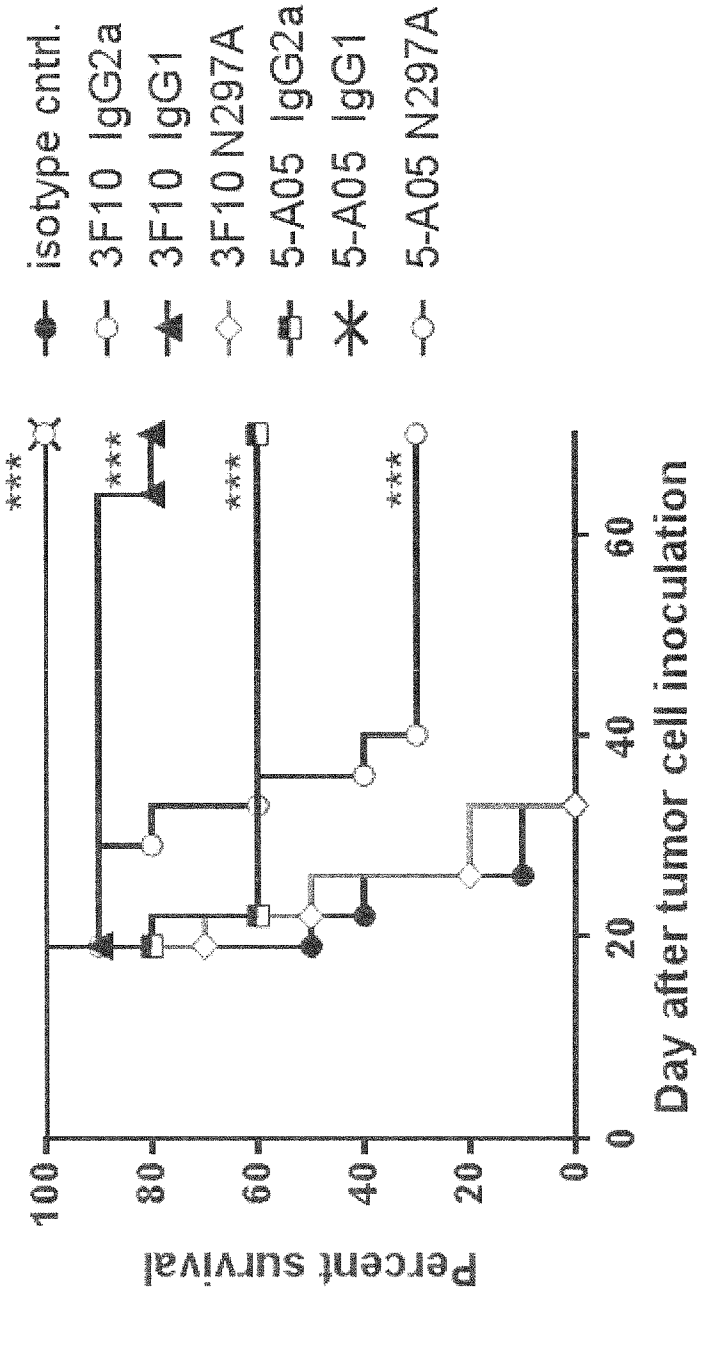

A
Isotype control, mIgG2a
FcγR binding
A>I
mIgG2a
I>A
mIgG1
FcγR defective
mIgG2a N297A
3F10
Ligand
blocker
5A05
Ligand
non-blocker
tumorsize(mm3)
tumorsize(mm)
0
1000
2000
day 1
day 4
day 7
day 11
day 14
day 18
day 21
day 25

Fig. 10


isotype cntrl.
3F10 IgG2a
3F10 IgG1
3F10 N297A
5-A05 IgG2a
5-A05 IgG1
5-A05 N297A
***
***
***
***
Percent survival
100
80
60
40
20
0
0
20
40
60
Day after tumor cell inoculation

B

Fig. 10, cont.

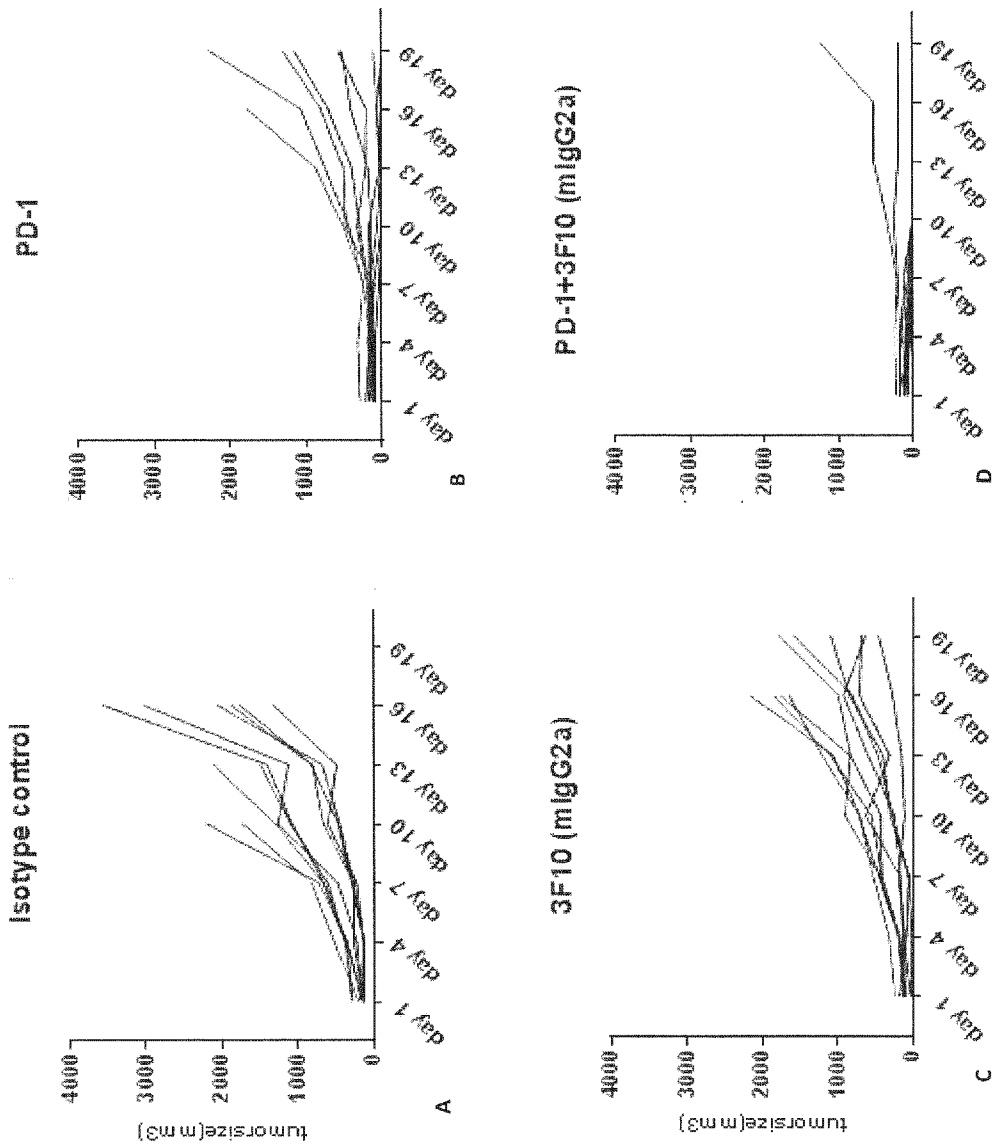
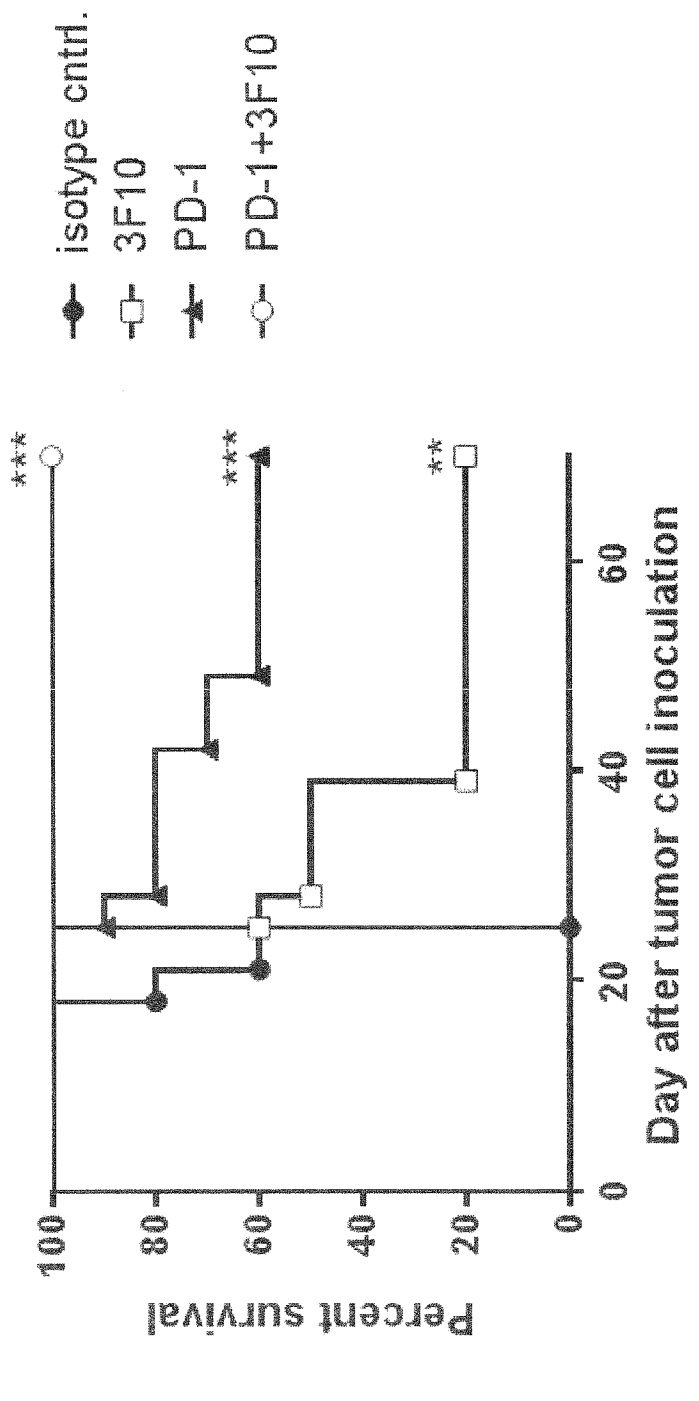

Isotype control
PD-1
3F10 (mIgG2a)
PD-1+3F10 (mIgG2a)
tumorsize(mm3)
4000
3000
2000
1000
0
day 1
day 4
day 7
day 10
day 13
day 16
day 19
A
B
C
D

Fig. 11


isotype cntrl.
3F10
PD-1
PD-1+3F10
***
***
**
Percent survival
100
80
60
40
20
0
0
20
40
60
Day after tumor cell inoculation

E

Fig. 11, cont.

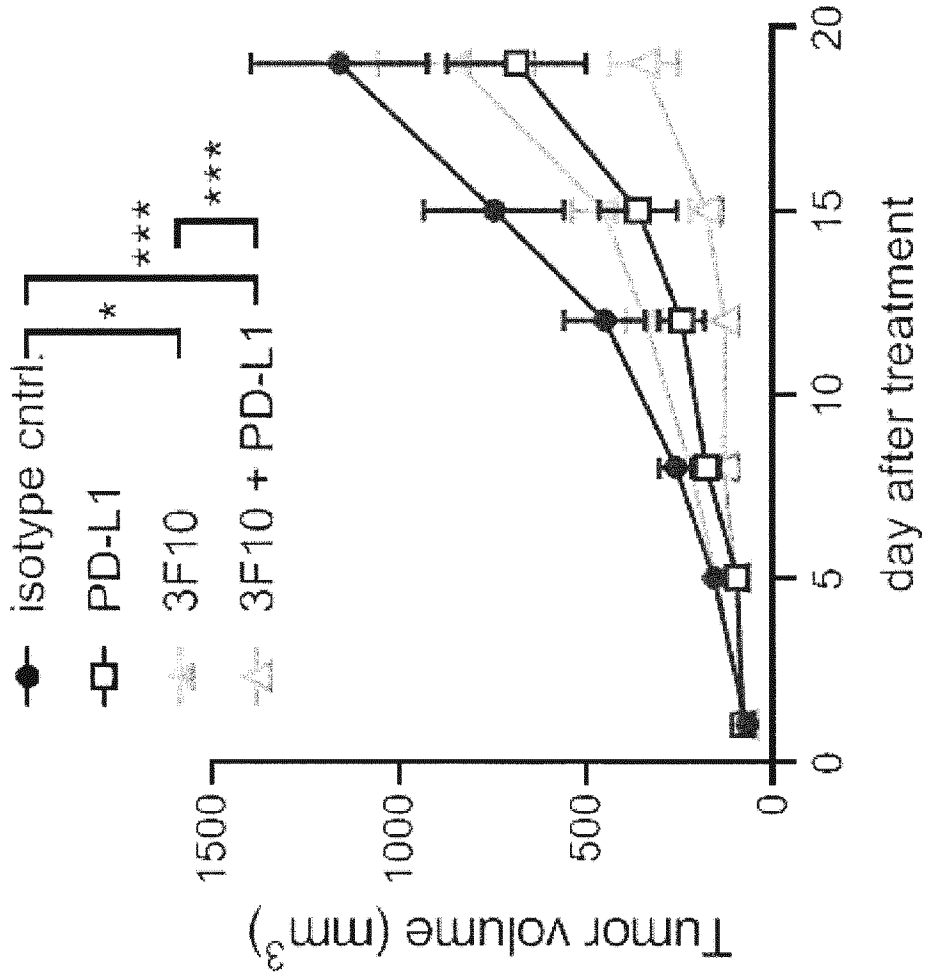
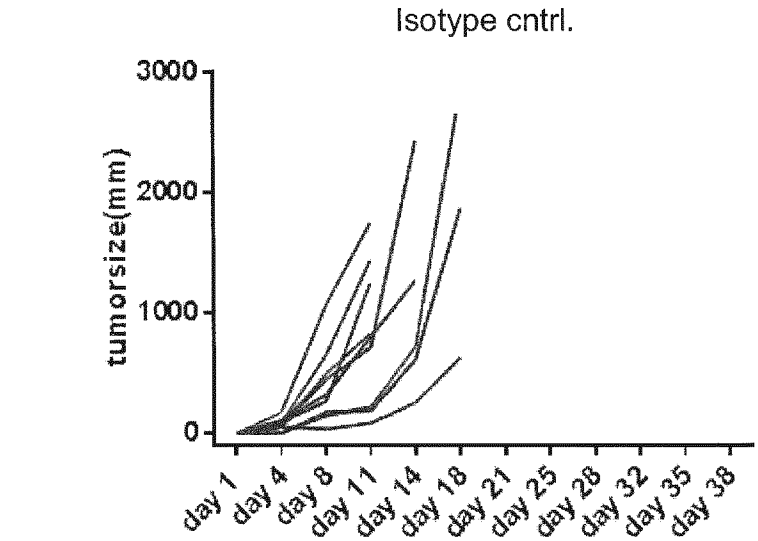
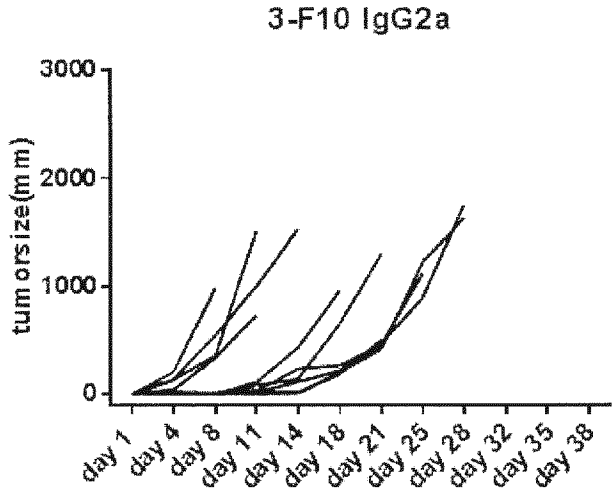

isotype cntrl.
PD-L1
3F10
3F10 + PD-L1
*
***
***
Tumor volume (mm³)
1500
1000
500
0
0
5
10
15
20
day after treatment

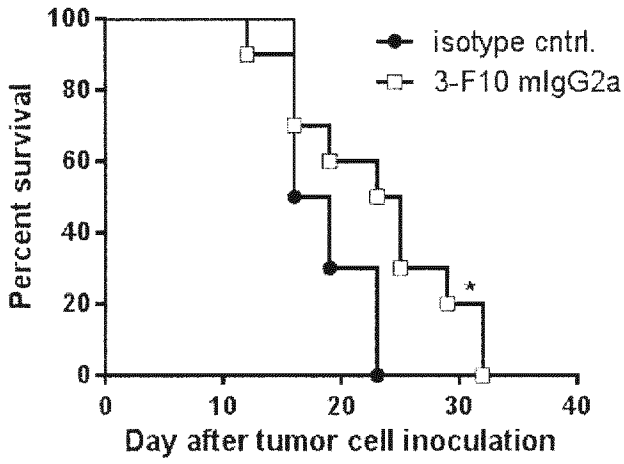
Fig. 12


Isotype cntrl.
tumorsize(mm)
3000
2000
1000
0
day 1
day 4
day 8
day 11
day 14
day 18
day 21
day 25
day 28
day 32
day 35
day 38

A


3-F10 IgG2a
tumorsize(mm)
3000
2000
1000
0
day 1
day 4
day 8
day 11
day 14
day 18
day 21
day 25
day 28
day 32
day 35
day 38

B


Percent survival
100
80
60
40
20
0
0
10
20
30
40
Day after tumor cell inoculation
isotype cntrl.
3-F10 mIgG2a
*

C

Fig. 13

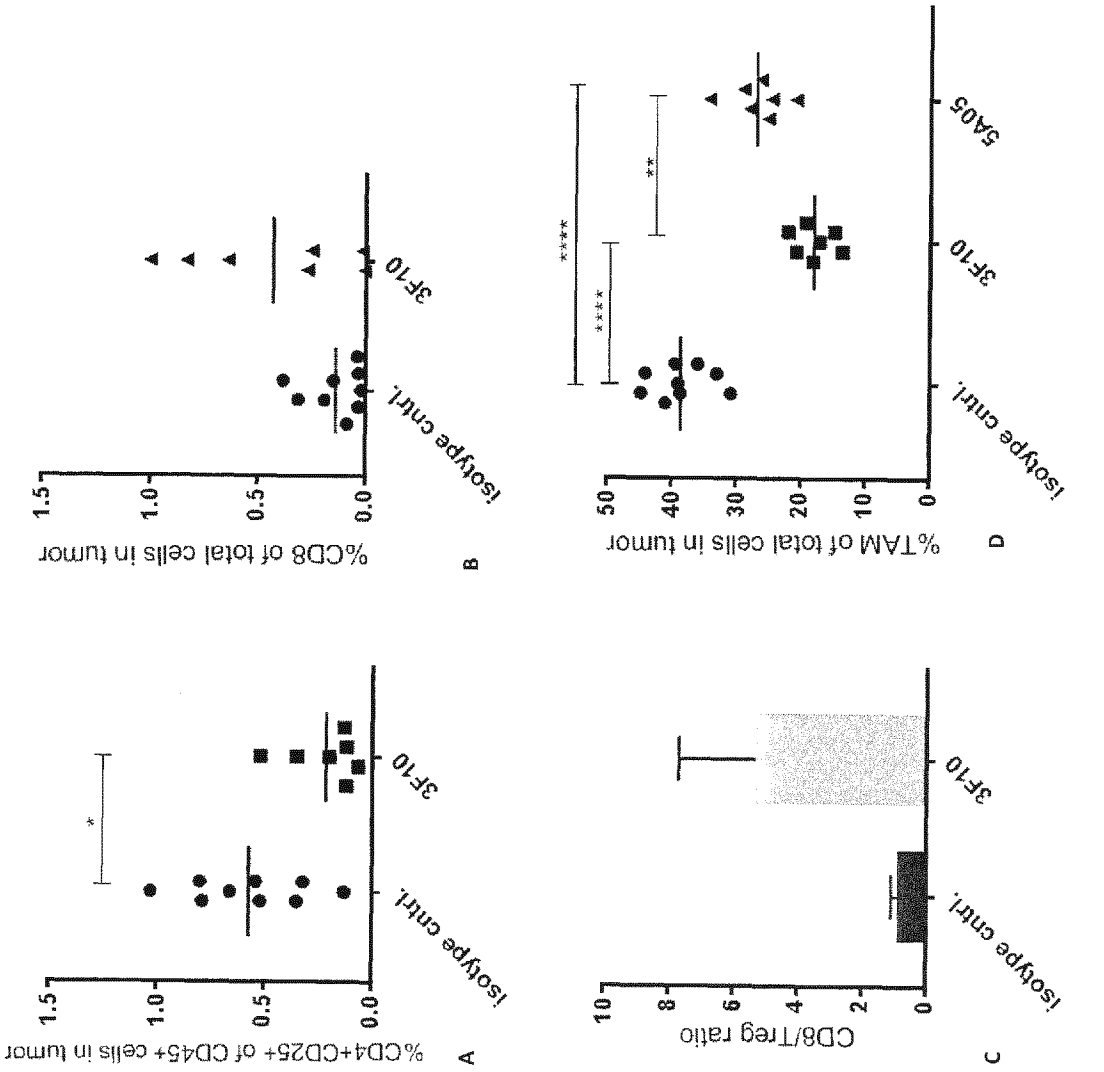
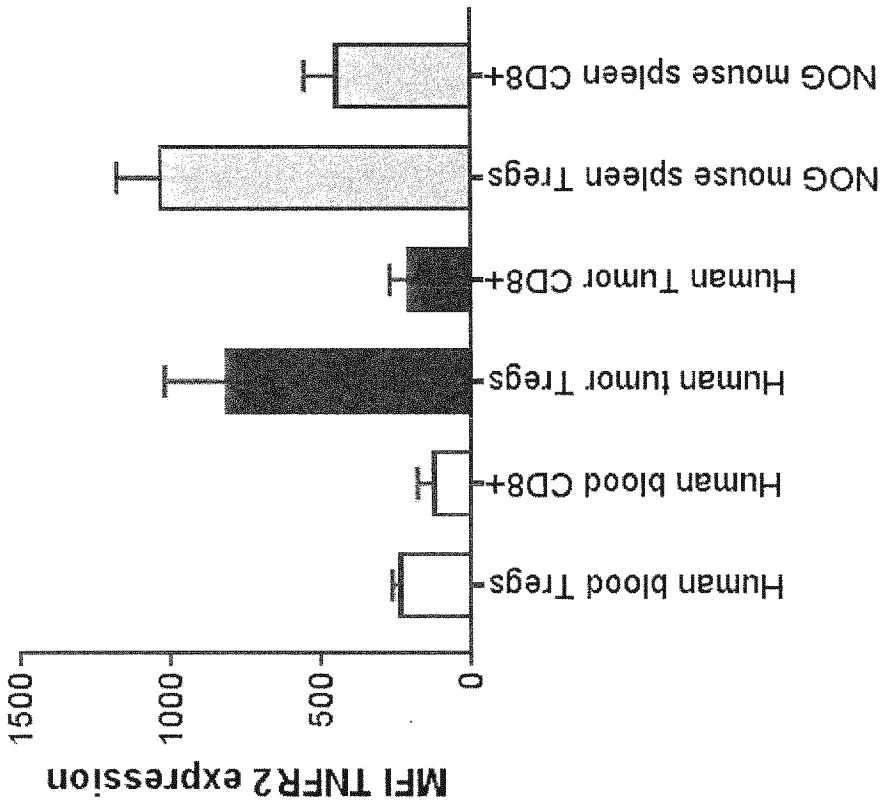

A
%CD4+CD25+ of CD45+ cells in tumor
1.5
1.0
0.5
0.0
*
isotype cntrl.
3F10
B
%CD8 of total cells in tumor
1.5
1.0
0.5
0.0
isotype cntrl.
3F10
C
CD8/Treg ratio
10
8
6
4
2
0
isotype cntrl.
3F10
D
%TAM of total cells in tumor
50
40
30
20
10
0
****
****
**
isotype cntrl.
3F10
5A05

Fig. 14

Fig. 15


MFI TNFR2 expression
1500
1000
500
0
Human blood Tregs
Human blood CD8+
Human tumor Tregs
Human Tumor CD8+
NOG mouse spleen Tregs
NOG mouse spleen CD8+

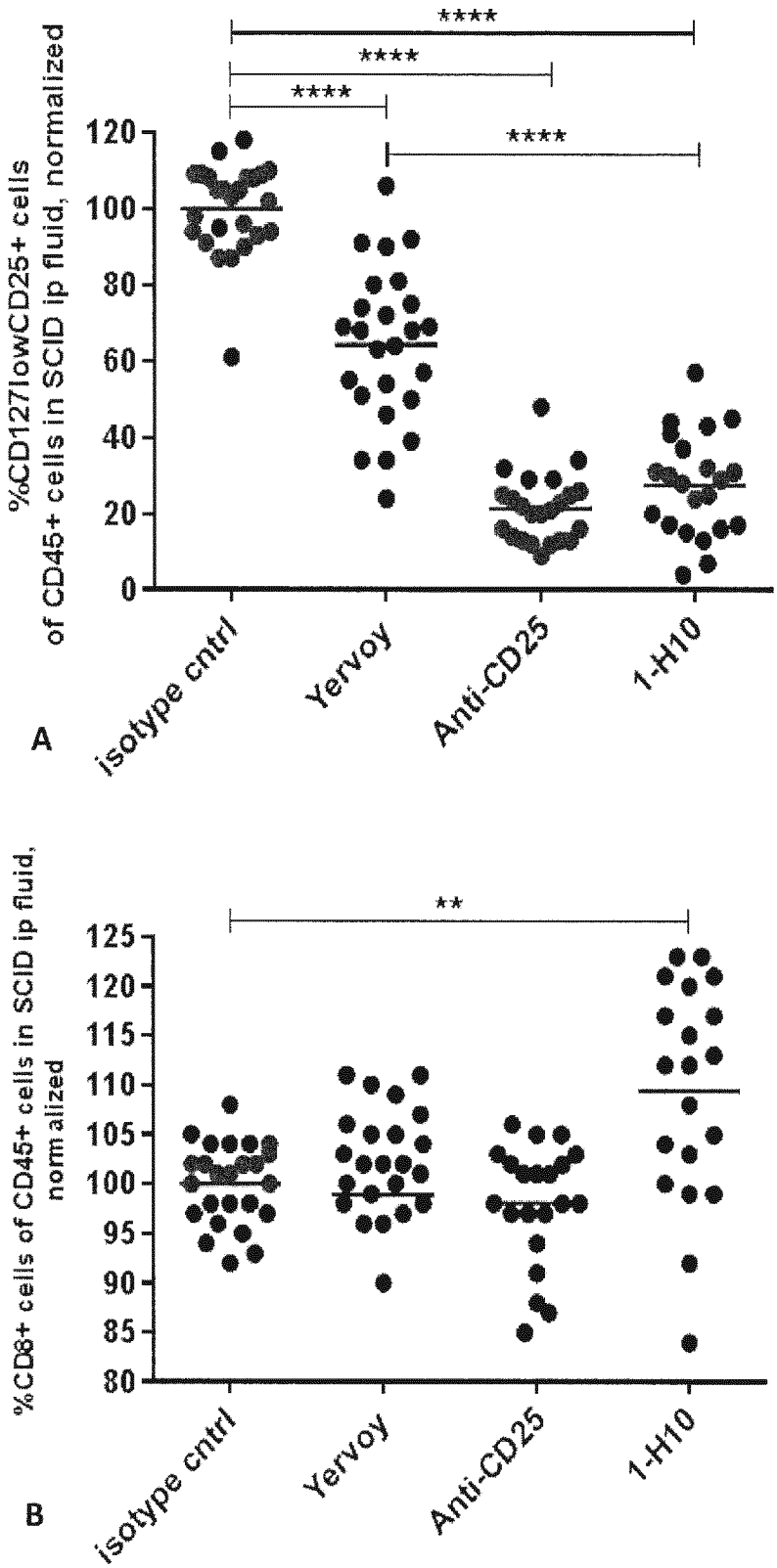
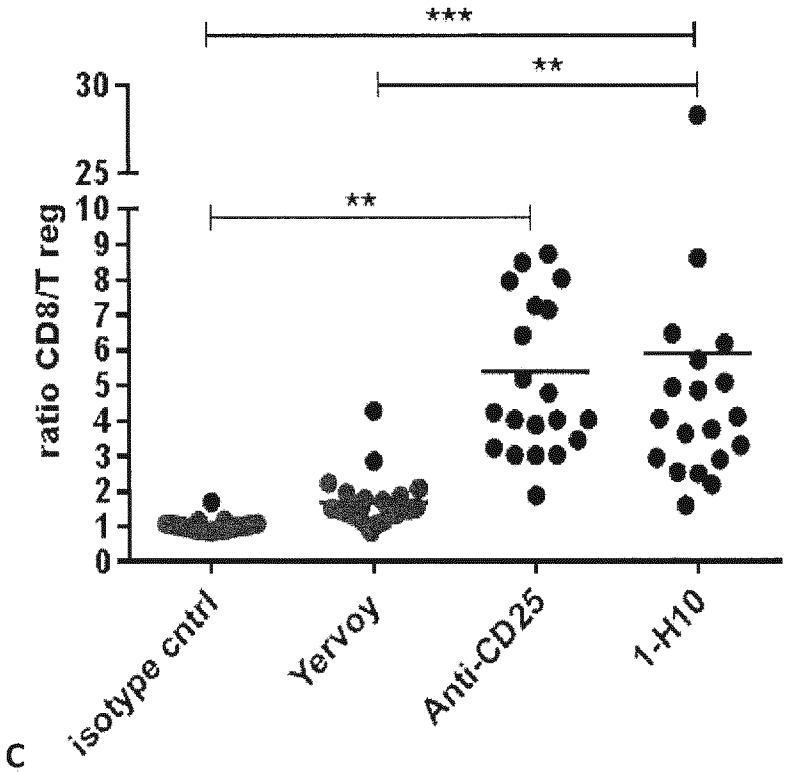
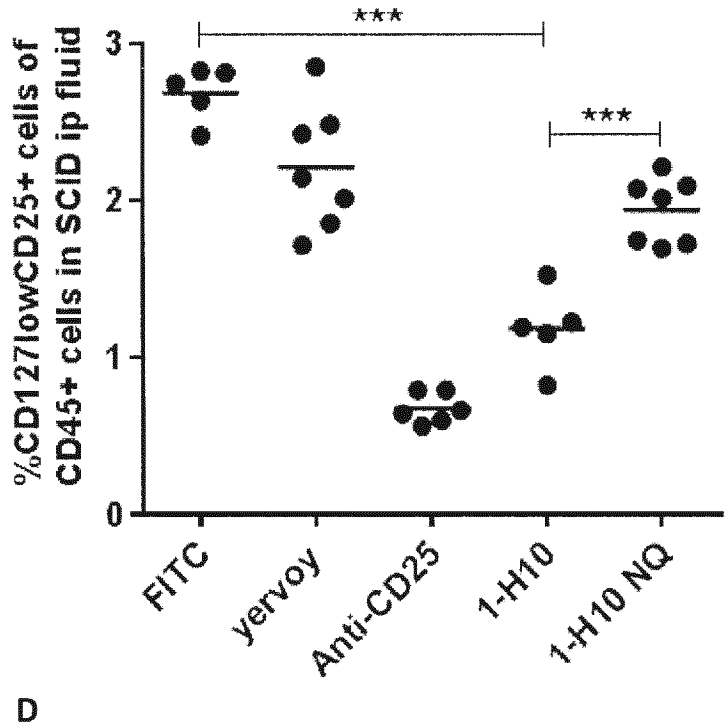

****
****
****
****
%CD127lowCD25+ cells of CD45+ cells in SCID ip fluid, normalized
120
100
80
60
40
20
0
isotype cntrl
Yervoy
Anti-CD25
1-H10
A
**
%CD8+ cells of CD45+ cells in SCID ip fluid, normalized
125
120
115
110
105
100
95
90
85
80
isotype cntrl
Yervoy
Anti-CD25
1-H10
B

Fig. 16



C



D

Fig. 16, cont.

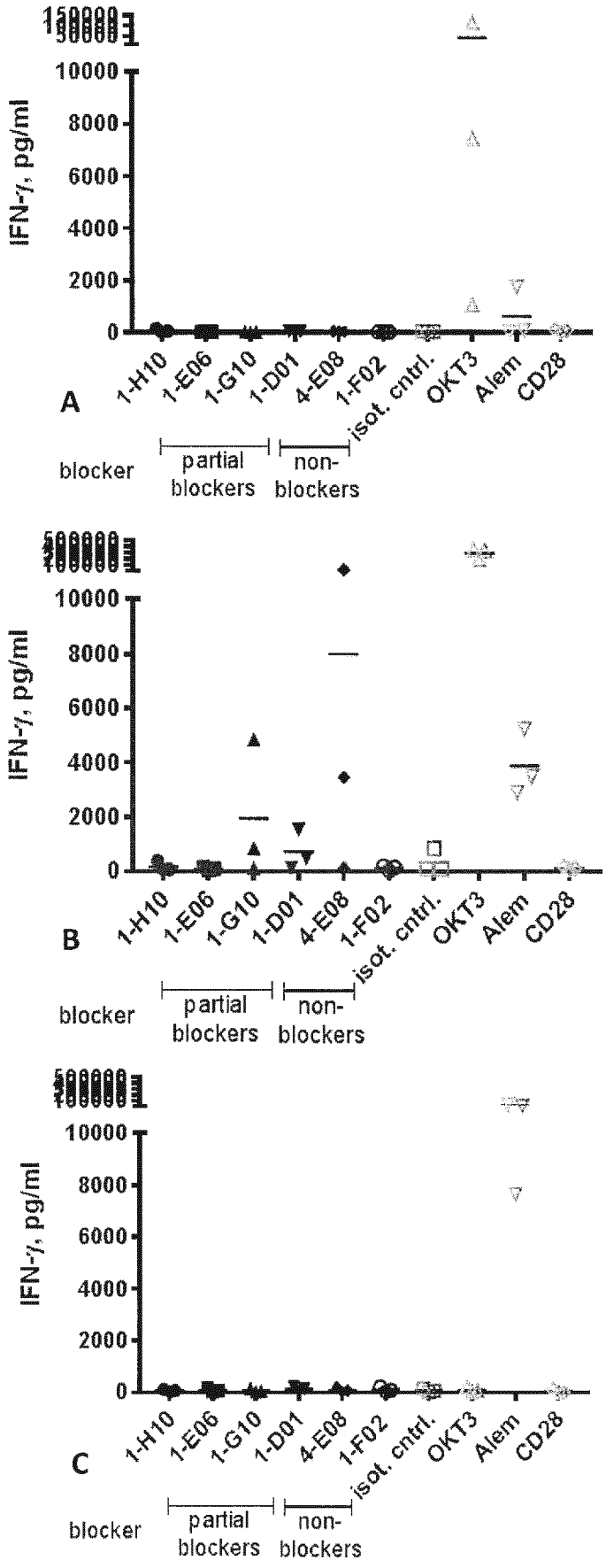

IFN-γ, pg/ml
10000
8000
6000
4000
2000
0
1-H10
1-E06
1-G10
1-D01
4-E08
1-F02
isot. cntrl.
OKT3
Alem
CD28
A
B
C
blocker
partial blockers
non-blockers

Fig. 17

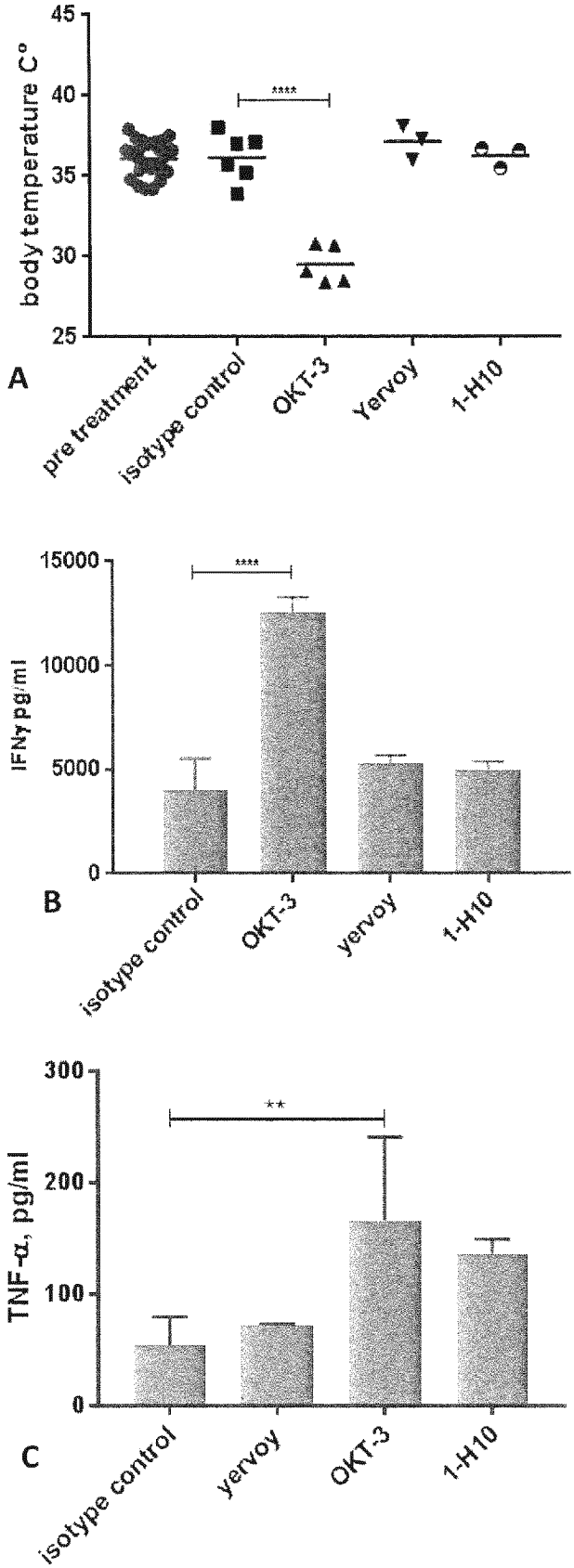

45
40
35
30
25
body temperature C°
****
A
pre treatment
isotype control
OKT-3
Yervoy
1-H10
15000
10000
5000
0
IFNγ pg/ml
****
B
isotype control
OKT-3
yervoy
1-H10
300
200
100
0
TNF-α, pg/ml
**
C
isotype control
yervoy
OKT-3
1-H10

Fig. 18

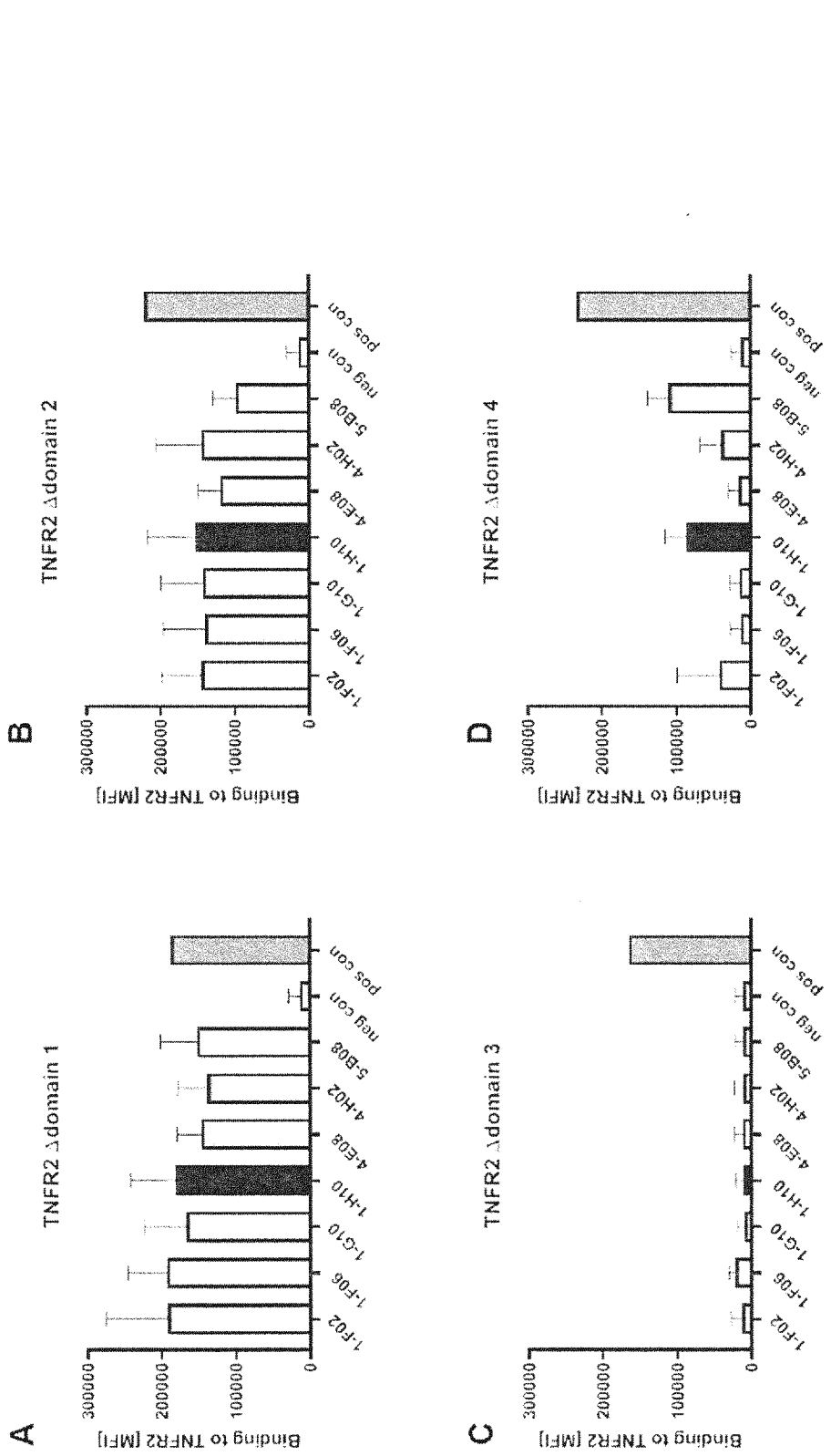

A
TNFR2 Δdomain 1
Binding to TNFR2 [MFI]
300000
200000
100000
0
1-F02
1-F06
1-G10
1-H10
4-E08
4-H02
5-B08
neg con
pos con
B
TNFR2 Δdomain 2
C
TNFR2 Δdomain 3
D
TNFR2 Δdomain 4

Fig. 19

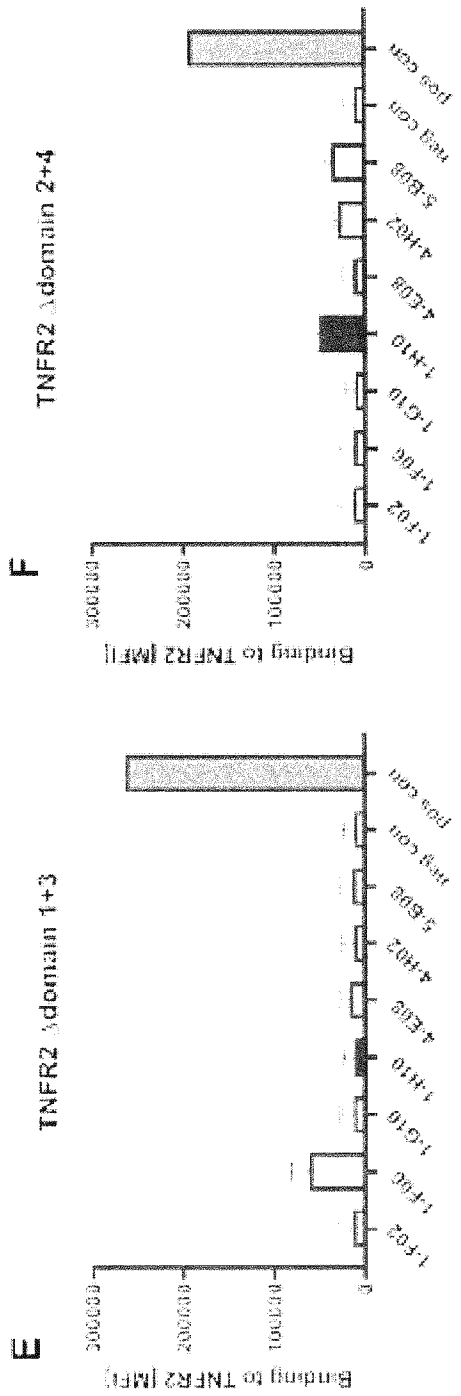

E
TNFR2 Δdomain 1+3
Binding to TNFR2 [MFI]
200000
100000
0
1-F02
1-F06
1-G10
1-H10
4-E06
4-H02
5-B06
neg con
pos con
F
TNFR2 Δdomain 2+4
Binding to TNFR2 [MFI]
200000
100000
0
1-F02
1-F06
1-G10
1-H10
4-E06
4-H02
5-B06
neg con
pos con

Fig. 19, cont.

TNFR2 domain 3

WT

TCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCK

Mouse sequence 1

ACEAGRYCALKTHSGCRLCAPLRKCRPGFGVARPGTETSDVVCK

aa 119-132

Mouse sequence 2

TCRPGWYCALSKQEGSCRQCMRLSKCGPGFGVARPGTETSDVVCK

aa 134-144

Mouse sequence 3

TCRPGWYCALSKQEGCRLCAPLRKCRPGFGVASSRAPNGNVLCK

aa 151-160

Mouse sequence 4

TCRPGWYCALSTHSGSCRQCMRLSKCGPGFGVARPGTETSDVVCK

aa 130-144

Fig. 20

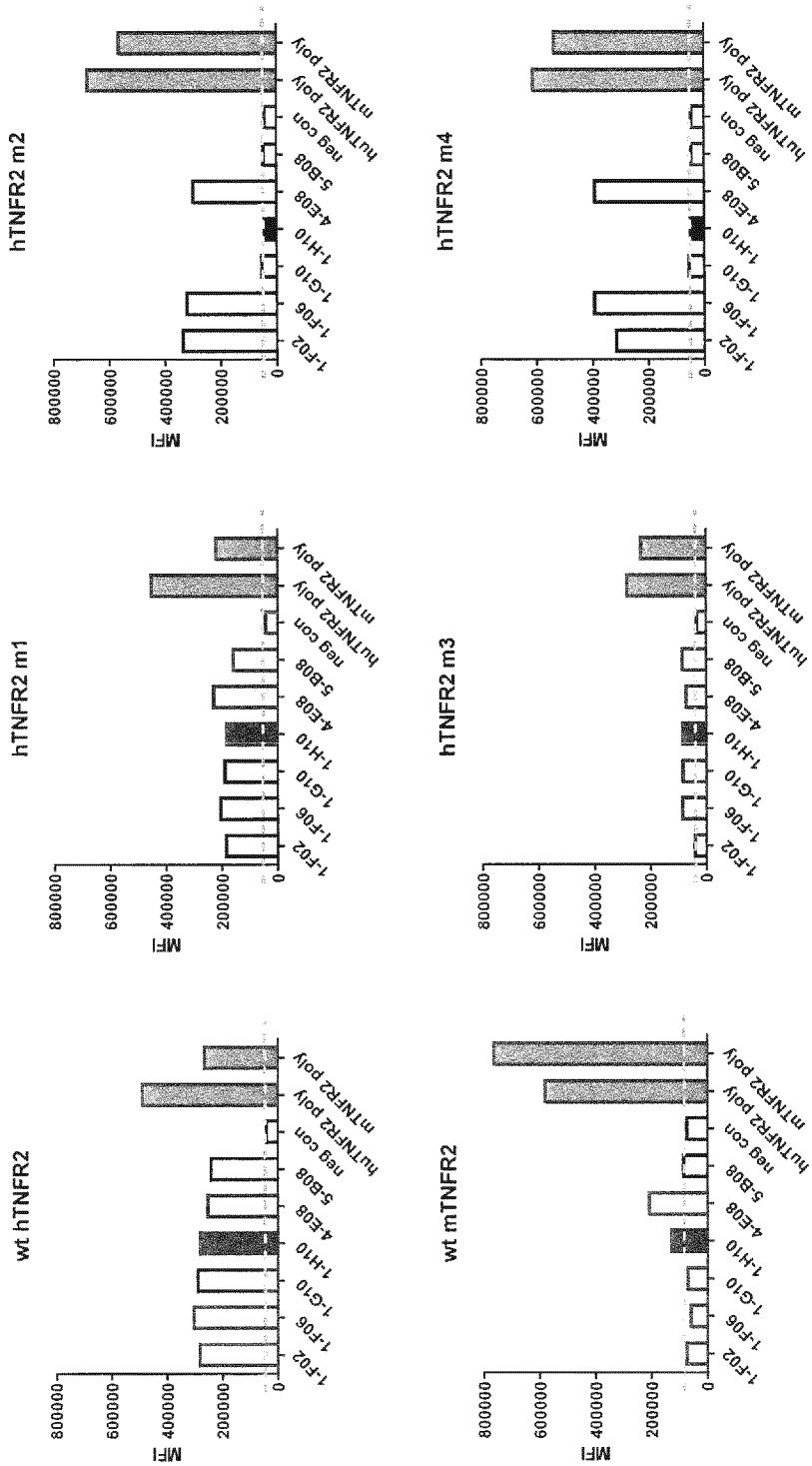

wt hTNFR2
hTNFR2 m1
hTNFR2 m2
wt mTNFR2
hTNFR2 m3
hTNFR2 m4
MFI
800000
600000
400000
200000
0
1-F02
1-F06
1-G10
1-H10
4-E08
5-B08
neg con
huTNFR2 poly
mTNFR2 poly

Fig. 21