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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 December 2016 (22.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/202414 A1

(51) International Patent Classification:
*G01N 33/68* (2006.01) *C07D 401/14* (2006.01)
*C07K 14/705* (2006.01) *C07D 471/00* (2006.01)
*C07K 14/525* (2006.01) *C07K 16/24* (2006.01)

(21) International Application Number:
PCT/EP2015/074527

(22) International Filing Date:
22 October 2015 (22.10.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1510758.4 18 June 2015 (18.06.2015) GB

(71) Applicant: **UCB BIOPHARMA SPRL** [BE/BE]; Allée de la Recherche 60, B-1070 Brussels (BE).

(72) Inventors: **O'CONNELL, James Philip**; IPD, UCB Celltech, 208 Bath Road, Slough Berkshire SL1 3WE (GB). **PORTER, John Robert**; IPD, UCB Celltech, 208 Bath Road, Slough Berkshire SL1 3WE (GB). **LAWSON, Alastair**; IPD, UCB Celltech, 208 Bath Road, Slough Berkshire SL1 3WE (GB). **LIGHTWOOD, Daniel John**; IPD, UCB Celltech, 208 Bath Road, Slough Berkshire SL1 3WE (GB). **WOOTTON, Rebecca Jayne**; IPD, UCB Celltech, 208 Bath Road, Slough Berkshire SL1 3WE (GB).

(74) Agent: **DUCKWORTH, Timothy John**; 14 South Square, Gray's Inn, London Greater London WC1R 5JJ (GB).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published**:

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

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

WO 2016/202414 A1

(54) **Title**: ANTIBODY

(57) **Abstract**: It has been demonstrated that certain compounds bind to TNF and stabilise a conformation of trimeric TNF that binds to the TNF receptor. Antibodies which selectively bind to complexes of such compounds with TNF superfamily members are disclosed. These antibodies may be used to detect further compounds with the same activity, and as target engagement biomarker.

# ANTIBODY

## Field of the Invention

This invention relates to antibodies which may be used to screen for small molecule modulators of the TNF superfamily that form complexes with TNF superfamily members. In particular, the invention relates to antibodies which selectively bind to such complexes, and uses of such antibodies. The present invention also relates to assays for identifying new modulators of the TNF superfamily using said antibodies.

## Background of the Invention

The Tumour Necrosis Factor (TNF) superfamily is a family of proteins that share a primary function of regulating cell survival and cell death. Members of the TNF superfamily share a common core motif, which consists of two antiparallel β-pleated sheets with antiparallel β-strands, forming a "*jelly roll*" β-structure. Another common feature shared by members of the TNF superfamily is the formation of homo- or heterotrimeric complexes. It is these trimeric forms of the TNF superfamily members that bind to, and activate, specific TNF superfamily receptors.

TNFα is the archetypal member of the TNF superfamily. Dysregulation of TNFα production has been implicated in a number of pathological conditions of significant medical importance. For example, TNFα has been implicated in rheumatoid arthritis, inflammatory bowel diseases (including Crohn's disease), psoriasis, Alzheimer's disease (AD), Parkinson's disease (PD), pain, epilepsy, osteoporosis, asthma, systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Other members of the TNF superfamily have also been implicated in pathological conditions, including autoimmune disease.

Conventional antagonists of TNF superfamily members are macromolecular and act by inhibiting the binding of the TNF superfamily member to its receptor. Examples of conventional antagonists include anti-TNFα antibodies, particularly monoclonal antibodies, such as infliximab (Remicade®), adalimumab (Humira®) and certolizumab pegol (Cimzia®), or soluble TNFα receptor fusion proteins, such as etanercept (Enbrel®).

**Summary of the Invention**

The present inventors have identified classes of small molecular entities (SME) that modulate TNFα. These compounds act by binding to the homotrimeric form of TNFα, and inducing and/or stabilising a conformational change in the homotrimer of TNFα. For example, homotrimers of TNFα with the compound bound can bind to TNFα receptors, but are less able, or unable, to initiate signalling downstream of the TNFα receptor. These compounds can be used in the treatment of conditions mediated by TNFα.

The present inventors have developed antibodies that bind selectively to complexes comprising such compounds and a TNF superfamily member. These antibodies may be used to identify further compounds that are capable of inhibiting TNFα in this manner, and may also be used as target engagement biomarkers.

Accordingly, the present invention provides an antibody that selectively binds to a complex comprising (i) a trimeric protein that is a TNF superfamily member and (ii) a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor.

The present invention also provides an antibody that selectively binds to a complex comprising (i) a human TNFα and (ii) a compound selected from the group consisting of compounds (1)-(6), or salts or solvates thereof.

The invention further provides

- An antibody which competes for binding to TNFα with, or binds to the same epitope on TNFα as, other antibodies of the invention.
- An isolated polynucleotide encoding an antibody of the invention.
- An antibody of the invention for use in a method of treatment of the human or animal body by therapy.
- A pharmaceutical composition comprising an antibody of the invention and a pharmaceutically acceptable adjuvant and/or carrier.
- Use of an antibody of the invention as a target engagement biomarker for the detection of a compound-trimer complex in a sample obtained from a subject; wherein said antibody is detectable and said complex comprises a

trimeric protein that is a TNF superfamily member and a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor.

- A method of detecting target engagement of a compound to a trimeric TNF superfamily member, whereby the compound-trimer complex binds to the requisite receptor and modulates the signalling induced by the trimer through the receptor, said method comprising:

(a) obtaining a sample from a subject administered said compound;

(b) contacting an antibody of the invention to said sample and a control sample, wherein said antibody is detectable;

(c) determining the amount of binding of said detectable antibody to said sample and said control sample,

wherein binding of said detectable antibody to said sample greater than binding of said detectable antibody to said control sample indicates target engagement of said compound to said trimeric TNF superfamily member.

- Use of an antibody of the invention in screening for a compound that elicits a conformational change in a trimeric TNF superfamily member, wherein said conformational change modulates the signalling of the requisite TNF superfamily receptor on binding of the trimeric TNF superfamily member.
- A complex comprising a trimeric protein that is a TNF superfamily member and a compound that is bound thereto, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein said complex binds to an antibody of the invention with a $K_{D\text{-}ab}$ of 1 nM or less.
- A TNFα trimer, said TNFα trimer being able to bind TNFR1, but wherein signalling from said bound TNFR1 is attenuated or antagonised, wherein said TNFα trimer binds to either or both of the following antibodies with a a $K_{D\text{-}ab}$ of 1 nM or less:

(i) an antibody with a heavy chain of SEQ ID NO: 27 and a light chain of SEQ ID NO: 26; or

- (ii) an antibody with a heavy chain of SEQ ID NO: 12 and a light chain of SEQ ID NO: 11.
- A compound that is capable of binding to a trimeric protein that is a TNF superfamily member to form a complex, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein the compound-trimer complex binds to an antibody of the invention with a $K_{D\text{-}ab}$ of 1 nM or less.
- A complex as defined above, a trimer as defined above, or a compound according as defined above for use in a method of therapy practised on the human or animal body.
- A method of identifying a compound that is capable of binding to a trimeric protein that is a TNF superfamily member and modulating signalling of the trimeric protein through the receptor, comprising the steps of:
  (a) performing a binding assay to measure the binding affinity of a test compound-trimer complex comprising a trimeric protein that is a TNF superfamily member and a test compound to an antibody that selectively binds to said complex;
  (b) comparing the binding affinity as measured in step (a) with the binding affinity of a different compound-trimer complex known to bind with high affinity to the antibody referred to in step (a); and
  (c) selecting the compound present in the compound-trimer complex of step (a) if its measured binding affinity is acceptable when considered in the light of the comparison referred to in step (b).

**Brief Description of the Figures**

Fig. 1 highlights residues N168, I194, F220 and A221 on the crystal structure of human TNFα .

Fig. 2 shows results of HPLC experiments with the CA185_01974 mFab and compound (1). Peaks corresponding to excess Fab appear at a 1.5x and 2.0x excess. The stoichiometry was therefore determined to be 1 Fab: 1 TNFα trimer.

Fig. 3 shows results of HPLC experiments with the CA185_01979 mFab and

compound (1). Again, peaks corresponding to excess Fab appear at a 1.5x and 2.0x excess. The stoichiometry was therefore also determined to be 1 Fab: 1 TNFα trimer.

Fig. 4 presents results of total TNFα ELISA with compounds (3), (4) and (5) using a commercial anti-TNFα polyclonal antibody.

Fig. 5 presents results of conformation specific TNFα ELISA with CA185_01974.0 and compounds (3), (4) and (5). Apo TNFα gave no signal in this assay, demonstrating the specific nature of the binding of antibody CA185_01974 to compound-bound TNFα.

Fig. 6 shows FACS histogram plots of staining with CA185_01974 and CA185_01979 at 1 and 10 μg/ml. These plots demonstrate that the antibodies only recognise TNFα which has been pre-incubated with compound (1). There is no staining with the DMSO control.

Fig. 7 shows FACS histogram plots of staining with CA185_01974 for a parental NS0 cell line and an engineered NS0 cell line, which overexpresses membrane TNFα. Cells were incubated with compound (1) or DMSO and stained with the antibody Fab fragment. Again, results indicate no staining for the DMSO control (for either the parental or engineered cell line). In the presence of compound (1) staining is, however, observed for the engineered cell line.

Fig. 8 shows sensograms for the determination of affinity values for CA185_01974 using cynomolgus TNFα. Controls (top panels) contained cynomolgus TNFα and DMSO. The bottom panels then present duplicated experiments for cynomolgus TNFα complexed with compound (4).

Fig. 9 shows sensograms for the determination of affinity values for CA185_01974 using human TNFα. Controls (top panels) contained human TNFα and DMSO. The bottom panels then present duplicated experiments for human TNFα complexed with compound (4).

Fig. 10 shows the structures of compounds (1)-(6).

**Brief description of the sequence listing**

SEQ ID NO: 1 shows the LCDR1 of CA185_01974.0.

SEQ ID NO: 2 shows the LCDR2 of CA185_01974.0.

SEQ ID NO: 3 shows the LCDR3 of CA185_01974.0.

SEQ ID NO: 4 shows the HCDR1 of CA185_01974.0.

SEQ ID NO: 5 shows the HCDR2 of CA185_01974.0.

SEQ ID NO: 6 shows the HCDR3 of CA185_01974.0.

SEQ ID NO: 7 shows the amino acid sequence of the LCVR of CA185_01974.0.

SEQ ID NO: 8 shows the amino acid sequence of the HCVR of CA185_01974.0.

SEQ ID NO: 9 shows the DNA sequence of the LCVR of CA185_01974.0.

SEQ ID NO: 10 shows the DNA sequence of the HCVR of CA185_01974.0.

SEQ ID NO: 11 shows the amino acid sequence of the kappa light chain of CA185_01974.0.

SEQ ID NO: 12 shows the amino acid sequence of the mIgG1 heavy chain of CA185_01974.0.

SEQ ID NO: 13 shows the amino acid sequence of the mFab (no hinge) heavy chain of CA185_01974.0.

SEQ ID NO: 14 shows the DNA sequence of the kappa light chain of CA185_01974.0.

SEQ ID NO: 15 shows the DNA sequence of the mIgG1 heavy chain of CA185_01974.0.

SEQ ID NO: 16 shows the DNA sequence of the mFab (no hinge) heavy chain of CA185_01974.0.

SEQ ID NO: 17 shows the LCDR2 of CA185_01979.0.

SEQ ID NO: 18 shows the LCDR3 of CA185_01979.0.

SEQ ID NO: 19 shows the HCDR1 of CA185_01979.0.

SEQ ID NO: 20 shows the HCDR2 of CA185_01979.0.

SEQ ID NO: 21 shows the HCDR3 of CA185_01979.0.

SEQ ID NO: 22 shows the amino acid sequence of the LCVR of CA185_01979.0.

SEQ ID NO: 23 shows the amino acid sequence of the HCVR of CA185_01979.0.

SEQ ID NO: 24 shows the DNA sequence of the LCVR of CA185_01979.0.

SEQ ID NO: 25 shows the DNA sequence of the HCVR of CA185_01979.0.

SEQ ID NO: 26 shows the amino acid sequence of the kappa light chain of CA185_01979.0.

SEQ ID NO: 27 shows the amino acid sequence of the mIgG1 heavy chain of CA185_01979.0.

SEQ ID NO: 28 shows the amino acid sequence of the mFab (no hinge) heavy chain of CA185_01979.0.

SEQ ID NO: 29 shows the DNA sequence of the kappa light chain of CA185_01979.0.

SEQ ID NO: 30 shows the DNA sequence of the mIgG1 heavy chain of CA185_01979.0.

SEQ ID NO: 31 shows the DNA sequence of the mFab (no hinge) heavy chain of CA185_01979.0.

SEQ ID NO: 32 shows the amino acid sequence of rat TNFα.

SEQ ID NO: 33 shows the amino acid sequence of mouse TNFα.

SEQ ID NO: 34 shows the amino acid sequence of human TNFα.

SEQ ID NO: 35 shows the amino acid sequence of the soluble form of human TNFα.

SEQ ID NO: 36 shows the amino acid sequence of the soluble form of human TNFα, but without the initial "S" (which is a cloning artefact in SEQ ID NO: 35)

## Detailed Description of the Invention

*Modulators of TNF superfamily members*

The present inventors have identified test compounds that bind to trimeric forms of the TNF superfamily members. These compounds are small molecular entities (SMEs) that have a molecular weight of 1000 Da or less, generally 750 Da or less, more suitably 600 Da or less. The molecular weight may be in the range of about 50 – about 1000 Da, or about 100 – about 1000 Da. These compounds stabilise a conformation of the trimeric TNF superfamily member that binds to the requisite TNF superfamily receptor and modulate the signalling of the receptor. Examples of such compounds include compounds of formulae (1)-(6).

The stabilising effect of compounds of the invention on trimeric forms of TNF

superfamily members may be quantified by measuring the thermal transition midpoint (Tm) of the trimers in the presence and absence of the compound. Tm signifies the temperature at which 50% of the biomolecules are unfolded. Compounds which stabilise TNF superfamily member trimers will increase the Tm of the trimers. Tm may be determined using any appropriate technique known in the art, for example using differential scanning calorimetry (DSC) or fluorescence probed thermal denaturation assays.

The compounds may bind inside the central space present within the TNF superfamily member trimer (i.e. the core of the trimer).

These compounds may turn the TNF superfamily member into a TNF superfamily receptor antagonist. These compounds are therefore capable of blocking the TNF superfamily member signalling without having to compete with the high affinity interaction between the TNF superfamily member and its receptor.

Alternatively, the compounds may stabilise a conformation of the trimeric TNF superfamily member that binds to the requisite TNF superfamily receptor and enhances the signalling of the receptor. These compounds are therefore capable of increasing the TNF superfamily member signalling without having to compete with the high affinity interaction between the TNF superfamily member and its receptor.

Where herein the compounds are described as antagonists, it will be understood that the compounds may equally be agonists and increase signalling by a TNF superfamily receptor that is bound to a complex of a TNF superfamily member trimer and such an agonist compound. Similarly, where other disclosure refers to antagonistic compounds, methods of identifying such compounds and uses of such compounds, this disclosure may refer equally to agonist compounds.

The compounds described herein are allosteric modulators that bind to the natural agonists of the TNF superfamily receptors, i.e. to trimeric forms of TNF superfamily members and drive these trimers to adopt a conformation that still binds to the requisite TNF superfamily receptor and modulates signalling by the receptor. By modulating, it will be understood that the compound may have an antagonistic effect and so decrease signalling by a TNF superfamily receptor, or else a stimulatory effect and so increase or enhance signalling by a TNF superfamily receptor.

These compounds may convert the natural TNF superfamily member agonists

into antagonists. In contrast, conventional TNF superfamily member antagonists bind to the TNF superfamily member or the TNF superfamily receptor and prevent the binding of the TNF superfamily member to the requisite receptor. In the alternative, the compounds may increase signalling by a TNF superfamily receptor when the TNF superfamily member is bound compared to the level of signalling by the TNF superfamily receptor when the TNF superfamily member is bound in the absence of the compound. The compounds may therefore convert the natural TNF superfamily member agonists into so-called "*super-agonists*". The compounds may therefore also be known as allosteric modulators of ligand activity (AMLAs).

The compounds are not limited in terms of their chemical formula or structure, provided that they bind to at least one TNF superfamily member and stabilise a conformation of the trimeric TNF superfamily member that binds to the requisite TNF superfamily receptor and modulate the signalling of the TNF superfamily receptor. The compounds can therefore be identified using the antibodies and methods described herein. The compounds may comprise a benzimidazole moiety or an isostere thereof.

The compounds may increase the binding affinity of TNF superfamily members (in the form of a compound-trimer complex) to the requisite receptor compared to the binding affinity of the TNF superfamily members to the requisite receptor in the absence of the compounds.

The compounds bind to the trimeric forms of TNF superfamily members. Such compounds may bind specifically (or selectively) to the trimeric forms of one or more TNF superfamily members. A compound may bind specifically (or selectively) to only one of the TNF superfamily members, but not to any other TNF superfamily members. A compound may also bind specifically to two, three, four or up to all of the TNF superfamily members. By specific (or selective), it will be understood that the compounds bind to the molecule or molecules of interest, in this case the trimeric form of the TNF superfamily member, with no significant cross-reactivity to any other molecule, which may include other members of the TNF superfamily. Cross-reactivity may be assessed by any suitable method, for example surface plasmon resonance. Cross-reactivity of a compound for the trimeric form of a TNF superfamily member with a molecule other than the trimeric form of that particular

TNF superfamily member may be considered significant if the compound binds to the other molecule at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 100% as strongly as it binds to the trimeric form of the TNF superfamily member of interest. For example, cross reactivity may be considered significant if the compound binds to the other molecule about 5% - about 100%, typically about 20% - about 100%, or about 50% - about 100% as strongly as it binds to the trimeric form of the TNF superfamily member of interest. A compound that is specific (or selective) for the trimeric form of a TNF superfamily member may bind to another molecule at less than about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% or 20% the strength that it binds to the trimeric form of the TNF superfamily member (down to zero binding). The compound suitably binds to the other molecule at less than about 20%, less than about 15%, less than about 10% or less than about 5%, less than about 2% or less than about 1% the strength that it binds to the trimeric form of the TNF superfamily member (down to zero binding).

The rates at which a test compound binds to a TNF superfamily member is referred to herein as the "*on*" rate" $k_{on-c}$ and the rate at which the test compound dissociates from the TNF superfamily member is referred to herein as the "*off*" rate or $k_{off-c}$. As used herein, the symbol "$K_{D-c}$" denotes the binding affinity (dissociation constant) of a test compound for a TNF superfamily member. $K_{D-c}$ is defined as $k_{off-c}/k_{on-c}$. Test compounds may have slow "*on*" rates, which can be measured in minutes by mass spectral analysis of the TNF superfamily member and compound-trimer complex peak intensities. $K_{D-c}$ values for a test compound can be estimated by repeating this measurement at different TNF superfamily member: compound-trimer complex ratios. Typically, binding of compounds of the invention to TNF superfamily trimers is characterized by fast "*on*" rates, ideally about $10^7$ $M^{-1}s^{-1}$, with slow "*off*" rate, for example values typically of $10^{-3}$ $s^{-1}$, $10^{-4}$ $s^{-1}$, or no measurable "*off*" rate.

As used herein, the symbol "$k_{on-r}$" denotes the rate (the "*on*" rate) at which a compound-trimer complex binds to a TNF superfamily receptor. As used herein, the symbol "$k_{off-r}$" denotes the rate (the "*off*" rate) at which a compound-trimer complex dissociates from a TNF superfamily receptor. As used herein, the symbol "$K_{D-r}$" denotes the binding affinity (dissociation constant) of a compound-trimer complex for

a superfamily receptor. $K_{D-r}$ is defined as $k_{off-r}/k_{on-r}$.

The $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the presence of the test compound (i.e. in the form of a compound-trimer complex) may be at least about 1.5 times, 2 times, 3 times, 4 times, 5 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times lower than the $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the absence of the test compound. The $K_{D-r}$ value of the compound-trimer complex for binding to the TNF superfamily member may be decreased at least about 1.5 times, generally at least about 3 times, more suitably at least about 4 times the $K_{D-r}$ value of the TNF superfamily trimer binding to the TNF superfamily receptor in the absence of the test compound, i.e. the binding affinity of the compound-trimer complex for the TNF superfamily may be increased at least about 1.5-fold, generally at least about three-fold, more suitably at least about four-fold compared to the binding affinity of the TNF superfamily trimer to the TNF superfamily receptor in the absence of test compound.

A compound described herein may increase the binding affinity of the TNF superfamily member to its receptor by about 2 times, 3 times, 4 times, 5 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times or more compared to the binding affinity of the TNF superfamily member to its receptor in the absence of the compound.

The binding affinity may be given in terms of binding affinities ($K_{D-r}$) and may be given in any appropriate units, such as μM, nM or pM. The smaller the $K_{D-r}$ value, the larger the binding affinity of the TNF superfamily member to its receptor.

The $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the presence of the compound may be at least about 1.5 times, 2 times, 3 times, 4 times, 5 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times lower than the $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the absence of the test compound.

The decrease in the $K_{D-r}$ value of the compound-trimer complex for binding to the TNF superfamily receptor compared to the $K_{D-r}$ value of the TNF superfamily trimer alone binding to the TNF superfamily receptor may result from an increase in the on rate ($k_{on-r}$) of the compound-trimer complex binding to the TNF superfamily

receptor compared to the TNF superfamily trimer alone, and/or a decrease in the off rate ($k_{off-r}$) compared to the TNF superfamily trimer alone. The on rate ($k_{on-r}$) of the compound-trimer complex binding to the TNF superfamily receptor is generally increased compared to the TNF superfamily trimer alone. The off rate ($k_{off-r}$) of the compound-trimer complex binding to the TNF superfamily receptor is generally decreased compared to the TNF superfamily trimer alone. Most suitably, the on rate ($k_{on-r}$) of the compound-trimer complex binding to the TNF superfamily receptor is increased, and the off-rate ($k_{off-r}$) of the compound-trimer complex binding to the TNF superfamily receptor is decreased, compared to the TNF superfamily trimer alone. The $k_{on-r}$ value of the compound-trimer complex to the requisite TNF superfamily receptor may be increased by at least about 1.5-fold or at least about two-fold and suitably at least about three fold compared to the $k_{on-r}$ value of the TNF superfamily trimer binding to its receptor in the absence of the compound and/or the $k_{off-r}$ value of the compound-trimer complex to the requisite TNF superfamily receptor may be decreased by at least about 1.2-fold, at least about 1.6-fold, at least about two-fold, more suitably at least about 2.4-fold compared to the $k_{off-r}$ value of the TNF superfamily trimer binding to its receptor in the absence of the compound.

The on-rate for compound binding to TNF superfamily trimer ($k_{on-c}$) is typicallyfaster than the on-rate for compound-trimer complex binding to TNF superfamily receptor ($k_{on-r}$). The off-rate for compound-trimer complex binding to TNF superfamily receptor ($k_{off-r}$) is also typically faster than the off-rate for compound binding to TNF superfamily trimer ($k_{off-c}$). Mostsuitably, the on-rate for compound binding to TNF superfamily trimer ($k_{on-c}$) is faster than the on-rate for compound-trimer complex binding to TNF superfamily receptor ($k_{on-r}$), and the off-rate for compound-trimer complex binding to TNF superfamily receptor ($k_{off-r}$) is faster than the off-rate for compound binding to TNF superfamily trimer ($k_{off-c}$).The $K_{D-c}$ value of the compound for binding to TNF superfamily trimer is generally lower than the $K_{D-r}$ value of the compound-trimer complex for binding to TNF superfamily receptor, i.e. the compound has a higher affinity for the trimer than the compound-trimer complex has for the receptor.

The $k_{on-r}$, $k_{off-r}$, and $K_{D-r}$ values for both the compound-trimer complex and the TNF superfamily trimer to the requisite TNF superfamily receptor may be determined

using any appropriate technique, for example surface plasmon resonance, mass spectrometry and isothermal calorimetry. The $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the presence of the test compound may be 1 μM, 100 nM, 10 nM, 5 nM, 1 nM, 100 pM, 10 pM or less (typically down to a lower value of about 1 pM). The $K_{D-r}$ value of the TNF superfamily member for binding to its receptor in the presence of the test compound (i.e. in a compound-trimer complex) may be 1nM or less. The $K_{D-r}$ value of a compound-trimer complex for binding to the requisite TNF superfamily receptor may be less than 600 pM, more preferably less than 500 pM, less than 400 pM, less than 300 pM, less than 200 pM, less than 100 pM or less than 50 pM (again down to a lower value of about 1 pM). The $K_{D-r}$ value of a compound-trimer complex for binding to the requisite TNF superfamily receptor may be less than about 200 pM (to about 1 pM).

Compounds may be identified by an assay which comprises determining the $K_{D-r}$ of the trimeric form of the TNF superfamily member in a sample of the TNF superfamily member and the compound; comparing the $K_{D-r}$ of the trimeric form of the TNF superfamily member in the sample with a control sample; and selecting a compound.

The compounds stabilise the trimeric form of the TNF superfamily member. Stabilisation is considered to occur if a test compound increases the proportion of trimer compared to the amount of trimer observed for a sample containing the TNF superfamily member and the destabilising agent in the absence of the test compound. The test compound may increase the amount of trimer by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400% or more compared to the amount of trimer present in a sample containing the TNF superfamily member and the destabilising agent in the absence of the test compound.

The test compound may also increase the amount of trimer compared to that observed for a sample of the TNF superfamily member in the absence of both the destabilising agent and the test compound. The test compound may increase the amount of trimer by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400% or more compared to the amount of trimer present in a sample containing the TNF

superfamily member in the absence of both the destabilising agent and the test compound.

The test compound may increase the amount of the TNF superfamily member bound to its receptor by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400% or more compared to the amount of the TNF superfamily member bound to its receptor in a sample containing the TNF superfamily member in the absence of the test compound.

The test compounds may enhance the stability of the trimeric form of the TNF superfamily member. Enhanced stability of the trimeric form of the TNF superfamily member is considered to occur if a test compound increases the thermal transition midpoint ($T_m$) of the trimeric form of the TNF superfamily member compared to the $T_m$ of the trimeric form of the TNF superfamily member observed for a sample containing the TNF superfamily member and the destabilising agent in the absence of the test compound. The $T_m$ of the trimeric form of the TNF superfamily member is the temperature at which 50% of the biomolecules are unfolded. The $T_m$ of the trimeric form of the TNF superfamily member in the presence and/or absence of the test compound may be measured using any appropriate technique known in the art, for example using differential scanning calorimetry (DSC) or fluorescence probed thermal denaturation assays.

The test compound may increase the $T_m$ of the trimeric form of the TNF superfamily member by at least 1 ˚C, at least 2 ˚C, at least 5 ˚C, at least 10 ˚C, at least 15 ˚C, at least 20 ˚C or more compared to the $T_m$ of the trimeric form of the TNF superfamily member in a sample containing the TNF superfamily member in the absence of the test compound. The test compound may increase the $T_m$ of the trimeric form of the TNF superfamily member by at least 1 °C, typically by at least 10 °C and more suitably by between 10 ˚C and 20 ˚C.

The compounds may completely or partially inhibit signalling through a TNF receptor when a TNF superfamily member in the form of a compound-trimer complex binds to the receptor. The compound may act to reduce signalling through a TNF superfamily receptor by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%. Alternatively, the compounds may increase signalling through a TNF

receptor when a TNF superfamily member in the form of a compound-trimer complex binds to the receptor. The compound may act to increase signalling through a TNF superfamily receptor by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or 200%. Any change in the level of signalling may be measured by any appropriate technique, including measuring reporter gene activity by alkaline phosphatase or luciferase, NF-κB translocation using machines such as the Cellomics Arrayscan, phosphorylation of downstream effectors, recruitment of signalling molecules, or cell death.

The compounds may modulate at least one of the downstream effects of signalling through a TNF receptor when a TNF superfamily member in the form of a compound-trimer complex binds to the receptor. Such effects are discussed herein and include TNF superfamily-induced IL-8, IL17A/F, IL2 and VCAM production, TNF superfamily-induced NF-κB activation and neutrophil recruitment. Standard techniques are known in the art for measuring the downstream effects of TNF superfamily members. The compounds may modulate at least 1, 2, 3, 4, 5, 10 or up to all of the downstream effects of signalling through a TNF receptor.

The activity of the compounds may be quantified using standard terminology, such as $IC_{50}$ or half maximal effective concentration ($EC_{50}$) values. $IC_{50}$ values represent the concentration of a compound that is required for 50% inhibition of a specified biological or biochemical function. $EC_{50}$ values represent the concentration of a compound that is required for 50% of its maximal effect. The compounds may have $IC_{50}$ or $EC_{50}$ values of 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 1 nM, 100 pM or less (down to a lower value of about 10 pM or 1 pM). $IC_{50}$ and $EC_{50}$ values may be measured using any appropriate technique, for example cytokine production can be quantified using ELISA. $IC_{50}$ and $EC_{50}$ values can then be generated using a standard 4-parameter logistic model also known as the sigmoidal dose response model.

As mentioned above, examples of compounds which are capable of binding to TNF and modulating signalling are compounds of formulae (1)-(6).

*Modulator-TNF superfamily member complexes*

The present inventors have found that binding of the compounds described

herein to trimeric forms of TNF superfamily members results in a conformational change in the TNF superfamily trimer. In particular, the TNF superfamily member trimer takes on a deformed or distorted conformation when bound by a compound as disclosed herein.

For example, when compounds (1)-(6) are bound the soluble domain of human TNFα the TNF retains its trimeric structure but the A and C subunits move away from each other and C rotates to generate a cleft between these subunits.

Without being bound by theory, it is believed that, in the absence of a compound, trimeric TNF superfamily members, including trimeric TNFα, are capable of binding to three separate dimeric TNF superfamily member receptors. Each of the dimeric TNF superfamily member receptors is capable of binding to two separate TNF superfamily trimers. This results in the aggregation of multiple TNF superfamily member trimers and TNF superfamily member receptor dimers, creating signalling rafts that initiate downstream signalling.

When trimeric TNFα is bound to the compound, the conformation of the resulting complex is deformed. Accordingly, without being bound by theory, it is believed that, in the presence of a compound as disclosed herein, trimeric TNF superfamily members, including trimeric TNFα, are only capable of binding to two separate dimeric TNF superfamily member receptors. The fact that only two, rather than three, separate dimeric TNF superfamily member receptors bind to the trimeric TNF superfamily member reduces or inhibiting the aggregation of multiple TNF superfamily member trimers and TNF superfamily member receptor dimers. This reduces or inhibits the formation of signalling rafts and so reduces or inhibits downstream signalling.

The antibodies of the invention may be used to detect TNF superfamily members with a distorted conformation as a result of the binding of a compound as disclosed herein. Typically the TNF superfamily member with a distorted or deformed conformation is a trimeric TNF superfamily member. However, antibodies of the invention may also bind to other forms of the TNF superfamily member. For example, antibodies of the invention may bind to TNF superfamily monomers.

The TNF superfamily member is typically TNFα, and may be trimeric TNFα (particularly $TNF\alpha_S$).

Accordingly, the invention provides a complex comprising a trimeric protein that is a TNF superfamily member and a compound that is bound thereto, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein said complex binds to an antibody of the invention with an affinity of at least 1 nM (i.e. 1 nM or less, down to about 1 pM). The TNF superfamily member is typically TNFα, more particularly $TNF\alpha_s$.

Furthermore, the antibody generally binds to the complex with an affinity of that is at least about 100 times lower (the affinity is improved at least about 100 times), suitably about 200 times lower, relative to the affinity for binding to the compound in the absence of the TNF timer and/or for binding to the TNF trimer in the absence of compound.

The present invention further provides a compound that is capable of binding to a trimeric protein that is a TNF superfamily member to form a complex, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein the compound-trimer complex binds to an antibody of the invention with a $K_{D\text{-}ab}$ of 1 nM or less (down to about 1 pM). The TNF superfamily member is typically TNFα, most particularly $TNF\alpha_s$.

The antibody typically binds to the complex with an affinity of that is at least about 100 times lower (the affinity is improved at least about 100 times), more suitably about 200 times lower, relative to the affinity for binding to the compound in the absence of the TNF timer and/or for binding to the TNF trimer in the absence of compound.

The compound-trimer complex may bind to any antibody of the invention. Particularly, the compound-trimer complex may bind to an antibody comprising the amino acid sequences disclosed herein.

A compound or complex described herein may be used in the treatment and/or prophylaxis of a pathological condition. Accordingly, provided is a compound or complex of the invention for use in a method of therapy practiced on the human or animal body. The invention also provides a method of therapy comprising the administration of a compound or complex of the invention to a subject. The

compound or complex of the invention may be used in any therapeutic indication and/or pharmaceutical composition described herein.

*Antibodies*

The invention provides antibodies that selectively bind to at least one compound-trimer complex comprising at least one compound disclosed herein and a trimeric TNF superfamily member.

Typcially, selective binding of an antibody of the invention to a compound-(trimer) complex is measured relative to the binding of the antibody to the compound in the absence of the TNF superfamily member, or to the TNF superfamily member in the absence of the compound or to other (different) compound-(trimer) complexes.

In particular, the invention provides an antibody that selectively binds to a complex comprising (i) a trimeric protein that is a TNF superfamily member and (ii) a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor. Typically said antibody binds selectively to said complex relative to its binding to the TNF superfamily member in the absence of the compound or to the compound in the absence of the TNF superfamily member.

The compound may be any compound described above, including compounds (1)-(6) (or salts or solvates thereof). As discussed further below, the TNF superfamily member may be any of the superfamily members, but is typically TNFα. More particularly, the TNFα is human TNFα, especially soluble TNFα ($TNF\alpha_S$). The $TNF\alpha_S$ may have the sequence of SEQ ID NO: 35 or SEQ ID NO: 36, or may be a variant of SEQ ID NO: 35 or SEQ ID NO: 36. Such variants typically retain at least about 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94% or 95%identity to SEQ ID NO: 35 or SEQ ID NO: 36 (or even about 96%, 97%, 98% or 99% identity). In other words, such variants may retain about 60% - about 99% identity to SEQ ID NO:35 or SEQ ID NO:36, suitably about 80% - about 99% identity to SEQ ID NO:35 or SEQ ID NO:36, more suitably about 90% - about 99% identity to SEQ ID NO:35 or SEQ ID NO:36 and most suitably about 95% - about 99% identity to SEQ ID NO:35 or SEQ ID NO:36. Variants are described further below.

The term "corresponding sequence" indicates that the TNFα may have the wild-type amino sequence of any known animal or human TNFα, in particular human TNFα, for instance SEQ ID NO: 36. It may be soluble TNFα (sTNFα) or membrane-bound TNFα, or both. Soluble homotrimeric TNFα (sTNF) is released from membrane-bound homotrimeric TNFα (mTNF) via proteolytic cleavage by the metalloprotease TNF alpha converting enzyme (TACE / ADAM17; though other proteinases can also release sTNF such as ADAM10, ADAM19, matrix metalloproteinase 7 and proteinase 3 which may yield corresponding soluble TNFα sequences that may be extended or truncated by 1, 2, 3, 4, or 5 amino acids relative to a TACE cleaved sTNFα such as SEQ ID NO: 36). The soluble 52 kDa trimeric sTNF takes on a triangular pyramid shape. A human sequence encompassed by the term mTNF is shown in SEQ ID NO: 34, and a human sequence encompassed by the term sTNF (the product of the action of TACE on SEQ ID NO: 34) is shown in SEQ ID NO: 36. Corresponding sequences of rat and mouse mTNFα are presented in SEQ ID NO:32 and 33, respectively. Corresponding sequences of TNFα from other animals (or known variants of the human sequence) may be readily overlaid with the SEQ ID NO:36 sequence and given the same amino acid numbering as for SEQ ID NO:36 (used in the numbering of TNFα amino acids herein). For instance, the sequence from various animals may be found within the Uniprot database (www.uniprot.org) including human sequences P01375 and Q5STB3. The corresponding sTNFα sequences may be the 157 amino acid C-terminal end of the mTNFα sequence (as SEQ ID NO:36) or may be longer or shorter by one, two or three amino acids (the rat and mouse sequences being 156 amino acids). The corresponding sTNFα sequence may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acid substitutions relative to SEQ ID NO:36. The corresponding sTNFα sequence may have 80, 90, 95, 96, 97, 98, or 99% amino acid sequence identity to SEQ ID NO:36 over the length of SEQ ID NO:36.

As discussed above, although the present disclosure generally relates to binding of antibodies of the invention to TNF superfamily member trimers, antibodies of the invention may also bind to other forms of the TNF superfamily member. To illustrate, the Examples of the present application demonstrate that the CA185_0179

antibody binds to trimeric TNF. However, as shown in Figure 1 (crystal structure of the CA185_0179 antibody bound to a TNFα monomer in the presence of compound (1)) the antibody also appears to bind to monomeric TNFα. Without being bound by theory, in the presence of the compound it is believed that the soluble domain of the TNF retains its trimeric structure. However, the A and C subunits move away from each other (and the C subunit rotates) to generate a cleft between these two subunits. Thus although antibodies of the invention bind to distorted trimers, it is also possible that the antibodies can still bind if the trimeric structure is forced apart into monomers.

With regards to the "A" and "C" subunits, when looking at a crystal structure of a TNFα trimer from the side it is approximately shaped like a pyramid/cone. When you look down the trimer axis with the N- and C-termini of the monomer ends pointing towards you then you are looking at the "fat" end of the trimer. In the distorted structure with compound, a cleft opens between A and C subunits in which, without being bound by theory, the Ab of the invention binds.

Which chain is A, B or C may be ascertained by measuring three distances between three C-alpha atoms of three identical residues – e.g. P117 in each chain (G121 is also appropriate).

The three distances form a triangle which is equilateral in apo TNF but distorted when compound is bound. The shortest distance is between BC and the longest between AC (for instance AC=13.8 Å, AB=12.3 Å, BC=10.2 Å); thus looking down through the axis of the molecule with N/C termini pointing towards you the longest distance defines C then A chains going anti-clockwise, then B and C again continuing anti-clockwise.

The invention therefore also provides antibodies that selectively bind to a complex comprising human TNFα and a compound selected from the group consisting of compounds (1)-(6), or salts or solvates thereof. The human TNFα is typically soluble TNFα (TNFαs). The TNFαs may comprise the sequence of SEQ ID NO: 35 or SEQ ID NO: 36, or a variant thereof. Such variants may retain at least about 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:35 or SEQ ID NO: 36 (see above and methods of identifying variants are described below). The TNFα may be trimeric.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An antibody refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or $V_H$) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or $V_L$) and a light chain constant region. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR).

The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.,* effector cells) and the first component (Clq) of the classical complement system.

An antibody of the invention may be a monoclonal antibody or a polyclonal antibody, and will typically be a monoclonal antibody. An antibody of the invention may be a chimeric antibody, a CDR-grafted antibody, a nanobody, a human or humanised antibody or an antigen-binding portion of any thereof. For the production of both monoclonal and polyclonal antibodies, the experimental animal is typically a non-human mammal such as a goat, rabbit, rat or mouse but the antibody may also be raised in other species.

Polyclonal antibodies may be produced by routine methods such as immunisation of a suitable animal, with the antigen of interest. Blood may be subsequently removed from the animal and the IgG fraction purified.

Antibodies generated against compound-trimer complexes of the invention may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, e.g. a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized.

However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.,* 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al.,* Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies of the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by for example the methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93(15): 7843-7848l; WO92/02551; WO2004/051268 and WO2004/106377.

The antibodies of the present invention can also be generated using various phage display methods known in the art and include those disclosed by Brinkman *et al.* (in J. Immunol. Methods, 1995, 182: 41-50), Ames *et al.* (J. Immunol. Methods, 1995, 184:177-186), Kettleborough *et al.* (Eur. J. Immunol. 1994, 24:952-958), Persic *et al.* (Gene, 1997 187 9-18), Burton *et al.* (Advances in Immunology, 1994, 57:191-280) and WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

Fully human antibodies are those antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, but not necessarily from the same antibody. Examples of fully human antibodies may include antibodies produced, for example by the phage display methods described above and antibodies produced by mice in which the murine immunoglobulin variable and optionally the constant region genes have been replaced by their human counterparts e.g. as described in general terms in EP 0546073, US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,661,016, US 5,770,429, EP 0438474 and EP 0463151.

Alternatively, an antibody according to the invention may be produced by a method comprising: immunising a non-human mammal with an immunogen

comprising a compound-trimer complex of a trimeric TNF superfamily member and a compound disclosed herein; obtaining an antibody preparation from said mammal; deriving therefrom monoclonal antibodies that selectively recognise said complex and screening the population of monoclonal antibodies for monoclonal antibodies that bind to the TNF superfamily member only in the presence of the compound.

The antibody molecules of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a fragment or antigen-binding portion thereof. The term "antigen-binding portion" of an antibody refers to one or more fragments of an antibody that retain the ability to selectively bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. The antibodies and fragments and antigen binding portions thereof may be, but are not limited to Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO 2005/003169, WO 2005/003170 and WO 2005/003171 and Fab-dAb fragments described in International patent application WO2009/040562. Multi-valent antibodies may comprise multiple specificities or may be monospecific (see for example WO 92/22853 and WO 05/113605). These antibody fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic

uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required.

An antibody of the invention may be prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.,* a mouse) that is transgenic or transchromosomal for the immunoglobulin genes of interest or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody of interest, *e.g.,* from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

An antibody of the invention may be a human antibody or a humanised antibody. The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

Such a human antibody may be a human monoclonal antibody. Such a human monoclonal antibody may be produced by a hybridoma that includes a B cell obtained from a transgenic nonhuman animal, *e.g.,* a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Human antibodies may be prepared by *in vitro* immunisation of human lymphocytes followed by transformation of the lymphocytes with Epstein-Barr virus.

The term “human antibody derivatives” refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to CDR-grafted antibody molecules in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

As used herein, the term 'CDR-grafted antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine or rat monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al*., 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the CDR-grafted antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs or specificity determining residues described above. Thus, provided in one embodiment is a neutralising CDR-grafted antibody wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al., supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available for

example at: http://www.vbase2.org/ (see Retter *et al*, Nucl. Acids Res. (2005) 33 (supplement 1), D671-D674).

In a CDR-grafted antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a CDR-grafted antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995).

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or surface carries no net electrical charge. In one embodiment the antibody or fragment according to the present disclosure has an

isoelectric point (pI) of at least 7. In one embodiment the antibody or fragment has an isoelectric point of at least 8, such as 8.5, 8.6, 8.7, 8.8 or 9. In one embodiment the pI of the antibody is 8. Programs such as ** ExPASY http://www.expasy.ch/tools/pi_tool.html (see Walker, The Proteomics Protocols Handbook, Humana Press (2005), 571-607), may be used to predict the isoelectric point of the antibody or fragment.

The antibody of the invention may comprise at least one, at least two or all three heavy chain CDR sequences of SEQ ID NOS: 4 to 6 (HCDR1/HCDR2/HCDR3 respectively). These are the HCDR1/HCDR2/HCDR3 sequences of the CA185_01974 antibody of the Examples.

Furthermore, the antibody of the invention may comprise at least one, at least two or all three light chain CDR sequences of SEQ ID NOS: 1 to 3 (LCDR1/LCDR2/LCDR3 respectively). These are the LCDR1/LCDR2/LCDR3 sequences of the CA185_01974 antibody of the Examples.

The antibody of the invention suitably comprises at least a HCDR3 sequence of SEQ ID NO: 6.

Typically, the antibody of the invention comprises at least one heavy chain CDR sequence selected from SEQ ID NOS: 4 to 6 and at least one light chain CDR sequence selected from SEQ ID NOS 1 to 3. The antibody of the invention may comprise at least two heavy chain CDR sequences selected from SEQ ID NOS: 4 to 6 and at least two light chain CDR sequences selected from SEQ ID NOS: 1 to 3. The antibody of the invention typically comprises all three heavy chain CDR sequences of SEQ ID NOS: 4 to 6 (HCDR1/HCDR2/HCDR3 respectively) and all three light chain CDR sequences SEQ ID NOS: 1 to 3 (LCDR1/LCDR2/LCDR3 respectively). The antibodies may be chimeric, human or humanised antibodies.

The antibody of the invention may also comprise at least one, at least two or all three heavy chain CDR sequences of SEQ ID NOS: 19 to 21 (HCDR1/HCDR2/HCDR3 respectively). These are the HCDR1/HCDR2/HCDR3 sequences of the CA185_01979 antibody of the Examples.

The antibody typically comprises a HCDR3 sequence of SEQ ID NO: 21.

The antibody of the invention may also comprise at least one, at least two or all three light chain CDR sequences of SEQ ID NOS: 1, 17, 18

(LCDR1/LCDR2/LCDR3 respectively). These are the LCDR1/LCDR2/LCDR3 sequences of the CA185_01979 antibody of the Examples

Typically, the antibody of the invention comprises at least one heavy chain CDR sequence selected from SEQ ID NOS: 19 to 21 and at least one light chain CDR sequence selected from SEQ ID NOS: 1, 17, 18. The antibody of the invention may comprise at least two heavy chain CDR sequences selected from SEQ ID NOS: 19 to 21 and at least two light chain CDR sequences selected from SEQ ID NOS: 1, 17, 18. The antibody of the invention typically comprises all three heavy chain CDR sequences of SEQ ID NOS: 19 to 21 (HCDR1/HCDR2/HCDR3 respectively) and all three light chain CDR sequences SEQ ID NOS: 1, 17, 18 (LCDR1/LCDR2/LCDR3 respectively). The antibodies may be chimeric, human or humanised antibodies.

The antibody of the invention may comprise any combination of CDR sequences of the CA185_01974 antibody and the CA185_01979 antibody. In particular, the antibody of the invention may comprise least one HCDR sequence selected from SEQ ID NOs: 4-6 and 19-21 and/or at least one LCDR sequence selected from SEQ ID NOs: 1-3, 17 and 18.

The antibody may comprise:

- a HCDR1 selected from SEQ ID NOs: 4 and 19; and/or
- a HCDR2 selected from SEQ ID NOs: 5 and 20; and/or
- a HCDR3 selected from SEQ ID NOs: 6 and 21; and/or
- a LCDR1 of SEQ ID NO: 1; and/or
- a LCDR2 selected from SEQ ID NOs: 2 and 17; and/or
- a LCDR3 selected from SEQ ID NOs: 3 and 18.

The antibody of the invention may comprise a heavy chain variable region (HCVR) sequence of SEQ ID NO: 8 (the HCVR of CA185_01974). The antibody of the invention may comprise a light chain variable region (LCVR) sequence of SEQ ID NO: 7 (the LCVR of CA185_01974). The antibody of the invention suitably comprises the heavy chain variable region sequence of SEQ ID NO: 8 and the light chain variable region sequence of SEQ ID NO: 7.

The antibody of the invention may also comprise a heavy chain variable region (HCVR) sequence of SEQ ID NO: 23 (the HCVR of CA185_01979). The antibody of the invention may comprise a light chain variable region (LCVR) sequence of SEQ ID

NO: 22 (the LCVR of CA185_01979). The antibody of the invention suitably comprises the heavy chain variable region sequence of SEQ ID NO: 23 and the light chain variable region sequence of SEQ ID NO: 22.

Again, the antibody of the invention may comprise a combination of heavy and light chain variable regions from the CA185_01974 and CA185_01979 antibodies. In other words, the antibody of the invention may comprise a heavy chain variable region of SEQ ID NO: 8 or 23 and/or a light chain variable region of SEQ ID NO: 7 or 22.

The antibody of the invention may comprise a heavy chain (H-chain) sequence of SEQ ID NO: 12 (CA185_01974 mIgG1) or 13 (CA185_01974 mFab (no hinge)). The antibody of the invention may comprise a light chain (L-chain) sequence of SEQ ID NO: 11 (CA185_01974 kappa light chain). The antibody of the invention typically comprises the heavy chain sequence of SEQ ID NO: 12/13 and the light chain sequence of SEQ ID NO: 11. The antibodies may be chimeric, human or humanised antibodies.

The antibody of the invention may comprise a heavy chain sequence of SEQ ID NO: 27 (CA185_01979 mIgG1) or 28 (CA185_01979 mFab (no hinge)). The antibody of the invention may comprise a light chain sequence of SEQ ID NO: 26 (CA185_01979 kappa light chain). Generally, the antibody of the invention comprises the heavy chain sequence of SEQ ID NO: 27/28 and the light chain sequence of SEQ ID NO: 26. The antibodies may be chimeric, human or humanised antibodies. Again, sequences from CA185_01974 and CA185_01979 may be combined.

The antibody may alternatively be or may comprise a variant of one of the specific sequences recited above. For example, a variant may be a substitution, deletion or addition variant of any of the above amino acid sequences.

A variant antibody may comprise 1, 2, 3, 4, 5, up to 10, up to 20 or more (typically up to a maximum of 50) amino acid substitutions and/or deletions from the specific sequences discussed above. “Deletion” variants may comprise the deletion of individual amino acids, deletion of small groups of amino acids such as 2, 3, 4 or 5 amino acids, or deletion of larger amino acid regions, such as the deletion of specific amino acid domains or other features. "Substitution" variants typically involve the

replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

| | | | |
|---|---|---|---|
| Ala | aliphatic, hydrophobic, neutral | Met | hydrophobic, neutral |
| Cys | polar, hydrophobic, neutral | Asn | polar, hydrophilic, neutral |
| Asp | polar, hydrophilic, charged (-) | Pro | hydrophobic, neutral |
| Glu | polar, hydrophilic, charged (-) | Gln | polar, hydrophilic, neutral |
| Phe | aromatic, hydrophobic, neutral | Arg | polar, hydrophilic, charged (+) |
| Gly | aliphatic, neutral | Ser | polar, hydrophilic, neutral |
| His | aromatic, polar, hydrophilic, charged (+) | Thr | polar, hydrophilic, neutral |
| Ile | aliphatic, hydrophobic, neutral | Val | aliphatic, hydrophobic, neutral |
| Lys | polar, hydrophilic, charged(+) | Trp | aromatic, hydrophobic, neutral |
| Leu | aliphatic, hydrophobic, neutral | Tyr | aromatic, polar, hydrophobic |

"Derivatives" or "variants" generally include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof. Amino acids used in the sequences may also be derivatized or modified, e.g. labelled, providing the function of the antibody is not significantly adversely affected.

Derivatives and variants as described above may be prepared during synthesis of the antibody or by post- production modification, or when the antibody is in recombinant form using the known techniques of site- directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

Variant antibodies may have an amino acid sequence which has more than about 60%, or more than about 70%, e.g. 75 or 80%, preferably more than about 85%, e.g. more than about 90 or 95% amino acid identity to the amino acid sequences disclosed herein (particularly the HCVR/LCVR sequences and the H- and L-chain sequences). Furthermore, the antibody may be a variant which has more than about

60%, or more than about 70%, e.g. about 75 or 80%, typically more than about 85%, e.g. more than about 90 or 95% amino acid identity to the HCVR/LCVR sequences and the H- and L-chain sequences disclosed herein, whilst retaining the exact CDRs disclosed for these sequences. Variants may retain at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the HCVR/LCVR sequences and to the H- and L-chain sequences disclosed herein (in some circumstances whilst retaining the exact CDRs).

Variants typically retain about 60% - about 99% identity, about 80% - about 99% identity, about 90% - about 99% identity or about 95% - about 99% identity. This level of amino acid identity may be seen across the full length of the relevant SEQ ID NO sequence or over a part of the sequence, such as across about 20, 30, 50, 75, 100, 150, 200 or more amino acids, depending on the size of the full length polypeptide.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson *et al.*, 1994, supra) with the following parameters:

Pairwise alignment parameters -Method: accurate, Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10;

Multiple alignment parameters -Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: GPSNDQEKR. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

The present invention thus provides antibodies having specific sequences and variants which maintain the function or activity of these chains.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain variable regions(s) of an antibody molecule of the present invention. Thus, the present invention provides an isolated DNA sequence of SEQ ID NO: 10, which encodes the heavy chain variable region of SEQ ID NO: 8. The invention also provides an isolated DNA sequence of SEQ ID NO: 9, which encodes the light chain variable region of SEQ ID NO: 7.

The present invention also provides an isolated DNA sequence of SEQ ID NO: 25, which encodes the heavy chain variable region of SEQ ID NO: 23. The invention also provides an isolated DNA sequence of SEQ ID NO: 24, which encodes the light chain variable region of SEQ ID NO: 22.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule of the present invention. Suitably, the DNA sequence encodes the heavy or the light chain of an antibody molecule of the present invention. Thus, the present invention provides an isolated DNA sequence of SEQ ID NO: 15 or 16, which encode the heavy chains of SEQ ID NOs: 12 and 13 respectively. The invention also provides an isolated DNA sequence of SEQ ID NO: 14, which encodes the light chain of SEQ ID NO: 11.

The present invention also provides an isolated DNA sequence of SEQ ID NO: 30 or 31, which encode the heavy chains of SEQ ID NOs: 27 and 28 respectively. The invention also provides an isolated DNA sequence of SEQ ID NO: 29, which encodes the light chain of SEQ ID NO: 26.

A suitable polynucleotide sequence may alternatively be a variant of one of these specific polynucleotide sequences. For example, a variant may be a substitution, deletion or addition variant of any of the above nucleic acid sequences. A variant polynucleotide may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40, up to 50, up to 75 or more nucleic acid substitutions and/or deletions from the sequences given in the sequence listing. Generally, a variant has 1– 20, 1-50, 1-75 or 1-100 substitutions and/or deletions.

Suitable variants may be at least about70% homologous to a polynucleotide of any one of nucleic acid sequences disclosed herein, typically at least about 80 or 90% and more suitablyat least about 95%, 97% or 99% homologous thereto. Variants may retain at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity. Variants typically retain about 60% - about 99% identity, about 80% - about 99% identity, about 90% - about 99% identity or about 95% - about 99% identity. Homology and identity at these levels is generally present at least with respect to the coding regions of the polynucleotides. Methods of measuring homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of nucleic acid identity. Such homology

may exist over a region of at least about 15, at least about 30, for instance at least about 40, 60, 100, 200 or more contiguous nucleotides (depending on the length). Such homology may exist over the entire length of the unmodified polynucleotide sequence.

Methods of measuring polynucleotide homology or identity are known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (e.g. used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, p387-395).

The PILEUP and BLAST algorithms can also be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S.F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89:10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the

smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, typically less than about 0.1, suitably less than about 0.01, and most suitably less than about 0.001. For example, the smallest sum probability may be in the range of about 1 - about 0.001, often about 0.01 - about 0.001.

The homologue may differ from a sequence in the relevant polynucleotide by less than about 3, 5, 10, 15, 20 or more mutations (each of which may be a substitution, deletion or insertion). For example, the homologue may differ by 3-50 mutations, often 3-20 mutations. These mutations may be measured over a region of at least 30, for instance at least about 40, 60 or 100 or more contiguous nucleotides of the homologue.

In one embodiment, a variant sequence may vary from the specific sequences given in the sequence listing by virtue of the redundancy in the genetic code. The DNA code has 4 primary nucleic acid residues (A, T, C and G) and uses these to "spell" three letter codons which represent the amino acids the proteins encoded in an organism's genes. The linear sequence of codons along the DNA molecule is translated into the linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly degenerate, with 61 codons coding for the 20 natural amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons. A variant polynucleotide of the invention may therefore encode the same polypeptide sequence as another polynucleotide of the invention, but may have a different nucleic acid sequence due to the use of different codons to encode the same amino acids.

The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be

synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

Screening methods as described herein may be used to identify suitable antibodies that are capable of binding to a compound-trimer complex. Thus, the screening methods described herein may be carried out to test antibodies of interest.

Antibodies of the invention can be tested for binding to a compound-trimer complex by, for example, standard ELISA or Western blotting. An ELISA assay can also be used to screen for hybridomas that show positive reactivity with the target protein. The binding selectivity of an antibody may also be determined by monitoring binding of the antibody to cells expressing the target protein, for example by flow cytometry. Thus, a screening method of the invention may comprise the step of identifying an antibody that is capable of binding a compound-trimer complex by carrying out an ELISA or Western blot or by flow cytometry.

Antibodies of the invention selectively (or specifically) recognise at least one compound-trimer complex, i.e. epitopes within a compound-trimer complex. An

antibody, or other compound, "selectively binds" or "selectively recognises" a protein when it binds with preferential or high affinity to the protein for which it is selective but does not substantially bind, or binds with low affinity, to other proteins. The selectivity of an antibody of the invention for a target a compound-trimer complex may be further studied by determining whether or not the antibody binds to other related compound-trimer complexes as discussed above or whether it discriminates between them.

An antibody of the invention may bind specifically (or selectively) to compound-trimer complexes comprising the trimeric forms of one or more TNF superfamily members. For example, an antibody may bind to compound-trimer complexes comprising TNFα, compound-trimer complexes comprising TNFβ and compound-trimer complexes comprising CD40L. Alternatively, an antibody may bind specifically (or selectively) to compound-trimer complexes comprising only one of the TNF superfamily members, but not to compound-trimer complexes comprising any other TNF superfamily members. For example, an antibody may bind to compound-trimer complexes comprising TNFα, but not to compound-trimer complexes comprising TNFβ or compound-trimer complexes comprising CD40L. An antibody may bind specifically (or selectively) to compound-trimer complexes comprising up to two, three, four or up to all of the TNF superfamily members.

By specific (or selective), it will be understood that the antibody binds to the compound-trimer complexes of interest with no significant cross-reactivity to any other molecule, which may include test compounds in the absence of a TNF superfamily trimer or TNF superfamily member trimers in the absence of a test compound. Cross-reactivity may be assessed by any suitable method described herein. Cross-reactivity of an antibody for a compound-trimer complex with a molecule other than the compound-trimer complex may be considered significant if the antibody binds to the other molecule at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 100% as strongly as it binds to the compound-trimer complex of interest. An antibody that is specific (or selective) for the compound-trimer complex may bind to another molecule at less than about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25% or 20% the strength that it binds to the compound-trimer complex.

The antibody may bind to the other molecule at less than about 20%, less than about 15%, less than about 10% or less than about 5%, less than about 2% or less than about 1% the strength that it binds to the compound-trimer complex. The antibody specifically (or selectively) binds to a compound-trimer complex compared with (i) the trimeric form of the TNF superfamily member in the absence of the compound and/or (ii) the compound in the absence of the TNF superfamily member trimer.

The rates at which an antibody binds to a compound-trimer complex is referred to herein as the "*on*" rate" $k_{on\text{-}ab}$ and the rate at which the antibody dissociates from the compound-trimer complex is referred to herein as the "*off*" rate or $k_{off\text{-}ab}$. As used herein, the symbol "$K_{D\text{-}ab}$" denotes the binding affinity (dissociation constant) of an antibody for a compound-trimer complex. $K_{D\text{-}ab}$ is defined as $k_{off\text{-}ab}/k_{on\text{-}ab}$. Antibodies may have slow "*on*" rates, which can be measured in minutes by mass spectral analysis of the compound-trimer complex and antibody peak intensities. $K_{D\text{-}ab}$ values for an antibody can be estimated by repeating this measurement at different antibody: compound-trimer complex ratios.

The $K_{D\text{-}ab}$ value of the antibody for binding to a compound-trimer complex may be at least about 1.5 times, 2 times, 3 times, 4 times, 5 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times or 400 times lower, or lower, than the $K_{D\text{-}ab}$ value of the antibody for binding to the trimeric TNF superfamily member in the absence of the compound and/or the $K_{D\text{-}ab}$ value of the antibody for binding to the compound in the absence of the trimeric TNF superfamily member. The $K_{D\text{-}ab}$ value of the antibody for binding to a compound-trimer complex may be decreased at least about 10 times, at least about 100 times, at least about 200 times, at least about 300 times the $K_{D\text{-}ab}$ value of the TNF superfamily trimer binding to the TNF superfamily receptor in the absence of the test compound, i.e. the binding affinity of the antibody for the compound-trimer complex is typically increased at least about 10-fold, suitably at least about 100-fold, more suitably at least about 200-fold, most suitably at least about 300-fold compared to the binding affinity of the antibody to the trimeric TNF superfamily member in the absence of the compound and/or the binding affinity of the antibody to the compound in the absence of the trimeric TNF superfamily member.

The binding affinity may be given in terms of binding affinities ($K_{D\text{-}ab}$) and

may be given in any appropriate units, such as μM, nM or pM. The smaller the $K_{D\text{-}ab}$ value, the larger the binding affinity of the antibody to the compound-trimer complex.

The $K_{D\text{-}ab}$ value of the antibody for binding to the compound-trimer complex may be at least about 1.5 times, 2 times, 3 times, 4 times, 5 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times lower, or even lower than the $K_{D\text{-}ab}$ value of the antibody for binding to the trimeric TNF superfamily member in the absence of the compound and/or the $K_{D\text{-}ab}$ value of the antibody for binding to the compound in the absence of the trimeric TNF superfamily member.

The decrease in the $K_{D\text{-}ab}$ value of the antibody for binding to the compound-trimer complex compared to the $K_{D\text{-}ab}$ value of the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the $K_{D\text{-}ab}$ value of the antibody for binding to the compound in the absence of the trimeric TNF superfamily member may result from an increase in the on rate ($k_{on\text{-}ab}$) of the antibody binding to the compound-trimer complex compared to the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member; and/or a decrease in the off rate ($k_{off\text{-}ab}$) compared to the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member.

The on rate ($k_{on\text{-}ab}$) of the antibody binding to the compound-trimer complex is generally increased compared to the on rate of the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member. The off rate ($k_{off\text{-}ab}$) of the antibody binding to the compound-trimer complex is generally decreased compared to the off rate of the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member. Most typically, the on rate ($k_{on\text{-}ab}$) of the antibody binding to the compound-trimer complex is increased, and the off-rate ($k_{off\text{-}ab}$) of the antibody binding to the compound-trimer complex is decreased, compared to the antibody binding to the

trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member.

The $k_{on-ab}$ value of the antibody binding to the compound-trimer complex may be increased by at least about 1.5-fold or at least two-fold and typically at least about three fold compared to the $k_{on-ab}$ value of the antibody binding to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member and/or the $k_{off-ab}$ value of the antibody binding to the compound-trimer complex may be decreased by at least about two-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold more suitablyat least about 90-fold compared to the $k_{off-ab}$ value of the antibody binding to to the trimeric TNF superfamily member in the absence of the compound and/or the antibody binding to the compound in the absence of the trimeric TNF superfamily member.

The $k_{on-ab}$, $k_{off-ab}$, and $K_{D-ab}$ values may be determined using any appropriate technique, for example surface plasmon resonance, mass spectrometry and isothermal calorimetry.

The $K_{D-ab}$ value of the antibody binding to a compound-trimer complex may be 1 nM, 900 pM, 700 pM, 500 pM, 100 pM, 10 pM or less (typically down to about 1 pM). Antibodies of the invention will desirably bind to the compound-trimer complexes of the invention with high affinity, for example in the picomolar range. The $K_{D-ab}$ value of the antibody binding to a compound-trimer complex may be 1 nM or less, 900 pM or less, 700 pM or less, 500 pM or less, 400 pM or less, 300 pM or less, 200 pM or less, 100 pM or less, 90 pM or less, 80 pM or less, 70 pM or less, 60 pM or less, 50 pM or less, 40 pM or less, 30 pM or less, 20 pM or less, 10 pM or less (again, down to about 1 pM).

Once a suitable antibody has been identified and selected, the amino acid sequence of the antibody may be identified by methods known in the art. The genes encoding the antibody can be cloned using degenerate primers. The antibody may be recombinantly produced by routine methods.

Antibodies of the invention may compete for binding to TNFα with, or bind to

the same epitope as, those defined above in terms of H-chain/L-chain, HCVR/LCVR or CDR sequences. In particular, an antibody may compete for binding to TNFα with, or bind to the same epitope as, an antibody which comprises a HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequence combination of SEQ ID NOs: 4/5/6/1/2/3 or SEQ ID NOs: 19/20/21/1/17/18. An antibody may compete for binding to TNFα with, or bind to the same epitope as, an antibody which comprises a HCVR and LCVR sequence pair of SEQ ID NOs: 8/7 or SEQ ID NOs: 23/22.

The term "epitope" is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference antibody of the invention, the reference antibody is allowed to bind to a protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the protein or peptide is assessed. If the test antibody is able to bind to the protein or peptide following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the reference antibody. On the other hand, if the test antibody is not able to bind to protein or peptide following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference antibody of the invention.

To determine if an antibody competes for binding with a reference antibody, the above-described binding methodology is performed in two orientations. In a first orientation, the reference antibody is allowed to bind to a protein/peptide under saturating conditions followed by assessment of binding of the test antibody to the

protein/peptide molecule. In a second orientation, the test antibody is allowed to bind to the protein/peptide under saturating conditions followed by assessment of binding of the reference antibody to the protein/peptide. If, in both orientations, only the first (saturating) antibody is capable of binding to the protein/peptide, then it is concluded that the test antibody and the reference antibody compete for binding to the protein/peptide. As will be appreciated by the skilled person, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50%, 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res, 1990:50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (e.g., peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

The antibodies of the invention may be used to identify compounds of the invention as described herein. The antibodies of the invention may also be used as target engagement biomarkers. A target engagement biomarker can be used to detect the engagement, i.e. the binding of a ligand to a target of interest. In the present case, the antibodies of the invention only bind to the complexes of compounds of the invention with trimeric forms of TNF superfamily members. Therefore, if an antibody of the invention is able to bind to a compound-trimer complex, this is

evidence that the ligand (compound) has bound to the target of interest (TNF superfamily member trimer). Antibodies of the invention can be modified to add a detectable marker as described herein. Therefore, engagement of a compound of the invention with a target TNF superfamily member may be detected using such an antibody.

The use of antibodies of the invention as target engagement biomarkers is potentially useful in a clinical or pre-clinical environment, where a sample may be taken from a subject being treated according to the present invention. The sample obtained from the subject may be treated with an antibody of the invention in order to determine whether the compound used to treat the subject has bound to the target TNF superfamily member. The sample obtained from the subject may be any appropriate tissue or fluid, such as blood, plasma or urine. The subject may be mammalian, typically human.

Accordingly, the invention provides the use of an antibody of the invention as a target engagement biomarker for the detection of a compound-trimer complex comprising a trimeric protein that is a TNF superfamily member and a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor in a sample obtained from a subject. The superfamily member is suitably TNFα and/or the modulation is antagonism of TNFR1 signalling.

Similarly, the present invention provides a method of detecting target engagement of a compound to a trimeric TNF superfamily member, whereby the compound-trimer complex binds to the requisite receptor and modulates the signalling induced by the trimer through the receptor, said method comprising:

(a) obtaining a sample from a subject administered said compound;

(b) contacting an antibody of the invention to said sample and a control sample, wherein said antibody is detectable;

(c) determining the amount of binding of said detectable antibody to said sample and said control sample,

wherein binding of said detectable antibody to said sample greater than binding of said detectable antibody to said control sample indicates target engagement of said

compound to said trimeric TNF superfamily member.

Methods of detecting antibodies, and measuring the amount of binding of an antibody to a target, are well known in the art. Typically, antibodies can be labelled. Such labels include enzymes, biotin/streptavidin, fluorescent proteins and fluorescent dyes.

Binding of an antibody to a target may be measured, for example, by an immunoassay method. Immunoassays include Western Blotting, ELISA, immunofluorescence, immunohistochemistry and flow cytometry. Any appropriate technique may be used to measure binding of the antibody to the TNF superfamily member.

In the method described above, binding of the detectable antibody to the sample from a subject who has been administered the compound is compared with binding of the antibody to a control sample. The control sample may be any appropriate sample. The control sample is typically a "negative control" which is representative of binding of the antibody to the TNF superfamily member in the absence of the compound. For example, the sample may be obtained from the patient prior to administration of the compound. The control may also be based on previously determined measurements e.g. from a number of samples from different subjects in the absence of compound. Measurements from about 5, 10, 20, 50 or 100 subjects may be used in determining the control value. The control may be an average value, or a range of all the values obtained.

The experimental conditions e.g. methods of detection are the same for the sample from a subject administered the compound, and for the control sample. The antibody is also the same in both cases.

Greater binding (increased binding) of the detectable antibody to the sample from the patient administered the compound compared with binding of the antibody to the control sample is indicative of target engagement of the compound to the trimeic TNF superfamily member. In other words, equivalent or lower binding (decreased binding) for the sample from the patient administered the compound relative to the control indicates that there is no target engagement of said compound. In other words, no significant difference in the two amounts indicates that there is no target engagement.

The skilled person can readily determine when there is increased binding relative to the control. For example when the control is a range of data, target engagement may be determined based on the spread of the data, the difference between the control data and the detected binding of the antibody in the sample in question, and calculated confidence levels. It is also possible to identify target engagement when the detected binding for the sample in question is higher than the maximum amount of binding detected in any negative control.

Target engagement may be detected if binding of the antibody is increased by about 30% or more relative to the highest amount in the control range. Target engagement may also be detected if binding of the antibody is increased by about 40% or more, or about 50% or more relative to the control range. The same applies when the control is an average value, or a single value based on a sample from the patient prior to administration of the compound. There is of course no upper limit to the percentage increase relative to the control.

An antibody of the invention may be used to screen for a compound that elicits a conformational change in a trimeric TNF superfamily member, wherein said conformational change modulates the signalling of the requisite TNF superfamily receptor on binding of the trimeric TNF superfamily member. The superfamily member is typically TNFα and/or the modulation is antagonism of TNFR1 signalling.

The antibodies of the present invention may be used in the treatment and/or prophylaxis of a pathological condition. Accordingly, provided is an antibody of the invention for use in a method of therapy practiced on the human or animal body. The invention also provides a method of therapy comprising the administration of an antibody of the invention to a subject. The antibody of the invention may be used in any therapeutic indication and/or pharmaceutical composition described herein.

*Antibody assays*

As described herein, the present invention provides antibodies that selectively bind to at least one compound-trimer complex described herein relative to their binding to the compound alone or to the TNF superfamily member in the absence of the compound. These antibodies may be used to identify further compounds or classes of compounds having the same properties.

Monoclonal antibodies may be generated against a TNF superfamily member using the standard techniques described herein. These anti-TNF superfamily member antibodies can then be screened for antibodies that bind to compound-trimer complexes of the invention, or for monoclonal antibodies for which binding to the TNF superfamily member is inhibited by compounds as described herein.

Alternatively, monoclonal antibodies can be generated against particular TNF superfamily member trimer-compound complexes. These antibodies can then be screened for monoclonal antibodies that selectively bind to the TNF superfamily member in the presence of the compound relative to their binding to the TNF superfamily member in the absence of the compound.

Once an antibody that selectively binds to at least one compound-trimer complex of the invention relative to its binding to the compound alone or to the TNF superfamily member in the absence of the compound has been generated, it can be used to screen for other compounds possessing the same activity as the test compounds.

Accordingly, the invention provides an assay for identifying a compound of the invention comprising the steps of:

a) performing a binding assay to measure the binding affinity of a test compound-trimer complex to an antibody of the invention;

b) comparing the binding affinity as measured in step (a) with the binding affinity of a different compound-trimer complex known to bind with high affinity to the antibody referred to in step (a); and

c) selecting the compound present in the compound-trimer complex of step (a) if its measured binding affinity is acceptable when considered in the light of the comparison referred to in step (b).

As will be appreciated, the "different" compound-trimer complex referred to in step (b) above will generally be a complex containing the same trimer as the compound-trimer complex of step (a), but a different compound. The compound may be any of compounds (1)-(6).

By "acceptable" in step (c) is meant that the binding affinity of the compound-trimer complex referred to in step (a) and the binding affinity of the different compound-trimer complex referred to in step (b) are approximately comparable.

Selective binding of said antibody to said complex is typically measured relative to the binding of said antibody to the TNF superfamily member in the absence of the compound or to the compound in the absence of the TNF superfamily member.

The binding affinity of the compound-trimer complex referred to in step (a) will generally be superior to the binding affinity of the different compound-trimer complex referred to in step (b). Suitably, the difference in the binding affinity of the compound-trimer complex referred to in step (a) relative to the binding affinity of the different compound-trimer complex referred to in step (b) will be within limits of 10-fold, 20-fold, 50-fold, 100-fold, 200-fold or 500-fold.

Libraries of compounds can be assayed using the antibodies of the invention. The library compounds can be incubated with said antibody in the presence and absence of a TNF superfamily member. A compound that forms part of a compound-trimer complex that binds to an antibody of the invention only in the presence of both the TNF superfamily member and the compound is a likely candidate to have the same activity as the compounds described herein. The assays disclosed herein may then be used to verify whether the test compound is a compound as described herein.

One or more of the antibodies of the invention may be used in the assay. A generic antibody that is capable of binding to complexes of any compound of the invention with a particular TNF superfamily member may be used in the antibody assay of the invention.

A panel of multiple antibodies of the present invention that are specific for different compound-trimer complexes may be used in the antibody assay of the invention. The panel of antibodies may include at least 5, at least 10, at least 15, at least 20, at least 30, at least 40 or at least 50 antibodies (for example up to 75 antibodies).

The antibody assay of the present invention may be a high throughput assay that is capable of screening a large number of test compounds over a short space of time to identify compounds of the present invention.

The TNF superfamily members and their receptors may be purified or present in mixtures, such as in cultured cells, tissue samples, body fluids or culture medium. Assays may be developed that are qualitative or quantitative, with the latter being useful for determining the binding parameters (affinity constants and kinetics) of the

test compound to trimeric forms of TNF superfamily members, and also of the binding parameters of the compound-trimer complex to the requisite TNF receptor.

The sample comprising the TNF superfamily member and the compound may further comprise a destabilising agent. Destabilising agents, also known as chaotropes, include low molar concentrations (e.g. 1M) of urea, guanidine or acetonitrile, high concentrations (e.g. 6M or higher) of these reagents will result in complete dissociation of the TNFα trimer and unfolding of the constituent TNFα monomeric subunits. The destabilising agent may be DMSO, typically at a concentration of 5%, 10% or higher.

The test compounds may have any/all of the properties discussed above.

*TNF Superfamily and their Receptors*

There are 22 TNF superfamily members currently known: TNFα (TNFSF1A), TNFβ (TNFSF1B), CD40L (TNFSF5), BAFF (TNFSF13B/BlyS), APRIL (TNFSF13), OX40L (TNFSF4), RANKL (TNFSF11/TRANCE), TWEAK (TNFSF12), TRAIL (TNFSF10), TL1A (TNFSF15), LIGHT (TNFSF14), Lymphotoxin, Lymphotoxin β (TNFSF3), 4-1BBL (TNFSF9), CD27L (TNFSF7), CD30L (TNFSF8), EDA (Ectodysplasin), EDA-A1 (Ectodysplasin A1), EDA-A2 (Ectodysplasin A2), FASL (TNFSF6), NGF and GITRL (TNFSF18).

The TNF superfamily member is typically TNFα. TNFα exists in both a soluble ($TNF\alpha_s$) and membrane-bound form ($TNF\alpha_m$). When TNFα is referred to herein this encompasses both the $TNF\alpha_s$ and $TNF\alpha_m$ forms. TNFα is most suitably in the $TNF\alpha_s$ form. The $TNF\alpha_s$ may comprise the sequence of SEQ ID NO: 35 or SEQ ID NO: 36, or a variant thereof (as described above).

The assays of the invention may be used to identify modulators of at least one of any TNF superfamily members, including the 22 known TNF superfamily members. Specifically, the assays of the invention may be used to identify compounds that bind to any TNF superfamily member, particularly to trimeric forms of TNF superfamily members, and that stabilise these trimers in a conformation that is capable of binding to the requisite TNF receptor, and which modulate signalling through said receptor. The assay of the invention is, in particular, used to identify modulators of TNFα or CD40L, especially TNFα, or even $TNF\alpha_s$.

The compound described herein may be a modulator of at least one of any TNF superfamily members, including the 22 known TNF superfamily members. In particular, the TNF superfamily member is TNFα or CD40L, especially TNFα or even $TNF\alpha_s$.

The compound-trimer complex of the invention may include the trimeric form of any TNF superfamily member, including the 22 known TNF superfamily members. The TNF superfamily member is typically TNFα or CD40L. The TNF superfamily member may be TNFα, most suitably $TNF\alpha_s$.

Members of the TNF superfamily bind to, and initiate signalling through TNF receptors. There are currently 34 known TNF receptors: 4-1BB (TNFRSF9/CD137), NGF R (TNFRSF16), BAFF R (TNFRSF13C), Osteoprotegerin (TNFRSF11B), BCMA (TNFRSF17), OX40 (TNFRSF4), CD27 (TNFRSF7), RANK (TNFRSF11A), CD30 (TNFRSF8), RELT (TNFRSF19L), CD40 (TNFRSF5), TACI (TNFRSF13B), DcR3 (TNFRSF6B), TNFRH3 (TNFRSF26), DcTRAIL R1 (TNFRSF23), DcTRAIL R2 (TNFRSF22), TNF-R1 (TNFRSF1A), TNF-R2 (TNFRSF1B), DR3 (TNFRSF25), TRAIL R1 (TNFRSF10A), DR6 (TNFRSF21), TRAIL R2 (TNFRSF10B), EDAR, TRAIL R3 (TNFRSF10C), Fas (TNFRSF6/CD95), TRAIL R4 (TNFRSF10D), GITR (TNFRSF18), TROY (TNFRSF19), HVEM (TNFRSF14), TWEAK R (TNFRSF12A), TRAMP (TNFRSF25), Lymphotoxin β R (TNFRSF3) and XEDAR.

The TNF receptor is suitably TNF-R1 (TNFR1) or TNF-R2 (TNFR2). When TNF-R is referred to herein this encompasses both TNF-R1 and TNF-R2, including the extracellular domain (ECD) of TNF-R1 and TNF-R2. The assays of the invention may be used to identify compounds that modulate the signalling of TNF superfamily members through any requisite TNF superfamily receptor. The assays of the invention may be used to identify compounds that modulate the signalling of TNF superfamily members through TNF-R1, TNF-R2 or CD40. The TNF superfamily member may be TNFα and the TNF receptor may be TNF-R1 or TNF-R2. In particular, the TNF superfamily member may be TNFα and the TNF receptor may be TNF-R1. More particularly, the TNF superfamily member msy be $TNF\alpha_s$ and the TNF receptor may be TNF-R1. The assays of the invention may be used to identify compounds which act by specifically modulate the signalling of TNF superfamily members through TNF-R1. In particular, the compounds may act by modulating the

signalling of TNF superfamily members through TNF-R1, but have no effect on signalling of TNF superfamily members through TNF-R2.

The compound-trimer complex of the invention may modulate TNF superfamily members signalling through at least one TNF receptor, including the 34 known TNF receptors. The TNF receptor is typically TNF-R1, TNF-R2 or CD40L.

In particular, the TNF superfamily member is TNFα and the TNF receptor is TNF-R1 or TNF-R2. The TNF superfamily member is more suitably TNFα and the TNF receptor is TNF-R1. Most suitably, the TNF superfamily member is $TNF\alpha_s$ and the TNF receptor is TNF-R1.

*Therapeutic Indications*

TNFα is the archetypal member of the TNF superfamily. TNFα is a pleiotropic cytokine that mediates immune regulation and inflammatory responses. *In vivo*, TNFα is also known to be involved in responses to bacterial, parasitic and viral infections. In particular, TNFα is known to have a role in rheumatoid arthritis (RA), inflammatory bowel diseases (including Crohn's disease), psoriasis, Alzheimer's disease (AD), Parkinson's disease (PD), pain, epilepsy, osteoporosis, asthma, sepsis, fever, Systemic lupus erythematosus (SLE) and Multiple Sclerosis (MS) and cancer. TNFα is also known to have a role in Amyotrophic Lateral Sclerosis (ALS), ischemic stroke, immune complex-mediated glomerulonephritis, lupus nephritis (LN), antineutrophil cytoplasmic antibodies (ANCA-) associated glomerulonephritis, minimal change disease, diabetic nephropathy (DN), acute kidney injury (AKI), obstructive uropathy, kidney allograft rejection, cisplatin-induced AKI and obstructive uropathy.

Other members of the TNF superfamily are known to be involved in autoimmune disease and immune deficiencies. In particular, members of the TNF superfamily are known to be involved in RA, SLE, cancer, MS, asthma, rhinitis, osteoporosis and multiple myeloma (MM). TL1A is known to play a role in organ transplant rejection.

A compound described herein may be used to treat, prevent or ameliorate any condition that that can be treated, prevented or ameliorated by a conventional TNF superfamily member modulator. The compound may be used alone or in combination

with a conventional TNF superfamily member modulator. Any condition that results, partially or wholly, from pathogenic signalling through a TNF receptor by a TNF superfamily member or from a deficiency in signalling through a TNF receptor by a TNF superfamily member may in principle be treated, prevented or ameliorated according to the present invention. Pathogenic signalling through a TNF receptor by a TNF superfamily member includes increased signalling through a TNF receptor over and above the normal physiological level of signalling, signalling through a TNF receptor which is initiated normally, but which fails to stop in response to normal physiological signals and signalling through a TNF receptor that is within the normal physiological range of magnitude, but which is initiated by non-physiological means. In a preferred embodiment, the invention relates to the treatment, prevention or amelioration of conditions mediated or influenced by TNFα or CD40L.

The compounds that interact with TNFα are accordingly beneficial in the treatment and/or prevention of various human ailments. These include autoimmune and inflammatory disorders; neurological and neurodegenerative disorders; pain and nociceptive disorders; and cardiovascular disorders.

Inflammatory and autoimmune disorders include systemic autoimmune disorders, autoimmune endocrine disorders and organ-specific autoimmune disorders. Systemic autoimmune disorders include systemic lupus erythematosus (SLE), psoriasis, vasculitis, polymyositis, scleroderma, multiple sclerosis, ankylosing spondylitis, rheumatoid arthritis and Sjögren's syndrome. Autoimmune endocrine disorders include thyroiditis. Organ-specific autoimmune disorders include Addison's disease, haemolytic or pernicious anaemia, glomerulonephritis (including Goodpasture's syndrome), Graves' disease, idiopathic thrombocytopenic purpura, insulin-dependent diabetes mellitus, juvenile diabetes, uveitis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), pemphigus, atopic dermatitis, autoimmune hepatitis, primary biliary cirrhosis, autoimmune pneumonitis, autoimmune carditis, myasthenia gravis, spontaneous infertility, osteoporosis, asthma and muscular dystrophy (including Duchenne muscular dystrophy).

Neurological and neurodegenerative disorders include Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, amyotrophic lateral sclerosis, spinal cord injury, head trauma, seizures and epilepsy.

Cardiovascular disorders include thrombosis, cardiac hypertrophy, hypertension, irregular contractility of the heart (e.g. during heart failure), and sexual disorders (including erectile dysfunction and female sexual dysfunction).

In particular, a compound may be used to treat or prevent inflammatory disorders, CNS disorders, immune disorders and autoimmune diseases, pain, osteoporosis, fever and organ transplant rejection. A compound may be used to treat or prevent rheumatoid arthritis, inflammatory bowel diseases (including Crohn's disease), psoriasis, Alzheimer's disease, Parkinson's disease, epilepsy, asthma, sepsis, systemic lupus erythematosus, multiple sclerosis, asthma, rhinitis, cancer and osteoporosis. A compound may be used to treat or prevent rheumatoid arthritis (RA), non specific inflammatory arthritis, erosive bone disease, chondritis, cartilage degeneration and/or destruction, juvenile inflammatory arthritis, Still's Disease (juvenile and/or adult onset), juvenile idiopathic arthritis, juvenile idiopathic arthritis (both oligoarticular and polyarticular forms), inflammatory bowel diseases (including Crohn's disease, ulcerative colitis, indeterminate colitis, pouchitis), psoriasis, psoriatic arthopathy, ankylosing spondylitis, Sjogren's Disease, Alzheimer's disease (AD), Behcet's Disease, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), ischemic stroke, pain, epilepsy, osteoporosis, osteopenia, anaemia of chronic disease, cachexia, diabetes, dyslipidemia, metabolic syndrome, asthma, chronic obstructive airways (or pulmonary) disease, sepsis, fever, respiratory distress syndrome, systemic lupus erythematosus (SLE), multiple sclerosis (MS) immune complex-mediated glomerulonephritis, lupus nephritis (LN), antineutrophil cytoplasmic antibodies (ANCA-) associated glomerulonephritis, minimal change disease, diabetic nephropathy (DN), acute kidney injury (AKI), obstructive uropathy, kidney allograft rejection, cisplatin-induced AKI and obstructive uropathy, eye diseases (including diabetic retinopathy, diabetic macular oedema, retinopathy of prematurity, age related macular degeneration, macular oedema, proliferative and/or non proliferative retinopathy, corneal vascularisation including neovascularization, retinal vein occlusion, various forms of uveitis and keratitis), thryoiditis, fibrosing disorders including various forms of hepatic fibrosis, various forms of pulmonary fibrosis, systemic sclerosis, scleroderma, cancer and cancer associated complications (including skeletal complications, cachexia and anaemia).

As discussed above, antibodies of the present invention may be used as target engagement biomarkers to assess the effectiveness of treatment with a compound or complex as described herein. In one embodiment, a sample taken from a subject treated with a compound or complex described herein may be contacted with an antibody of the invention. The antibody may then be used to determine the amount of TNF superfamily member-compound complex present within the sample. The amount of complex determined using the antibody may be related to the effectiveness of the treatment. For example, the more complex detected by the antibody of the invention, the more effective the treatment. The amount of complex determined using the antibody is directly proportional to the effectiveness of the treatment. For example, doubling the amount of complex determined using the antibody may be indicative of a doubling of the effectiveness of the treatment.

An antibody of the invention may be used to determine the amount of compound-trimer complex using any appropriate technique. Standard techniques are known in the art and are disclosed herein. For example, ELISA and Western blotting with an antibody of the invention may be used to determine the amount of compound-trimer complex.

The amount of the compound-trimer complex may be determined by measuring the mass of the compound-trimer complex, the concentration of the compound-trimer complex, and the molarity of the compound-trimer complex. This amount may be given in any appropriate units. For example, the concentration of the compound-trimer complex may be given in pg/ml, ng/ml or μg/ml. The mass of the compound-trimer complex may be given in pg, ng or μg.

The amount of the compound-trimer complex in a sample of interest may be compared with the level of the compound-trimer complex in another sample, such as a control sample, as described herein. In such a method, the actual amount of the compound-trimer complex, such as the mass, molar amount, concentration or molarity of the compound-trimer complex in the samples may be assessed. The amount of the compound-trimer complex may be compared with that in another sample without quantifying the mass, molar amount, concentration or molarity of the compound-trimer complex. Thus, the amount of the compound-trimer complex in a sample according to the invention may be assessed as a relative amount, such as a relative

mass, relative molar amount, relative concentration or relative molarity of the compound-trimer complex based on a comparison between two or more samples.

*Pharmaceutical Compositions, Dosages and Dosage Regimes*

An antibody, compound or complex of the invention may be provided in a pharmaceutical composition. The pharmaceutical composition that will normally be sterile and will typically include a pharmaceutically acceptable carrier and/or adjuvant. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically acceptable adjuvant and/or carrier.

As used herein, "*pharmaceutically acceptable carrier*" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier may be suitable for parenteral, e.g. intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Alternatively, the carrier may be suitable for non-parenteral administration, such as a topical, epidermal or mucosal route of administration. The carrier may be suitable for oral administration. Depending on the route of administration, the modulator may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compositions of the invention may include one or more pharmaceutically acceptable salts. A "*pharmaceutically acceptable salt*" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include acid addition salts and base addition salts.

Pharmaceutically acceptable carriers comprise aqueous carriers or diluents. Examples of suitable aqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, buffered water and saline. Examples of other carriers include ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. In many cases, it will be desirable to include isotonic agents, for example, sugars, polyalcohols such as

mannitol, sorbitol, or sodium chloride in the composition.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

Pharmaceutical compositions of the invention may comprise additional active ingredients.

Also within the scope of the present invention are kits comprising antibodies, compounds and/or complexes of the invention and instructions for use. The kit may further contain one or more additional reagents, such as an additional therapeutic or prophylactic agent as discussed above.

The compounds identified by the methods and/or antibodies of the invention and the antibodies of the present invention or formulations or compositions thereof may be administered for prophylactic and/or therapeutic treatments.

In therapeutic applicationscompounds are administered to a subject already suffering from a disorder or condition as described above, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an increase in frequency or duration of symptom-free periods. An amount adequate to accomplish this is defined as a "*therapeutically effective amount*".

In prophylactic applications, formulations are administered to a subject at risk of a disorder or condition as described above, in an amount sufficient to prevent or reduce the subsequent effects of the condition or one or more of its symptoms. An amount adequate to accomplish this is defined as a "*prophylactically effective amount*". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject.

A subject for administration may be a human or non-human animal. The term "*non-human animal*" includes all vertebrates, *e.g.,* mammals and non-mammals, such as non-human primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. Administration to humans is typical.

A compound or pharmaceutical composition of the invention may be administered via one or more routes of administration using one or more of a variety

of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Examples of routes of administration for compounds or pharmaceutical compositions of the invention include intravenous, intramuscular, intradermal, intraocular, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "*parenteral administration*" as used herein means modes of administration other than enteral and topical administration, usually by injection. Alternatively, compound or pharmaceutical composition of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration. The compound or pharmaceutical composition of the invention may be for oral administration.

A suitable dosage of a compound or pharmaceutical composition of the invention may be determined by a skilled medical practitioner. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A suitable dose may be, for example, in the range of from about 0.01μg/kg to about 1000mg/kg body weight, typically from about 0.1μg/kg to about 100mg/kg body weight, of the patient to be treated. For example, a suitable dosage may be from about 1μg/kg to about 10mg/kg body weight per day or from about 10 μg/kg to about 5 mg/kg body weight per day.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.,* a therapeutic response). For example, a single dose may be administered, several divided doses may be administered over time or the dose may be

proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Administration may be in single or multiple doses. Multiple doses may be administered via the same or different routes and to the same or different locations. Alternatively, doses can be via a sustained release formulation, in which case less frequent administration is required. Dosage and frequency may vary depending on the half-life of the antagonist in the patient and the duration of treatment desired.

As mentioned above, compounds or pharmaceutical composition of the invention may be co-administered with one or other more other therapeutic agents. For example, the other agent may be an analgesic, anaesthetic, immunosuppressant or anti-inflammatory agent.

Combined administration of two or more agents may be achieved in a number of different ways. Both may be administered together in a single composition, or they may be administered in separate compositions as part of a combined therapy. For example, the one may be administered before, after or concurrently with the other.

The following Examples illustrate the invention.

**Examples**

*Example 1 – Synthesis of compounds*

Synthesis of compound (1) is disclosed in WO 2013/186229 (Example 44).
Synthesis of compound (2) is disclosed in WO 2013/186229 (Example 89).
Synthesis of compound (3) is disclosed in WO 2014/009295 (Example 129).
Synthesis of compound (4) is disclosed in WO 2014/009295 (Example 173).
Synthesis of compound (5) is disclosed in WO 2014/009295 (Example 319).
Synthesis of compound (6) is disclosed in WO 2013/186229 (Example 490).

*Example 2- Antibody Derivation*

Following the immunisation of 5 Sprague Dawley rats with human TNFα in

complex with the benzimidazole compound (1), immune B cells were cultured in 96-well plates to induce clonal expansion and antibody secretion (Tickle, S. *et al.*, High throughput screening for high affinity antibodies Journal of Laboratory Automation 2009 14: 303-307). Culture supernatants were screened for IgG antibodies preferentially binding to human TNFα in complex with compound (1) (at a 50 fold molar excess), compared to apo human TNFα, in a homogeneous bead-based FMAT assay. Human TNFα (+/- compound (1)) was presented on bead surfaces (superavidin-coated Bangs Beads, catalogue number CP01N) by a capture system using a human TNF-Receptor I–Fc fusion protein (R&D Systems catalogue number 372-Rl-050), bound with biotinylated anti-human Fc (Jackson catalogue number 109-066-098).

Antibodies which demonstrated preferential binding to the TNFα-compound (1) complex were termed '*conformation-selective*' and were taken forward for cloning. The Fluorescent Foci method (US Patent 7993864/ Europe EP1570267B1) was used to identify and isolate antigen-specific B cells from positive wells, and specific antibody variable region genes were recovered from single cells by reverse transcription (RT)-PCR.

The amino acid sequences of two representative antibodies, CA185_01974 and CA185_01979, which demonstrated conformation-selective binding to both human and mouse TNFα + compound are shown below:

CA185_01974.0 (VR0001837)

Light chain variable region (LCVR) SEQ ID NO: 7 (CDRs underlined)

DIQMTQSPASLPASPEEIVTITCQASQDIGNWLSWYQQKPGKSPQLLIYGATSL
ADGVPSRFSASRSGTQYSLKISRLQVEDFGIFYCLQGQSTPYTFGAGTKLELK

Heavy chain variable region (HCVR) SEQ ID NO:8 (CDRs underlined)

DVQLVESGGGLVQPGRSLKLSCAASGFTFSAYYMAWVRQAPTKGLEWVASI
NYDGANTFYRDSVKGRFTVSRDNARSSLYLQMDSLRSEDTATYYCTTEA

YGYNSNWFGYWGQGTLVTVSS

CA185_01979.0 (VR0001842)

Light chain variable region (LCVR) SEQ ID NO: 22 (CDRs underlined)

DIQMTQSPASLSASLEEIVTITCQASQDIGNWLSWYQQKPGKSPHLLIYGTTSL ADGVPSRFSGSRSGTQYSLKISGLQVADIGIYVCLQAYSTPFTFGSGTKLEIK

Heavy chain variable region (HCVR) SEQ ID NO: 23 (CDRs underlined)

EVHLVESGPGLVKPSQSLSLTCSVTGYSITNSYWDWIRKFPGNKMEWMGYIN YSGSTGYNPSLKSRISISRDTSNNQFFLQLNSITTEDTATYYCARGTYGYNAYH FDYWGRGVMVTVSS

*Example 3 - Potential Epitope of the Derived Antibodies*

Given the ability of the rat-derived antibodies to bind to both human and mouse TNFα in the presence of compounds, detailed analysis of rat, mouse and human amino acid sequences, together with X-ray crystal structures of TNFα, was undertaken to see if a likely epitope could be determined.

**Rat UniProt P16599 (SEQ ID NO: 32)**

```
         10         20         30         40         50         60
MSTESMIRDV ELAEEALPKK MGGLQNSRRC LCLSLFSFLL VAGATTLFCL LNFGVIGPNK

         70         80         90        100        110        120
EEKFPNGLPL ISSMAQTLTL RSSSQNSSDK PVAHVVANHQ AEEQLEWLSQ RANALLANGM

       130        140        150        160        170        180
DLKDNQLVVP ADGLYLIYSQ VLFKGQGCPD YVLLTHTVSR FAISYQEKVS LLSAIKSPCP

       190        200        210        220        230
KDTPEGAELK PWYEPMYLGG VFQLEKGDLL SAEVNLPKYL DITESGQVYF GVIAL
```

**Mouse UniProt P06804 (SEQ ID NO: 33)**

```
        10         20         30         40         50         60
MSTESMIRDV ELAEEALPQK MGGFQNSRRC LCLSLFSFLL VAGATTLFCL LNFGVIGPQR

        70         80         90        100        110        120
DEKFPNGLPL ISSMAQTLTL RSSSQNSSDK PVAHVVANHQ VEEQLEWLSQ RANALLANGM

       130        140        150        160        170        180
DLKDNQLVVP ADGLYLVYSQ VLFKGQGCPD YVLLTHTVSR FAISYQEKVN LLSAVKSPCP

       190        200        210        220        230
KDTPEGAELK PWYEPIYLGG VFQLEKGDQL SAEVNLPKYL DFAESGQVYF GVIAL
```

**Human UniProt P01375 (SEQ ID NO: 34)**

```
        10         20         30         40         50         60
MSTESMIRDV ELAEEALPKK TGGPQGSRRC LFLSLFSFLI VAGATTLFCL LHFGVIGPQR

        70         80         90        100        110        120
EEFPRDLSLI SPLAQAVRSS SRTPSDKPVA HVVANPQAEG QLQWLNRRAN ALLANGVELR

       130        140        150        160        170        180
DNQLVVPSEG LYLIYSQVLF KGQGCPSTHV LLTHTISRIA VSYQTKVNLL SAIKSPCQRE

       190        200        210        220        230
TPEGAEAKPW YEPIYLGGVF QLEKGDRLSA EINRPDYLDF AESGQVYFGI IAL
```

From alignments and comparison of the rat, mouse and human TNFα UniProt sequences, examples of where the rat amino acid sequence differs from the human, and where the human and mouse sequences are identical in the mature, cleaved product include N168, I194, F220 and A221 (residues and numbering from the human sequence).

These residues are highlighted on the crystal structure of human TNFα (1TNF) (Figure 1). It is possible that any of these amino acids are included in the epitope targeted by the antibodies CA185_01974 and CA185_01979.

Following cloning of the antibody variable regions into mouse IgG and mouse Fab (no-hinge) vectors, the conformation-selective nature of the binding of antibodies CA185_01974 and CA185_01979 was confirmed, using a variety of test compounds bound to TNFα, in HPLC, BIAcore, ELISA and cell-based assays.

*Example 4 – High Performance Liquid Chromatography (HPLC) to Determine Antibody Characteristics*

Specific binding of mouse Fab fragments was demonstrated by complex formation between CA185_01974 and human TNFα complexed with compound (1) using size exclusion chromatography. Results are shown in Figure 2. As shown in this Figure, with a 0.5x molar excess of Fab the predominant peak corresponds to bound Fab and trimer-compound complex (although there is a small peak showing the presence of some trimer-compound complex not bound to Fab). At a 1.0x molar excess of Fab there is single higher molecular weight peak corresponding to Fab bound to trimer-compound complex. At 1.5x and 2x molar excesses of Fab, there is a growing lower molecular peak corresponding to unbound Fab.

The stoichiometry was therefore determined to be 1 Fab : 1 TNFα trimer, with excess Fab appearing at 1.5x and 2x molar excess.

Binding of CA185_01979 to human TNFα complexed with compound (1) was also investigated using size exclusion chromatography. Results are shown in Figure 3. As for CA185_01974, the stoichiometry was determined to be 1 Fab : 1 TNFα trimer, with excess Fab appearing at 1.5x and 2x molar excess.

*Example 5 - BIAcore Assays to Determine Antibody Characteristics*

Surface plasmon resonance was performed at 25 °C using a BIAcore T200 (GE Healthcare). Anti-Mouse Fc (Jackson 115-006-071) was immobilised on a CM5 Sensor Chip (GE Healthcare) via amine coupling chemistry to a capture level of ~6000 response units. HBS-EP buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% (v/v) surfactant P20 - GE Healthcare) +1% DMSO was used as the running buffer. A 10 µl injection of each IgG at 1 µg/ml was used for capture by the immobilised anti-mouse Fc to create the TNFα-binding surface. Human or mouse TNFα (in-house) at 50 nM was pre-incubated with 2 µM compound in HBS-EP+ (1%

DMSO) for 5 hours.

A 3 minute injection of human or mouse TNFα +/- test compound was passed over each captured IgG at a flow rate of 30µl/min. The surface was regenerated at a flow-rate of 10µl/min by a 60 s injection of 40 mM HCl x2 and a 30 s 5 mM NaOH. Double referenced background subtracted binding curves were analysed using the T200 Evaluation software (version 1.0) following standard procedures. Kinetic parameters were determined from the fitting algorithm.

The kinetic binding data for human and mouse TNFα in the presence and absence of test compounds from two chemical series are shown in Tables 1 and 2 below.

| Antibody | Human TNFα | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) |
|---|---|---|---|---|
| CA185_01974 | + compound (2) | $4.2x10^{5}$ | $3.9x10^{-5}$ | $9.4x10^{-11}$ |
| CA185_01974 | + compound 1 | $3.2x10^{5}$ | $3.8x10^{-5}$ | $1.2x10^{-10}$ |
| CA185_01974 | apo | $6.6x10^{4}$ | $1.3x10^{-3}$ | $1.9x10^{-8}$ |
| | | | | |
| CA185_01979 | + compound (2) | $5.7x10^{5}$ | $3.3x10^{-5}$ | $5.8x10^{-11}$ |
| CA185_01979 | + compound (1) | $4.7x10^{5}$ | $1.6x10^{-5}$ | $3.4x10^{-11}$ |
| CA185_01979 | apo | $1.1x10^{5}$ | $7.1x10^{-4}$ | $6.7x10^{-9}$ |

Table 1 – BIAcore data with human TNFα

Both CA185_01974 and CA185_01979 demonstrated >2 log selective binding for compound-distorted human TNFα, with representative test compounds from two chemical series.

| Antibody | Mouse TNFα | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) |
|---|---|---|---|---|
| CA185_01974 | + compound (2) | $6.7x10^{4}$ | $4.8x10^{-5}$ | $7.1x10^{-10}$ |

| | | | | |
|---|---|---|---|---|
| CA185_01974 | + compound (1) | $5.8x10^{4}$ | $8.8x10^{-5}$ | $1.5x10^{-9}$ |
| CA185_01974 | apo | $4.2x10^{4}$ | $4.9x10^{-3}$ | $1.2x10^{-7}$ |
| | | | | |
| CA185_01979 | + compound (2) | $1.9x10^{5}$ | $3.5x10^{-5}$ | $1.9x10^{-10}$ |
| CA185_01979 | + compound (1) | $1.6x10^{5}$ | $6.3x10^{-5}$ | $3.8x10^{-10}$ |
| CA185_01979 | apo | $7.2x10^{4}$ | $2.0x10^{-3}$ | $2.7x10^{-8}$ |

Table 2 – BIAcore data with mouse TNFα

Both CA185_01974 and CA185_01979 demonstrated >1.5 and >2 log selective binding for compound-distorted mouse TNFα, with representative test compounds from two chemical series.

*Example 6 - ELISAs to Determine Antibody Characteristics*

A sandwich ELISA was developed to measure the concentration of TNFα bound to compounds of the invention, using antibody CA185_01974.0 that specifically detects the conformation of TNFα when in complex with these compounds. Briefly, a microtitre plate was coated with CA185_01974.0 to immobilise TNFα in complex with a test compound. TNFα was incubated overnight at 28 °C with a 50x molar excess of the test compound. Following this overnight incubation, TNFα was serially diluted in neat human plasma depleted of endogenous TNFα, in the presence of heterophilic antibody blockers, and added to the coated plate. Curves were generated with a concentration range of 0.78 pg/ml – 50 pg/ml TNFα. A biotinylated polyclonal anti TNFα antibody was used to detect bound TNFα, with streptavidin-peroxidase and TMB substrate to give a colorimetric signal. Sensitivity of the assay was increased with the use of tyramide signal amplification, using the ELAST kit from Perkin Elmer, as an additional step between streptavidin-peroxidase and the substrate.

An ELISA was also developed to measure total TNFα (free TNFα + TNFα in complex with a test compound) in parallel. For this assay the coating antibody was replaced with a commercial anti-TNFα polyclonal antibody (Invitrogen AHC3812).

The sample incubation time was also increased to 3 hours. All other steps were identical to the conformation-specific assay. This enables the amount of TNFα in complex with a test compound to be calculated as a proportion of total TNFα.

Results for the total TNFα ELISA with compounds (3), (4) and (5) are shown in Figure 4.

Results of the conformation specific TNFα ELISA with CA185_01974.0 and compounds (3), (4) and (5) are shown in Figure 5. Apo TNFα gave no signal in this assay, demonstrating the specific nature of the binding of antibody CA185_01974 to compound-bound TNFα. The antibody was able to recognise TNFα bound by a variety of test compounds from different chemical series.

*Example 7 - Cell-based Assays to Determine Antibody Characteristics*

Recombinant antibodies were also tested for binding to compound-distorted TNFα in a FACS assay using human embryonic kidney (HEK) JumpIn cells, which overexpress TNF-RI after induction with doxycycline at 1 μg/ml for 2.5 hours. HEK cells were trypsinised and incubated for 2 h in medium to allow recovery of digested TNFRI levels. Human TNFα at 2 μg/mL was pre-incubated with 40 μM compound (1) or 0.4% DMSO for 1 h at 37 °C. The preincubation mix was added to the cells for 1 h on ice (dilution 1:4, final concentrations: 0.5 μg/mL human-TNFα +/- 10 μM compound (1) or 0.1% DMSO ). Cells were washed, fixed (1.5% PFA) and stained with 1 or 10 μg/mL antibody for 1h on ice. (Secondary antibody: anti-mouse-Alexa488), before anlaysis for receptor-bound TNFα.

As shown in Figure 6, FACS histogram plots of staining with CA185_01974 and CA185_01979 at 1 and 10 μg/ml demonstrate that the antibodies only recognise TNFα which has been pre-incubated with compound (1). There is no staining with the DMSO control.

In addition, specific binding of CA185_01974 and CA185-01979 Fab fragments was demonstrated with compound-distorted membrane-bound TNFα. An engineered NS0 cell line, which overexpresses membrane TNFα, due to knock-out of the TACE cleavage site was incubated with 0.001 – 10 μM compound (1) or 0.1% DMSO for 1h at 37 °C. Cells were washed, fixed and stained with antibody Fab fragments at 0.01 or 0.1 μg/ml for 1 hour on ice. (Secondary antibody was anti-

mouse Fab-DyeLight488 from Jackson ImmunoResearch).

FACS histogram plots of staining with CA185_01974 (Figure 7) and CA185_01979 Fab fragments demonstrate that the antibodies only recognise TNF which has been pre-incubated with compound (1). There is no staining with the DMSO controls.

*Example 8 – Antibody CA185_01974, shows a 300- fold selectivity for human TNF-compound (4) complex*

Compound (4) was incubated with human and cynomolgus TNF and titrated over mouse full length antibody CA185_01974 to determine an accurate affinity value. The experiment included the following controls: (i) human or cynomolgus TNF + DMSO over 1974; (ii) human or cynomolgus TNF + DMSO over no antibody; and (iii) human or cynomolgus TNF + compound (4) over no antibody. Each sample and control was carried out in duplicate and used four concentrations in each replicate.

As shown in Figures 8 and 9, background binding of hTNF + compound (4) and cTNF + compound (4) increased by 5-10RU over the course of the assay. This is seen in the higher response of h/cTNF+ compound (4) binding to CA185_01974 in the second duplicate. Binding of hTNF and cTNF in the absence of compound (4) was consistently very low.

Kinetics of hTNF + DMSO binding mouse full length IgG CA185_1974_P8 was very similar in this assay to previous single concentration analysis. Affinity of cynomolgus TNF for mouse full length IgG CA185_1974_P8 is similar, however the kinetics differ.

Table 3 gives the kinetics of each analyte binding to CA185_1974_P8. Table 4 gives the average values and the fold difference +/- compound (4) of TNF kinetics for CA185_1974_p8. Figure 8 gives the sensograms of both duplicates of cTNF +/- compound (4). Figure 9 gives the sensograms of both duplicates of hTNF +/- compound (4).

| Duplicate | Antibody | Analyte | ka (1/Ms) | kd (1/s) | KD (M) | KD (pM) |
|---|---|---|---|---|---|---|
| 1 | CA185_1974_P8 | cyno TNF | 1.03E+05 | 1.87E-03 | 1.83E-08 | 18270 |
| 2 | CA185_1974_P8 | cyno TNF | 1.25E+05 | 1.92E-03 | 1.54E-08 | 15350 |
| 1 | CA185_1974_P8 | cyno TNF +compound (4) | 1.84E+05 | 1.46E-05 | 7.91E-11 | 79.1 |
| 2 | CA185_1974_P8 | cyno TNF +compound (4) | 2.01E+05 | 2.06E-05 | 1.03E-10 | 103 |
| | | | | | | |
| 1 | CA185_1974_P8 | human TNF | 8.02E+04 | 1.77E-03 | 2.21E-08 | 22100 |
| 2 | CA185_1974_P8 | human TNF | 1.05E+05 | 1.67E-03 | 1.59E-08 | 15900 |
| 1 | CA185_1974_P8 | human TNF +compound (4) | 3.06E+05 | *1.00E-05* | 3.27E-11 | 32.7 |
| 2 | CA185_1974_P8 | human TNF +compound (4) | 3.07E+05 | 2.73E-05 | 8.88E-11 | 88.8 |

Table 3: Binding kinetics of hTNF and cTNF +/- compound (4) to the CA185_1974_P8 antibody

| Average of duplicates | ka (1/Ms) | kd (1/s) | KD (M) | KD (pM) |
|---|---|---|---|---|
| cyno TNF | 1.14E+05 | 1.90E-03 | 1.68E-08 | 16810 |
| | | | | |
| cyno TNF +compound (4) | 1.93E+05 | 1.76E-05 | 9.09E-11 | 90.92 |
| | | | | |
| Fold difference | 1.69 | 107.81 | 184.89 | 184.89 |
| human TNF | 9.27E+04 | 1.72E-03 | 1.90E-08 | 19000 |
| | | | | |
| human TNF +compound (4) | 3.06E+05 | 1.86E-05 | 6.08E-11 | 60.76 |
| | | | | |
| Fold difference | 3.31 | 92.43 | 312.70 | 312.70 |

Table 4: Average values and fold differences +/- compound (4) of hTNF and cTNF kinetics for CA185_1974_p8.

*Conclusions*

The antibodies CA185_01974 and CA185_01989 have been demonstrated specifically to bind to a compound-distorted state of TNFα, and will be useful target-

engagement biomarkers for compounds of the invention.

The antibodies have been shown to bind to a conformation of TNFα, which is specifically stabilised by compounds from different chemical series. It is envisaged that these antibodies will become standards in defining this, and closely related, biologically relevant conformations, of the TNFα trimer, which are stabilised by a wider range of chemical series than are described here. Based on the data shown, the human TNFα trimer could be considered to be stabilised in the defined, biologically relevant conformation described if either CA185_01974 or CA185_01989 antibody binds with a $K_D$ better than 1 nM in the BIAcore assay format described above.

*Example 9 - Compounds and complexes of Ma et al (2014) and Silvian et al (2011) have different characteristics to those of the present invention*

As described on page 12458 of Ma *et al.* (2014) JBC 289:12457-12466, C87 was discovered through virtual screening by attempting to find molecules which fit the space occupied by a 7 amino-acid peptide from loop2/domain2 of TNFR1 in its interaction with the external surface of TNFβ. The C87 compound from Ma *et al.* and the BIO8898 compound from Silvian *et al.* (2011) ACS Chemical Biology 6:636-647 were tested by the present inventors.

*Summary of findings*

The Biacore observations described in Ma *et al.* for C87 could not be repeated.

No evidence of TNF specific inhibition in cells was observed.

Additionally C87 was not observed to bind by mass spectrometry, which is sensitive to millimolar affinities.

Extensive crystallography trials only produced apo-TNF (TNF without compound).

In the fluorescence polarisation (FP) assay, C87 showed no significant inhibition above the interference level of the compound with the fluorescent read-out.

Thermofluor, which measures stabilisation of the thermal melting temperature of TNFα, did show a small stabilisation for C87.

In summary, no evidence was found that C87 binds in the centre of the trimer.

The overwhelming majority of the data suggested no direct interaction with TNFα. BIO8898 was also found not to bind to TNFα.

*Cells – TNF induced HEK NFKB reporter gene assay*

C87 was preincubated with TNFα for 1 hour prior to the addition to HEK-293 cells stably transfected with SEAP under the control of NFκB. An appropriate counter-screen was also tested in order to detect non-TNF related (off target) activity. The assay was incubated overnight before inhibition was measured compared to 100 % blocking by a control compound. The maximum C87 concentration was 10,000 nM, with a 3-fold serial dilution.

No inhibitory effect could be detected that could not be attributed to off-target activity.

*Biacore*

TNF was immobilised using an avi-tag linker and C87 was passed over the chip. In one experiment, a dose response of C87 from a highest concentration of 10 μM was performed. No binding was observed.

In a second experiment, the flow rate of C87 passing over the chip was reduced. A small shift was observed but overall binding was negligible.

The binding of C87 to TNF described in Ma *et al* was likely to be super-stoichiometric based on the RU value on the Y-axis. At standard TNF density on the chip this value was in the region of thirty times higher than expected for simple 1:1 binding.

In another experiment, BIO8898 was tested against the immobilised soluble form of CD40L and the soluble form of TNFα by SPR on a Biacore 4000 machine. A geomean IC50 of 17 μM was determined for binding against CD40L whereas no binding was detected at a concentration of up to 100 μM for TNFα in this assay.

*Mass spectrometry*

There was no evidence of C87 binding to human TNFα (20 μM) at a concentration of 400 μM. A species of lower molecular weight (~473 Da appears to bind at less than 5 % occupancy). C87 has a molecular weight of 503 Da. Based on

the occupancy at a concentration of 400 μM, an affinity of the low molecular weight species in excess of 1 mM is predicted.

*Crystallography*

Overall a large effort was put into crystallising C87 with TNFα, including testing conditions that routinely work with compounds described in the present application. This comprised setting up a large number of crystallization trials at different ligand concentrations, different protein concentrations, and different soaking times. A few crystals were observed that, on analysis, proved to be salt or TNF with no compound.

*Fluorescent polarization (FP)*

C87 was preincubated with TNFα for 1 hour prior to assay against the fluorescent compound (probe). Competition with the fluorescent compound either directly (binding at the same site) or indirectly (disrupting TNF) is detected by a reduction in FP.

Extrapolation of the inhibition curve produced an IC50 of about 100 μM. Fluorescence quenching was, however, observed at the highest concentrations of inhibitor which, when subtracted, resulted in negligible inhibition of C87 in this assay.

*Thermofluor*

Thermofluor measures the change of melting temperature (Tm) of TNFα due to compound either stabilising or disrupting the protein. A stabilization effect of 3.8 °C was observed at a concentration of 500 μM C87, suggesting the possibility of weak binding, which may not be specific.

## Sequence listing

**SEQ ID NO: 1 (LCDR1 of 1974)**

QASQDIGN

**SEQ ID NO: 2 (LCDR2 of 1974)**

GATSLAD

**SEQ ID NO: 3 (LCDR3 of 1974)**

LQGQSTPYT

**SEQ ID NO: 4 (HCDR1 of 1974)**

AYYMA

**SEQ ID NO: 5 (HCDR2 of 1974)**

ASINYDGANTFYRDSVKG

**SEQ ID NO: 6 (HCDR3 of 1974)**

EAYGYNSNWFGY

**SEQ ID NO: 7 (LCVR of 1974)**

DIQMTQSPASLPASPEEIVTITCQASQDIGNWLSWYQQKPGKSPQLLIYGATSLADGVPSRFSASRSGT
QYSLKISRLQVEDFGIFYCLQGQSTPYTFGAGTKLELK

**SEQ ID NO: 8 (HCVR of 1974)**

DVQLVESGGGLVQPGRSLKLSCAASGFTFSAYYMAWVRQAPTKGLEWVASINYDGANTFYRDSVKGRFT
VSRDNARSSLYLQMDSLRSEDTATYYCTTEAYGYNSNWFGYWGQGTLVTVSS

**SEQ ID NO: 9 (LCVR DNA of 1974)**

GACATCCAGATGACCCAGTCTCCTGCCTCCCTGCCTGCATCCCCGGAAGAAATTGTCACCATCACATGC
CAGGCAAGCCAGGACATTGGTAATTGGTTATCATGGTATCAGCAGAAACCAGGGAAATCGCCTCAGCTC
CTGATCTATGGTGCAACCAGCTTGGCAGATGGGGTCCCATCAAGGTTCAGCGCCAGTAGATCTGGCACA
CAGTACTCTCTTAAGATCAGCAGACTGCAGGTTGAAGATTTTGGAATCTTTTACTGTCTACAGGGTCAA
AGTACTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAACTGAAA

**SEQ ID NO: 10 (HCVR DNA of 1974)**

GACGTGCAGCTGGTGGAATCTGGAGGAGGCTTAGTGCAGCCTGGAAGGTCCCTGAAACTCTCCTGTGCA
GCCTCAGGATTCACTTTCAGTGCCTATTACATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGTCTGGAG
TGGGTCGCATCCATTAATTATGATGGTGCTAACACTTTCTATCGCGACTCCGTGAAGGGCCGATTCACT
GTCTCCAGAGATAATGCAAGAAGCAGCCTATACCTACAAATGGACAGTCTGAGGTCTGAGGACACGGCC
ACTTATTACTGTACAACAGAGGCTTACGGATATAACTCAAATTGGTTTGGTTACTGGGGCCAAGGCACT
CTGGTCACTGTCTCGAGC

**SEQ ID NO: 11 (1974 LC kappa full)**

DIQMTQSPASLPASPEEIVTITCQASQDIGNWLSWYQQKPGKSPQLLIYGATSLADGVPSRFSASRSGT
QYSLKISRLQVEDFGIFYCLQGQSTPYTFGAGTKLELKRTDAAPTVSIFPPSSEQLTSGGASVVCFLNN
FYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK

SFNRNEC

**SEQ ID NO: 12 (1974 HC mIgG1 full)**

DVQLVESGGGLVQPGRSLKLSCAASGFTFSAYYMAWVRQAPTKGLEWVASINYDGANTFYRDSVKGRFT
VSRDNARSSLYLQMDSLRSEDTATYYCTTEAYGYNSNWFGYWGQGTLVTVSSAKTTPPSVYPLAPGSAA
QTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH
PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWF
VDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSN
WEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

**SEQ ID NO: 13 (1974 HC mFabno hinge full)**

DVQLVESGGGLVQPGRSLKLSCAASGFTFSAYYMAWVRQAPTKGLEWVASINYDGANTFYRDSVKGRFT
VSRDNARSSLYLQMDSLRSEDTATYYCTTEAYGYNSNWFGYWGQGTLVTVSSAKTTPPSVYPLAPGSAA
QTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH
PASSTKVDKKIVPRDC

**SEQ ID NO: 14 (1974 LC DNA kappa full)**

GACATCCAGATGACCCAGTCTCCTGCCTCCCTGCCTGCATCCCCGGAAGAAATTGTCACCATCACATGC
CAGGCAAGCCAGGACATTGGTAATTGGTTATCATGGTATCAGCAGAAACCAGGGAAATCGCCTCAGCTC
CTGATCTATGGTGCAACCAGCTTGGCAGATGGGGTCCCATCAAGGTTCAGCGCCAGTAGATCTGGCACA
CAGTACTCTCTTAAGATCAGCAGACTGCAGGTTGAAGATTTTGGAATCTTTTACTGTCTACAGGGTCAA
AGTACTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAACTGAAACGTACGGATGCTGCACCAACTGTA
TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAAC
TTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAAC
AGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGAC
GAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAG
AGCTTCAACAGGAATGAGTGT

**SEQ ID NO: 15 (1974 HC DNA mIgG1 full)**

GACGTGCAGCTGGTGGAATCTGGAGGAGGCTTAGTGCAGCCTGGAAGGTCCCTGAAACTCTCCTGTGCA
GCCTCAGGATTCACTTTCAGTGCCTATTACATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGTCTGGAG
TGGGTCGCATCCATTAATTATGATGGTGCTAACACTTTCTATCGCGACTCCGTGAAGGGCCGATTCACT
GTCTCCAGAGATAATGCAAGAAGCAGCCTATACCTACAAATGGACAGTCTGAGGTCTGAGGACACGGCC
ACTTATTACTGTACAACAGAGGCTTACGGATATAACTCAAATTGGTTTGGTTACTGGGGCCAAGGCACT
CTGGTCACTGTCTCGAGTGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCC
CAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACC
TGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACT
CTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCAC
CCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGT
ACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTG
ACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTT
GTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGC
TCAGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAAC
AGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAG
GTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACA
GACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC
ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAAC
TGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAG
AGCCTCTCCCACTCTCCTGGTAAA

**SEQ ID NO: 16 (1974 HC DNA mFabno hinge full)**

GACGTGCAGCTGGTGGAATCTGGAGGAGGCTTAGTGCAGCCTGGAAGGTCCCTGAAACTCTCCTGTGCA
GCCTCAGGATTCACTTTCAGTGCCTATTACATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGTCTGGAG

TGGGTCGCATCCATTAATTATGATGGTGCTAACACTTTCTATCGCGACTCCGTGAAGGGCCGATTCACT
GTCTCCAGAGATAATGCAAGAAGCAGCCTATACCTACAAATGGACAGTCTGAGGTCTGAGGACACGGCC
ACTTATTACTGTACAACAGAGGCTTACGGATATAACTCAAATTGGTTTGGTTACTGGGGCCAAGGCACT
CTGGTCACTGTCTCGAGTGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCC
CAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACC
TGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCGGCTGTCCTGCAATCTGACCTCTACACT
CTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCAC
CCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGT

**SEQ ID NO: 17 (LCDR2 of 1979)**

GTTSLAD

**SEQ ID NO: 18 (LCDR3 of 1979)**

LQAYSTPFTF

**SEQ ID NO: 19 (HCDR1 of 1979)**

NSYWD

**SEQ ID NO: 20 (HCDR2 of 1979)**

YINYSGSTGYNPSLKS

**SEQ ID NO: 21 (HCDR3 of 1979)**

GTYGYNAYHFDY

**SEQ ID NO: 22 (LCVR of 1979)**

DIQMTQSPASLSASLEEIVTITCQASQDIGNWLSWYQQKPGKSPHLLIYGTTSLADGVPSRFSGSRSGT
QYSLKISGLQVADIGIYVCLQAYSTPFTFGSGTKLEIK

**SEQ ID NO: 23 (HCVR of 1979)**

EVHLVESGPGLVKPSQSLSLTCSVTGYSITNSYWDWIRKFPGNKMEWMGYINYSGSTGYNPSLKSRISI
SRDTSNNQFFLQLNSITTEDTATYYCARGTYGYNAYHFDYWGRGVMVTVSS

**SEQ ID NO: 24 (LCVR DNA of 1979)**

GACATCCAAATGACACAGTCTCCTGCCTCCCTGTCTGCATCTCTGGAAGAAATTGTCACCATTACATGC
CAGGCAAGCCAGGACATTGGTAATTGGTTATCATGGTATCAGCAGAAACCAGGGAAATCTCCTCACCTC
CTGATCTATGGTACCACCAGCTTGGCAGATGGGGTCCCATCAAGGTTCAGCGGCAGTAGATCTGGTACA
CAGTATTCTCTTAAGATCAGCGGACTACAGGTTGCAGATATTGGAATCTATGTCTGTCTACAGGCTTAT
AGTACTCCATTCACGTTCGGCTCAGGGACAAAGCTGGAAATAAAA

**SEQ ID NO: 25 (HCVR DNA of 1979)**

GAGGTGCACCTGGTGGAGTCTGGACCTGGCCTTGTGAAACCCTCACAGTCACTCTCCCTCACCTGTTCT
GTCACTGGTTACTCCATCACTAATAGTTACTGGGACTGGATCCGGAAGTTCCCAGGAAATAAAATGGAG
TGGATGGGATACATAAACTACAGTGGTAGCACTGGCTACAACCCATCTCTCAAAAGTCGAATCTCCATT
AGTAGAGACACATCGAACAATCAGTTCTTCCTGCAGCTGAACTCTATAACTACTGAGGACACAGCCACA
TATTACTGTGCACGAGGGACCTATGGGTATAACGCCTACCACTTTGATTACTGGGGCCGAGGAGTCATG
GTCACAGTCTCGAGC

**SEQ ID NO: 26 (1979 LC Kappa full)**

DIQMTQSPASLSASLEEIVTITCQASQDIGNWLSWYQQKPGKSPHLLIYGTTSLADGVPSRFSGSRSGT

QYSLKISGLQVADIGIYVCLQAYSTPFTFGSGTKLEIKRTDAAPTVSIFPPSSEQLTSGGASVVCFLNN
FYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK
SFNRNEC

**SEQ ID NO: 27 (1979 HC mIgG1 full)**

EVHLVESGPGLVKPSQSLSLTCSVTGYSITNSYWDWIRKFPGNKMEWMGYINYSGSTGYNPSLKSRISI
SRDTSNNQFFLQLNSITTEDTATYYCARGTYGYNAYHFDYWGRGVMVTVSSAKTTPPSVYPLAPGSAAQ
TNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP
ASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFV
DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQV
YTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNW
EAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

**SEQ ID NO: 28 (1979 HC mFabno hinge full)**

EVHLVESGPGLVKPSQSLSLTCSVTGYSITNSYWDWIRKFPGNKMEWMGYINYSGSTGYNPSLKSRISI
SRDTSNNQFFLQLNSITTEDTATYYCARGTYGYNAYHFDYWGRGVMVTVSSAKTTPPSVYPLAPGSAAQ
TNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP
ASSTKVDKKIVPRDC

**SEQ ID NO: 29 (1979 LC DNA Kappa full)**

GACATCCAAATGACACAGTCTCCTGCCTCCCTGTCTGCATCTCTGGAAGAAATTGTCACCATTACATGC
CAGGCAAGCCAGGACATTGGTAATTGGTTATCATGGTATCAGCAGAAACCAGGGAAATCTCCTCACCTC
CTGATCTATGGTACCACCAGCTTGGCAGATGGGGTCCCATCAAGGTTCAGCGGCAGTAGATCTGGTACA
CAGTATTCTCTTAAGATCAGCGGACTACAGGTTGCAGATATTGGAATCTATGTCTGTCTACAGGCTTAT
AGTACTCCATTCACGTTCGGCTCAGGGACAAAGCTGGAAATAAAACGTACGGATGCTGCACCAACTGTA
TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAAC
TTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAAC
AGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGAC
GAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAG
AGCTTCAACAGGAATGAGTGT

**SEQ ID NO: 30 (1979 HC DNA mIgG1 full)**

GAGGTGCACCTGGTGGAGTCTGGACCTGGCCTTGTGAAACCCTCACAGTCACTCTCCCTCACCTGTTCT
GTCACTGGTTACTCCATCACTAATAGTTACTGGGACTGGATCCGGAAGTTCCCAGGAAATAAAATGGAG
TGGATGGGATACATAAACTACAGTGGTAGCACTGGCTACAACCCATCTCTCAAAAGTCGAATCTCCATT
AGTAGAGACACATCGAACAATCAGTTCTTCCTGCAGCTGAACTCTATAACTACTGAGGACACAGCCACA
TATTACTGTGCACGAGGGACCTATGGGTATAACGCCTACCACTTTGATTACTGGGGCCGAGGAGTCATG
GTCACAGTCTCGAGTGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAA
ACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGG
AACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTG
AGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCG
GCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACA
GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACT
CCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTA
GATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCA
GTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGT
GCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTG
TACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGAC
TTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACT
CAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGG
GAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGC
CTCTCCCACTCTCCTGGTAAA

**SEQ ID NO: 31 (1979 HC DNA mFabno hinge full)**

GAGGTGCACCTGGTGGAGTCTGGACCTGGCCTTGTGAAACCCTCACAGTCACTCTCCCTCACCTGTTCT
GTCACTGGTTACTCCATCACTAATAGTTACTGGGACTGGATCCGGAAGTTCCCAGGAAATAAAATGGAG
TGGATGGGATACATAAACTACAGTGGTAGCACTGGCTACAACCCATCTCTCAAAAGTCGAATCTCCATT
AGTAGAGACACATCGAACAATCAGTTCTTCCTGCAGCTGAACTCTATAACTACTGAGGACACAGCCACA
TATTACTGTGCACGAGGGACCTATGGGTATAACGCCTACCACTTTGATTACTGGGGCCGAGGAGTCATG
GTCACAGTCTCGAGTGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAA
ACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGG
AACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCGGCTGTCCTGCAATCTGACCTCTACACTCTG
AGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCG
GCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGT

**SEQ ID NO: 32 – Rat TNFα**

MSTESMIRDVELAEEALPKKMGGLQNSRRCLCLSLFSFLLVAGATTLFCLLNFGVIGPNKEEKFPNGLP
LISSMAQTLTLRSSSQNSSDKPVAHVVANHQAEEQLEWLSQRANALLANGMDLKDNQLVVPADGLYLIY
SQVLFKGQGCPDYVLLTHTVSRFAISYQEKVSLLSAIKSPCPKDTPEGAELKPWYEPMYLGGVFQLEKG
DLLSAEVNLPKYLDITESGQVYFGVIAL

**SEQ ID NO: 33 – Mouse TNFα**

MSTESMIRDVELAEEALPQKMGGFQNSRRCLCLSLFSFLLVAGATTLFCLLNFGVIGPQRDEKFPNGLP
LISSMAQTLTLRSSSQNSSDKPVAHVVANHQVEEQLEWLSQRANALLANGMDLKDNQLVVPADGLYLVY
SQVLFKGQGCPDYVLLTHTVSRFAISYQEKVNLLSAVKSPCPKDTPEGAELKPWYEPIYLGGVFQLEKG
DQLSAEVNLPKYLDFAESGQVYFGVIAL

**SEQ ID NO: 34 – Human TNFα**

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSL
ISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQV
LFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDR
LSAEINRPDYLDFAESGQVYFGIIAL

**SEQ ID NO: 35 – Soluble form of human TNFα**

SVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQG
CPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEIN
RPDYLDFAESGQVYFGIIAL

**SEQ ID NO: 36 – Soluble form of human TNFα, but lacking the "S" cloning artefact of SEQ ID NO: 35**

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVL
FKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRL
SAEINRPDYLDFAESGQVYFGIIAL

# CLAIMS

1. An antibody that selectively binds to a complex comprising (i) a trimeric protein that is a TNF superfamily member and (ii) a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor.

2. The antibody of claim 1, wherein the compound antagonises the signalling induced by the trimer through the receptor.

3. The antibody of claim 2, wherein the compound increases the stability of the trimeric form of the TNF superfamily member compared to the stability of the trimeric form of the TNF superfamily member in the absence of the compound.

4. The antibody of claim 3, wherein the increase in stability results in an increase in the thermal transition midpoint ($T_m$) of the trimeric form of the TNF superfamily member of at least 1 °C.

5. The antibody of claim 4, wherein the increase in stability results in an increase in the thermal transition midpoint ($T_m$) of the trimeric form of the TNF superfamily member of at least 10 °C.

6. The antibody of claim 5, wherein the increase in the $T_m$ of the trimeric form of the TNF superfamily member is between 10 °C and 20 °C.

7. The antibody of any one of the preceding claims, wherein the compound increases the binding affinity of the TNF superfamily member to the requisite receptor compared to the binding affinity of the TNF superfamily member to its receptor in the absence of the compound.

8. The antibody of claim 7, wherein the compound increases the binding affinity of

the TNF superfamily member to the requisite receptor by increasing the on rate ($k_{on-r}$) and/or decreasing the off rate ($k_{off-r}$) compared to the $k_{on-r}$ and $k_{off-r}$ values for binding of the TNF superfamily member to its receptor in the absence of the compound.

9. The antibody of claim 8, wherein the compound increases the binding affinity of the TNF superfamily member to the requisite receptor by increasing the on rate ($k_{on-r}$) compared to the $k_{on-r}$ value for binding of the TNF superfamily member to its receptor in the absence of the compound.

10. The antibody of any one of claims 7 to 9, wherein the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound, wherein:

a) the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor by at least 10 times compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound;

b) the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite receptor in the presence of the compound is less than 10 nM.

11. The antibody of any one of claims 7 to 9, wherein the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound, wherein:

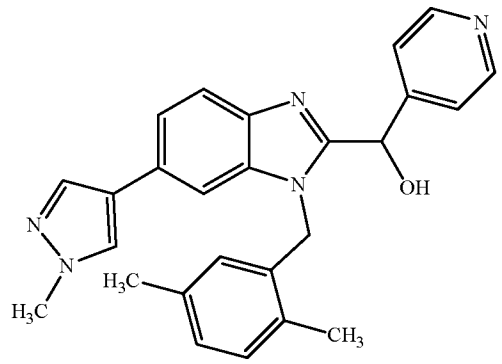
a) the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor by at least 4 times compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound;

b) the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite receptor in the presence of the compound is less than 600 pM.

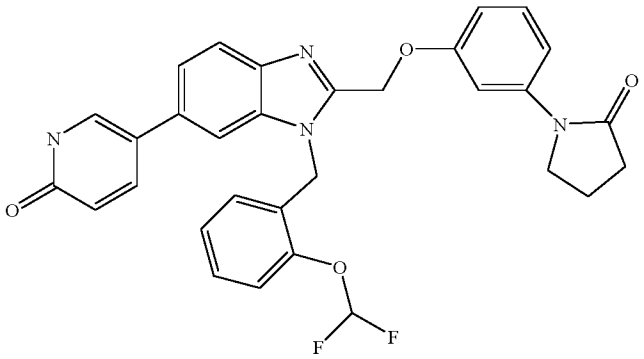
12. The antibody of claim 11, wherein the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite receptor in the presence of the compound is less than 200 pM.

13. The antibody of any one of the preceding claims, wherein said compound has an $IC_{50}$ value of 500 nM or less.

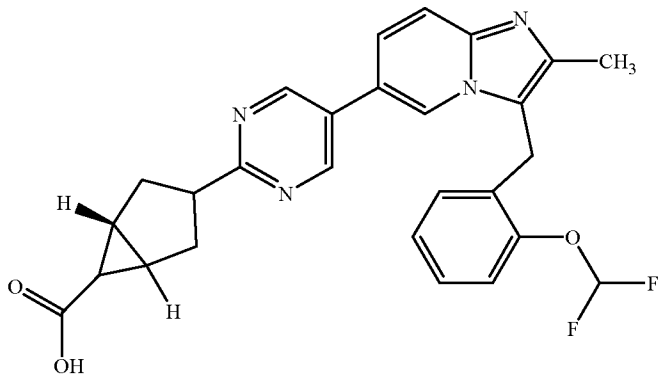
14. The antibody of any one of the preceding claims, wherein the compound is selected from the group consisting of compounds (1)-(6), or salts or solvates thereof:



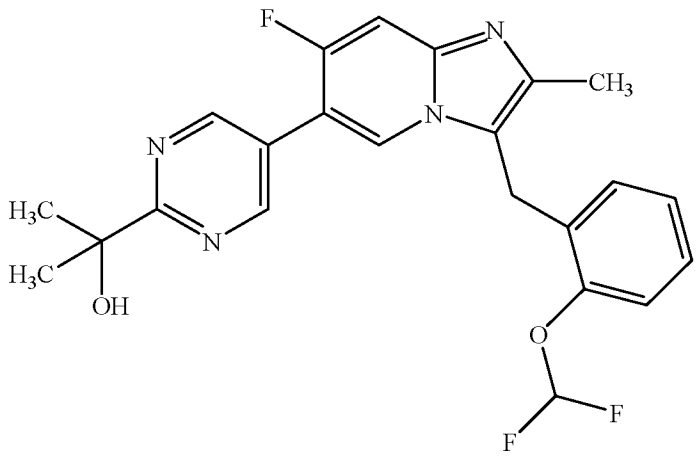
(1)



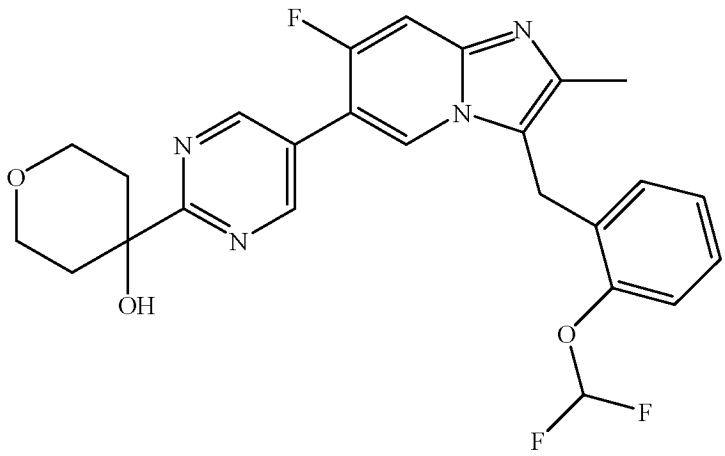
(2)



(3)

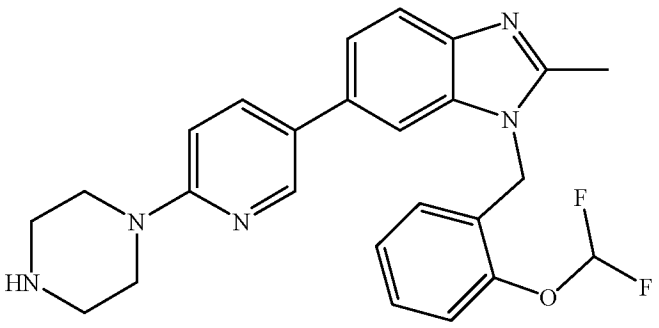


(4)



(5)

or



(6)

15. The antibody of any one of the preceding claims, wherein the antibody binds selectively to the trimer-compound complex compared with binding to the compound

in the absence of the TNF superfamily member trimer and/or binding to the TNF superfamily member trimer in the absence of the compound.

16. The antibody of claim 15, wherein the antibody binds to the trimer-compound complex with a $K_{D\text{-}ab}$ that is at least 100 times lower than the $K_{D\text{-}ab}$ for binding to the trimeric TNF superfamily member in the absence of compound and/or for binding to the compound in the absence of the TNF superfamily member.

17. The antibody of claim 16, wherein the antibody binds to the trimer-compound complex with a $K_{D\text{-}ab}$ that is at least 200 times lower than the $K_{D\text{-}ab}$ for binding to the trimeric TNF superfamily member in the absence of compound and/or for binding to the compound in the absence of the TNF superfamily member.

18. The antibody of any one of the preceding claims, wherein the TNF superfamily member is TNFα and the receptor is the TNF receptor.

19. The antibody of claim 18, wherein the receptor is TNFR1.

20. An antibody that selectively binds to a complex comprising (i) a human TNFα and (ii) a compound selected from the group consisting of compounds (1)-(6), or salts or solvates thereof.

21. The antibody of claim 20, wherein the TNFα is $TNF\alpha_S$.

22. The antibody of claim 21, wherein the $TNF\alpha_S$ comprises the sequence of SEQ ID NO: 35 or SEQ ID NO: 36, or a variant thereof.

23. The antibody of any one of claims 20 to 22, wherein the TNFα is trimeric.

24. The antibody of any one of claims 20 to 23, wherein the antibody binds selectively to the TNFα-compound complex compared with binding to the compound in the absence of the TNFα and/or binding to the TNFα in the absence of the

compound.

25. The antibody of claim 24, wherein the antibody binds to the TNFα-compound complex with a $K_{D\text{-}ab}$ that is at least 100 times lower than the $K_{D\text{-}ab}$ for binding to the TNFα in the absence of compound and/or for binding to the compound in the absence of the TNFα.

26. The antibody of claim 25, wherein the antibody binds to the TNFα-compound complex with a $K_{D\text{-}ab}$ that is at least 200 times lower than the $K_{D\text{-}ab}$ for binding to the TNFα in the absence of compound and/or for binding to the compound in the absence of the TNFα.

27. The antibody of any one of the preceding claims, which comprises at least one heavy chain complementarity determining region (HCDR) sequence selected from SEQ ID NOs: 4-6 and 19-21 and/or at least one light chain complementarity determining region (LCDR) sequence selected from SEQ ID NOs: 1-3, 17 and 18.

28. The antibody of claim 27, which comprises a HCDR3 sequence of SEQ ID NO: 6 or SEQ ID NO: 21.

29. The antibody of claim 27 or 28, which comprises HCDR1, HCDR2 and HCDR3 sequences and LCDR1, LCDR2, and LCDR3 sequences contained within a heavy chain variable region (HCVR) and light chain variable region (LCVR) pair of SEQ ID NOs: 8/7 or SEQ ID NOs: 23/22.

30. The antibody of claim 29, wherein the HCDR1/HCDR2/HCDR3 sequence combination is selected from SEQ ID NOs: 4/5/6 and SEQ ID NOs: 19/20/21, and/or the LCDR1/LCDR2/LCDR3 sequence combination is selected from SEQ ID NOs: 1/2/3 and SEQ ID NOs: 1/17/18.

31. The antibody of any one of claims 27 to 30, which comprises a HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequence combination of SEQ ID

NOs: 4/5/6/1/2/3 or SEQ ID NOs: 19/20/21/1/17/18.

32. The antibody of any one of claims 27 to 31, which comprises a heavy chain variable region (HCVR) sequence of SEQ ID NO: 8 or 23 and/or a light chain variable region (LCVR) sequence of SEQ ID NO: 7 or 22, or sequences which are at least 95% identical thereto.

33. The antibody of claim 32, which comprises a HCVR and LCVR sequence pair of SEQ ID NOs: 8/7 or SEQ ID NOs: 23/22, or sequences which are at least 95% identical thereto.

34. The antibody of claim 33, wherein:

(a) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of SEQ ID NOs: 4/5/6/1/2/3 and the remainder of the HCVR and LCVR comprise at least 95% identity to SEQ ID NOs: 8 and 7 respectively; or

(b) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of SEQ ID NOs: 19/20/21/1/17/18 and the the remainder of the HCVR and LCVR comprise at least 95% identity to SEQ ID NOs: 23 and 22 respectively.

35. The antibody of claim 32, which comprises a heavy chain of SEQ ID NO: 12, 13, 27 or 28 and/or a light chain of SEQ ID NO: 11 or 26, or sequences which are at least 95% identical thereto.

36. The antibody of claim 35, which comprises a heavy and light chain pair of SEQ ID NOs: 12/11, 13/11, 27/26 or 28/26, or sequences which are at least 95% identical thereto.

37. The antibody of claim 36, wherein:

(a) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of SEQ ID NOs: 4/5/6/1/2/3 and the remainder of the heavy and light chains comprise at least 95% identity to SEQ ID NOs: 12 and 11 respectively;

(b) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of

SEQ ID NOs: 4/5/6/1/2/3 and the remainder of the heavy and light chains comprise at least 95% identity to SEQ ID NOs: 13 and 11 respectively;

(c) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of SEQ ID NOs: 19/20/21/1/17/18 and the remainder of the heavy and light chains comprise at least 95% identity to SEQ ID NOs: 27 and 26 respectively; or

(d) the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 sequences consist of SEQ ID NOs: 19/20/21/1/17/18 and the remainder of the heavy and light chains comprise at least 95% identity to SEQ ID NOs: 28 and 26 respectively.

38. An antibody which competes for binding to TNFα with, or binds to the same epitope on TNFα as, an antibody as defined in any one of claims 27 to 37.

39 An antibody according to any one of the preceding claims, which is a humanised antibody.

40. An antibody according to any one of the preceding claims, which is a Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibody or an scFv.

41. An isolated polynucleotide encoding an antibody as defined in any one of claims 1 to 40.

42. An antibody as defined in any one of claims 1 to 40 for use in a method of treatment of the human or animal body by therapy.

43. A pharmaceutical composition comprising an antibody as defined in any one of claims 1 to 40 and a pharmaceutically acceptable adjuvant and/or carrier.

44. Use of an antibody as defined in any one of claims 1 to 40 as a target engagement biomarker for the detection of a compound-trimer complex in a sample obtained from a subject; wherein said antibody is detectable and said complex comprises a trimeric protein that is a TNF superfamily member and a compound that is capable of binding to a trimeric protein that is a TNF superfamily member, whereby

the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor.

45. A method of detecting target engagement of a compound to a trimeric TNF superfamily member, whereby the compound-trimer complex binds to the requisite receptor and modulates the signalling induced by the trimer through the receptor, said method comprising:

(a) obtaining a sample from a subject administered said compound;

(b) contacting an antibody as defined in any one of claims 1 to 40 to said sample and a control sample, wherein said antibody is detectable;

(c) determining the amount of binding of said detectable antibody to said sample and said control sample,

wherein binding of said detectable antibody to said sample greater than binding of said detectable antibody to said control sample indicates target engagement of said compound to said trimeric TNF superfamily member.

46. Use of an antibody as defined in any one of claims 1 to 40 in screening for a compound that elicits a conformational change in a trimeric TNF superfamily member, wherein said conformational change modulates the signalling of the requisite TNF superfamily receptor on binding of the trimeric TNF superfamily member.

47. A complex comprising a trimeric protein that is a TNF superfamily member and a compound that is bound thereto, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein said complex binds to an antibody as defined in any one of claims 1 to 40 with a $K_{D\text{-}ab}$ of 1 nM or less.

48. The complex of claim 47, wherein the TNF superfamily member is TNFα.

49. A TNFα trimer, said TNFα trimer being able to bind TNFR1, but wherein signalling from said bound TNFR1 is attenuated or antagonised, wherein said TNFα trimer binds to either or both of the following antibodies with a a $K_{D\text{-}ab}$ of 1 nM or

less:

(i) an antibody with a heavy chain of SEQ ID NO: 27 and a light chain of SEQ ID NO: 26; or

(ii) an antibody with a heavy chain of SEQ ID NO: 12 and a light chain of SEQ ID NO: 11.

50. A TNFα trimer according to claim 49, wherein the TNFα subunits comprise the amino acid sequence of SEQ ID NO: 36, or a corresponding sequence.

51. A compound that is capable of binding to a trimeric protein that is a TNF superfamily member to form a complex, whereby the compound-trimer complex binds to the requisite TNF superfamily receptor and modulates the signalling induced by the trimer through the receptor, wherein the compound-trimer complex binds to an antibody as defined in any one of claims 1 to 40 with a $K_{D\text{-}ab}$ of 1 nM or less.

52. The compound of claim 51, wherein the TNF superfamily member is TNFα.

53. A complex according to claim 47 or 48, a trimer according to claim 49 or 50, or a compound according to claim 51 or 52 for use in a method of therapy practised on the human or animal body.

54. The complex, trimer or compound for use according to claim 53, for use in the treatment and/or prevention of one or more of autoimmune and inflammatory disorders; neurological and neurodegenerative disorders; pain and nociceptive disorders; and cardiovascular disorders.

55. The complex, trimer or compound for use according to claim 54, for use in the treatment and/or prevention of one or more of rheumatoid arthritis, Crohn's disease, psoriasis, systemic lupus erythematosus, Alzheimer's disease, Parkinson's disease and epilepsy.

56. A method of treating and/or preventing one of more of autoimmune and

inflammatory disorders; neurological and neurodegenerative disorders; pain and nociceptive disorders; and cardiovascular disorders, by directly or indirectly administering to a patient in need thereof a complex according to claim 47 or 48, a trimer according to claim 49 or 50, or a compound according to claim 51 or 52.

57. The method of claim 56, wherein one or more of rheumatoid arthritis, Crohn's disease, psoriasis, systemic lupus erythematosus, Alzheimer's disease, Parkinson's disease and epilepsy are treated and/or prevented.

58. The complex, trimer or compound for use according to any one of claims 53-55, or the method of claim 56 or 57, wherein the therapy is on the human body or the patient is a human.

59. A method of identifying a compound that is capable of binding to a trimeric protein that is a TNF superfamily member and modulating signalling of the trimeric protein through the receptor, comprising the steps of:

(a) performing a binding assay to measure the binding affinity of a test compound-trimer complex comprising a trimeric protein that is a TNF superfamily member and a test compound to an antibody that selectively binds to said complex;

(b) comparing the binding affinity as measured in step (a) with the binding affinity of a different compound-trimer complex known to bind with high affinity to the antibody referred to in step (a); and

(c) selecting the compound present in the compound-trimer complex of step (a) if its measured binding affinity is acceptable when considered in the light of the comparison referred to in step (b).

60. The method of claim 59, wherein the antibody binds selectively to the TNF trimer-compound complex compared with binding to the compound in the absence of the TNF superfamily member trimer and/or binding to the TNF superfamily member trimer in the absence of the compound.

61. The method of claim 60, wherein the antibody binds to the TNF superfamily member trimer-compound complex with a $K_{D\text{-}ab}$ that is at least 100 times lower than the $K_{D\text{-}ab}$ for binding to the TNF superfamily member trimer in the absence of compound and/or for binding to the compound in the absence of the TNF superfamily member trimer.

62. The method of claim 61, wherein the antibody binds to the TNF superfamily member trimer-compound complex with a $K_{D\text{-}ab}$ that is at least 200 times lower than the $K_{D\text{-}ab}$ for binding to the TNF superfamily member trimer in the absence of compound and/or for binding to the compound in the absence of the TNF superfamily member trimer.

63. The method of any one of claims 59 to 62, wherein the antibody is as defined in any one of claims 1 to 40.

64. The method of any one of claims 59 to 63, which is a high throughput assay.

65. The method of any one of claims 59 to 64, wherein the test compound increases the stability of the trimeric form of the TNF superfamily member compared to the stability of the trimeric form of the TNF superfamily member in the absence of the compound.

66. The method of claim 65, wherein the increase in stability results in an increase in the thermal transition midpoint ($T_m$) of the trimeric form of the TNF superfamily member of at least 1 °C.

67. The method of claim 66, wherein the increase in stability results in an increase in the thermal transition midpoint ($T_m$) of the trimeric form of the TNF superfamily member of at least 10 °C.

68. The method of claim 67, wherein the increase in the $T_m$ of the trimeric form of the TNF superfamily member is between 10 °C and 20 °C.

69. The method of any one claims 59 to 68, wherein the test compound increases the binding affinity of the TNF superfamily member to the requisite receptor compared to the binding affinity of the TNF superfamily member to its receptor in the absence of the compound.

70. The method of claim 69, wherein the test compound increases the binding affinity of the TNF superfamily member to the requisite receptor by increasing the on rate ($k_{on-r}$) and/or decreasing the off rate ($k_{off-r}$) compared to the $k_{on-r}$ and $k_{off-r}$ values for binding of the TNF superfamily member to its receptor in the absence of the compound.

71. The method of claim 69, wherein the test compound increases the binding affinity of the TNF superfamily member to the requisite receptor by increasing the on rate ($k_{on-r}$) compared to the $k_{on-r}$ value for binding of the TNF superfamily member to its receptor in the absence of the compound.

72. The method of claim 69, 70 or 71, wherein the test compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound, wherein:

a) the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor by at least 10 times compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound;

b) the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite receptor in the presence of the compound is less than 10 nM.

73. The method of claim 69, 70 or 71, wherein the test compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound, wherein:

a) the compound decreases the $K_{D-r}$ of the TNF superfamily member to the requisite receptor by at least 4 times compared to the $K_{D-r}$ of the TNF superfamily member to its receptor in the absence of the compound;

b) the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite

receptor in the presence of the compound is less than 600 pM.

74. The method of claim 73, wherein the $K_{D-r}$ value of the TNF superfamily member for binding to the requisite receptor in the presence of the compound is less than 200 pM.

75. The method of any one claims 59 to 74, wherein said test compound has an $IC_{50}$ value of 500 nM or less.

76. The method of any one of claims 59 to 75, wherein the compound in step (b) is selected from the group consisting of compounds (1)-(6), or salts or solvates thereof.

77. The method of any one of claims 59 to 76, wherein the TNF superfamily member is TNFα and the receptor is the TNF receptor.

Fig. 1

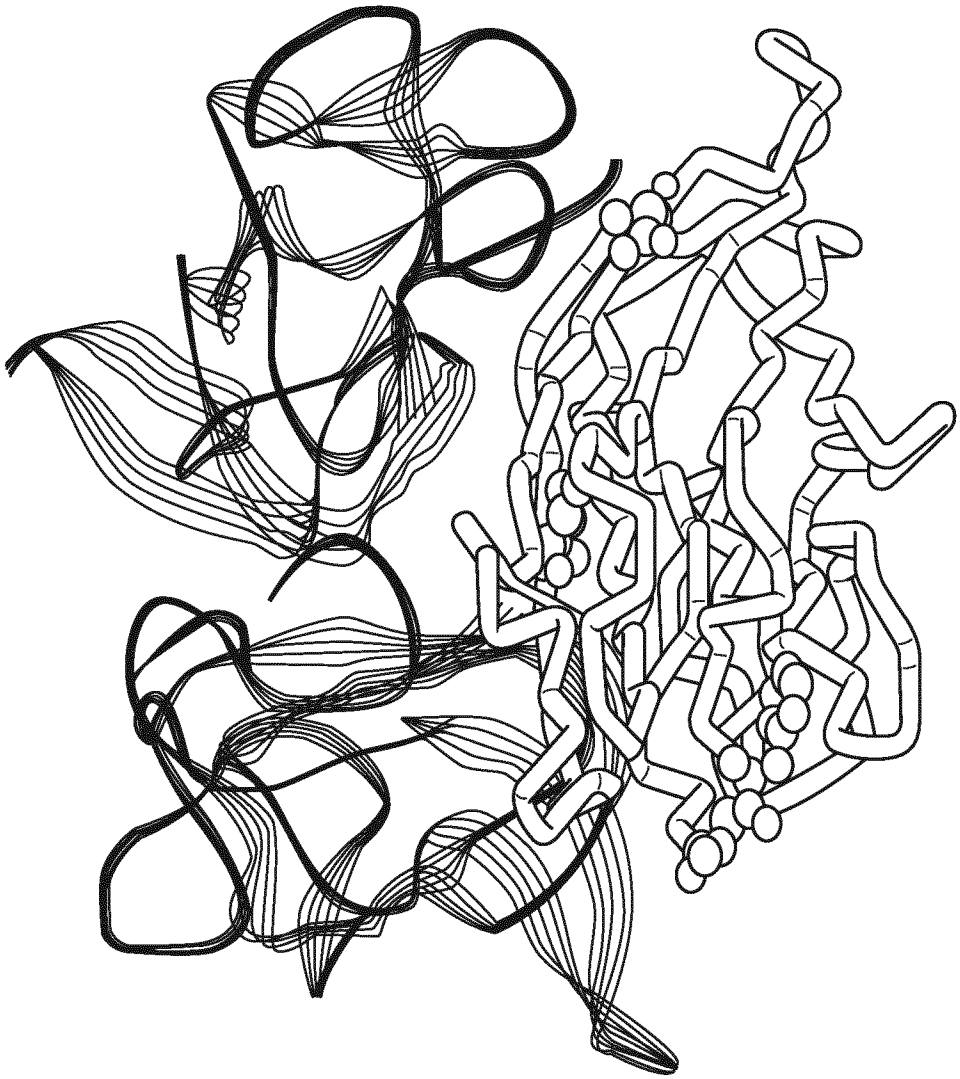
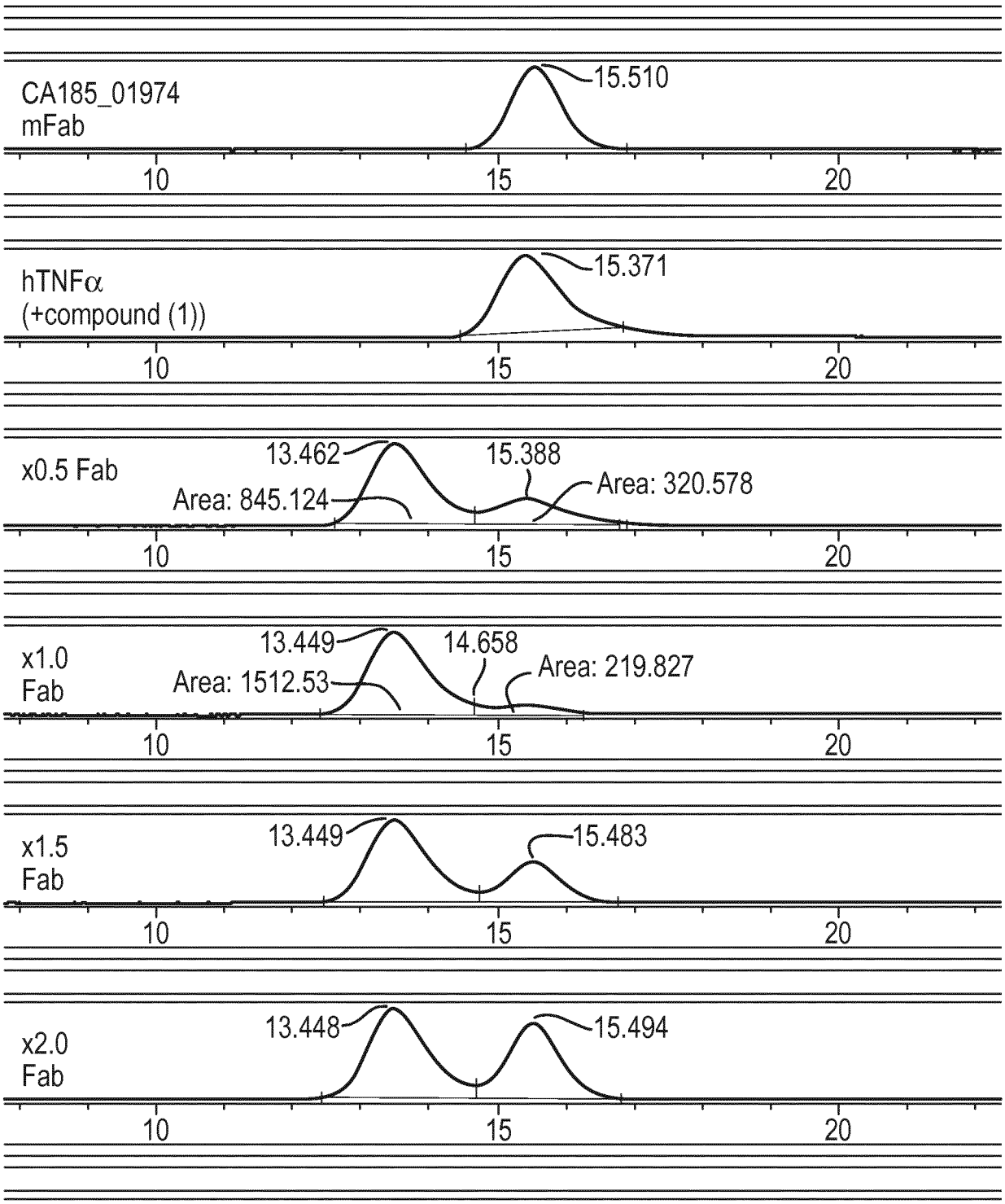


Fig. 2


CA185_01974
mFab
15.510
10
15
20
hTNFα
(+compound (1))
15.371
10
15
20
x0.5 Fab
13.462
15.388
Area: 845.124
Area: 320.578
10
15
20
x1.0
Fab
13.449
14.658
Area: 1512.53
Area: 219.827
10
15
20
x1.5
Fab
13.449
15.483
10
15
20
x2.0
Fab
13.448
15.494
10
15
20

# Fig. 3

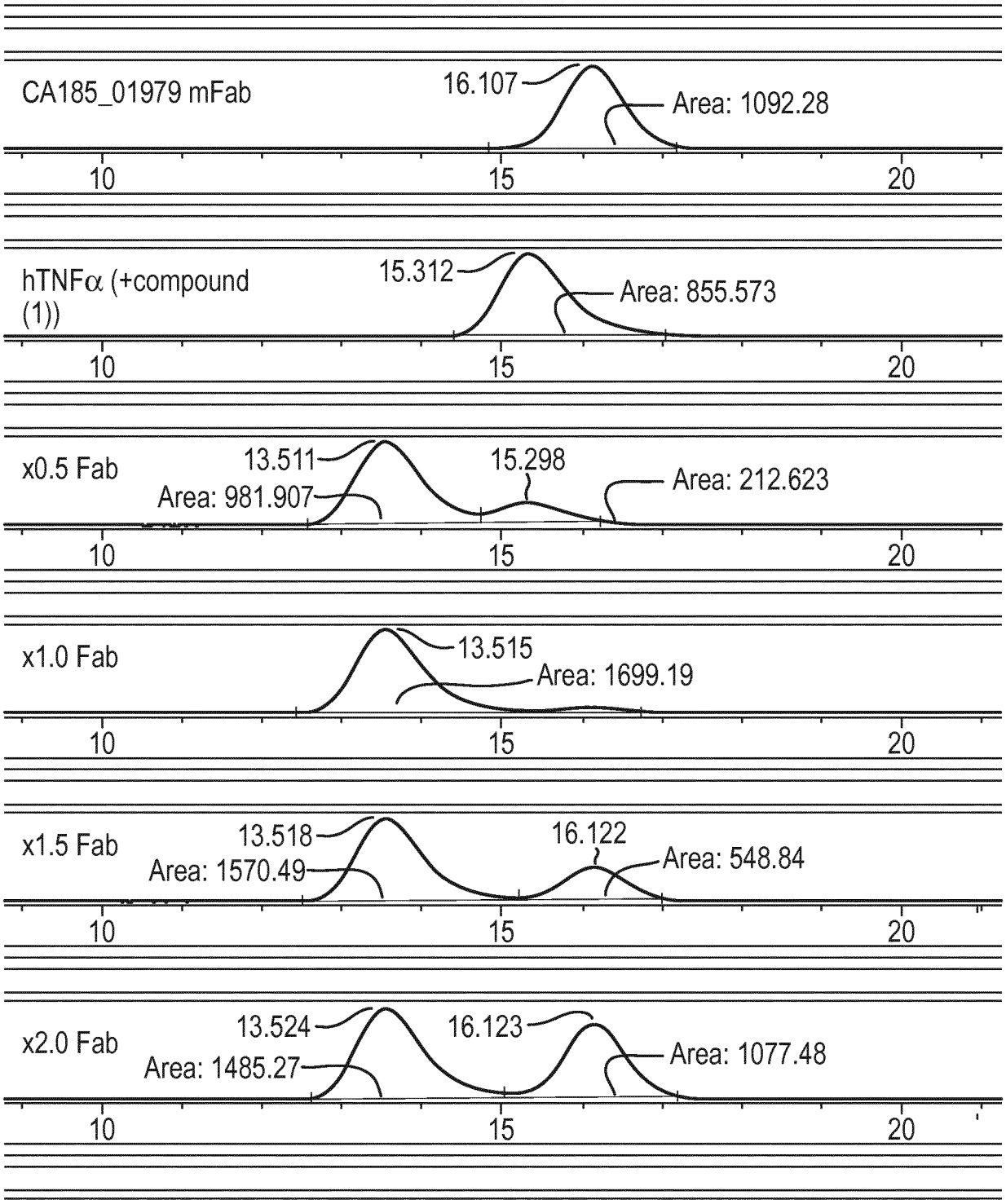

CA185_01979 mFab
16.107
Area: 1092.28
10
15
20
hTNFα (+compound (1))
15.312
Area: 855.573
10
15
20
x0.5 Fab
13.511
Area: 981.907
15.298
Area: 212.623
10
15
20
x1.0 Fab
13.515
Area: 1699.19
10
15
20
x1.5 Fab
13.518
Area: 1570.49
16.122
Area: 548.84
10
15
20
x2.0 Fab
13.524
Area: 1485.27
16.123
Area: 1077.48
10
15
20

Fig. 4

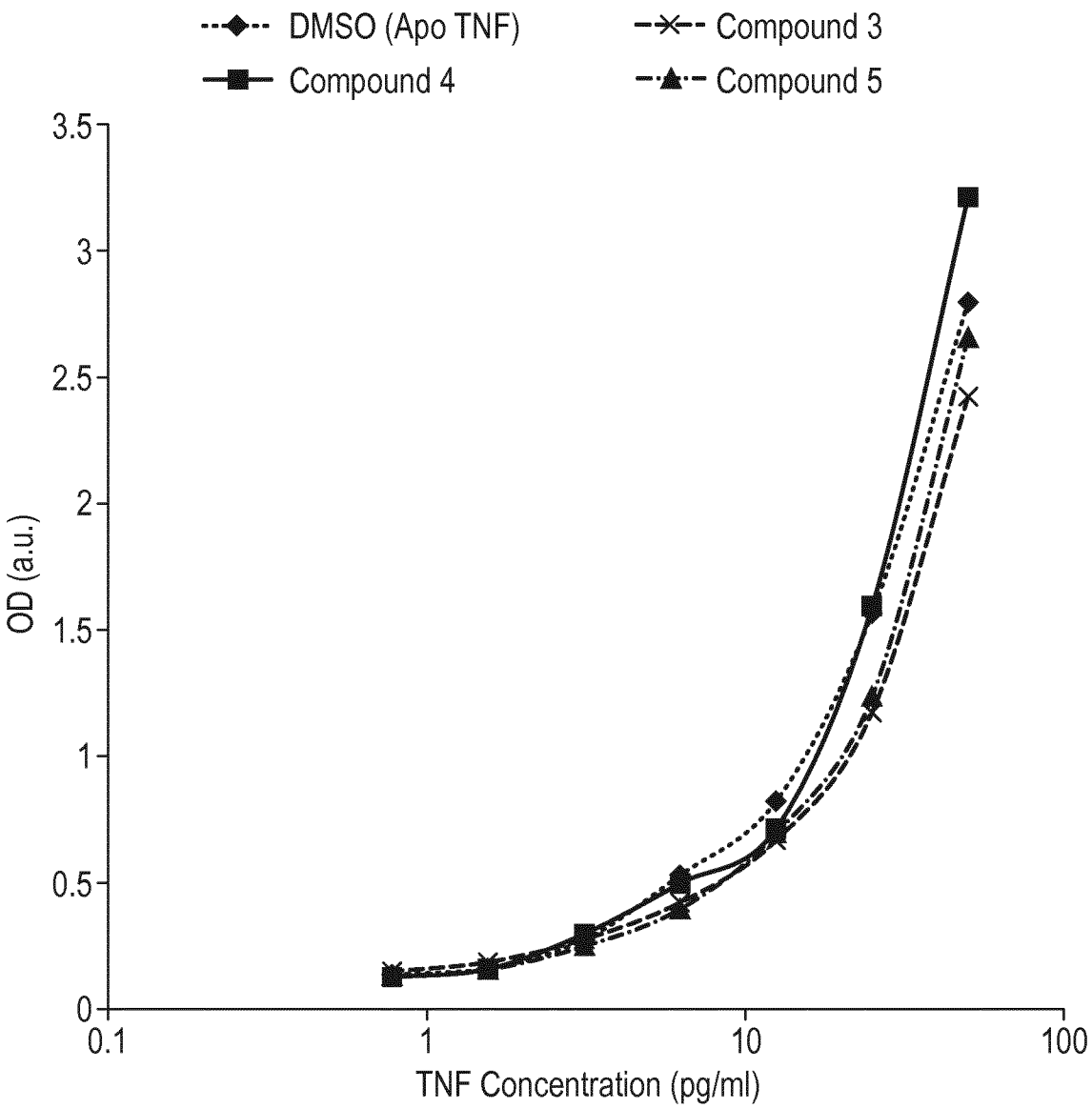

DMSO (Apo TNF)
Compound 3
Compound 4
Compound 5
OD (a.u.)
3.5
3
2.5
2
1.5
1
0.5
0
0.1
1
10
100
TNF Concentration (pg/ml)

Fig. 5

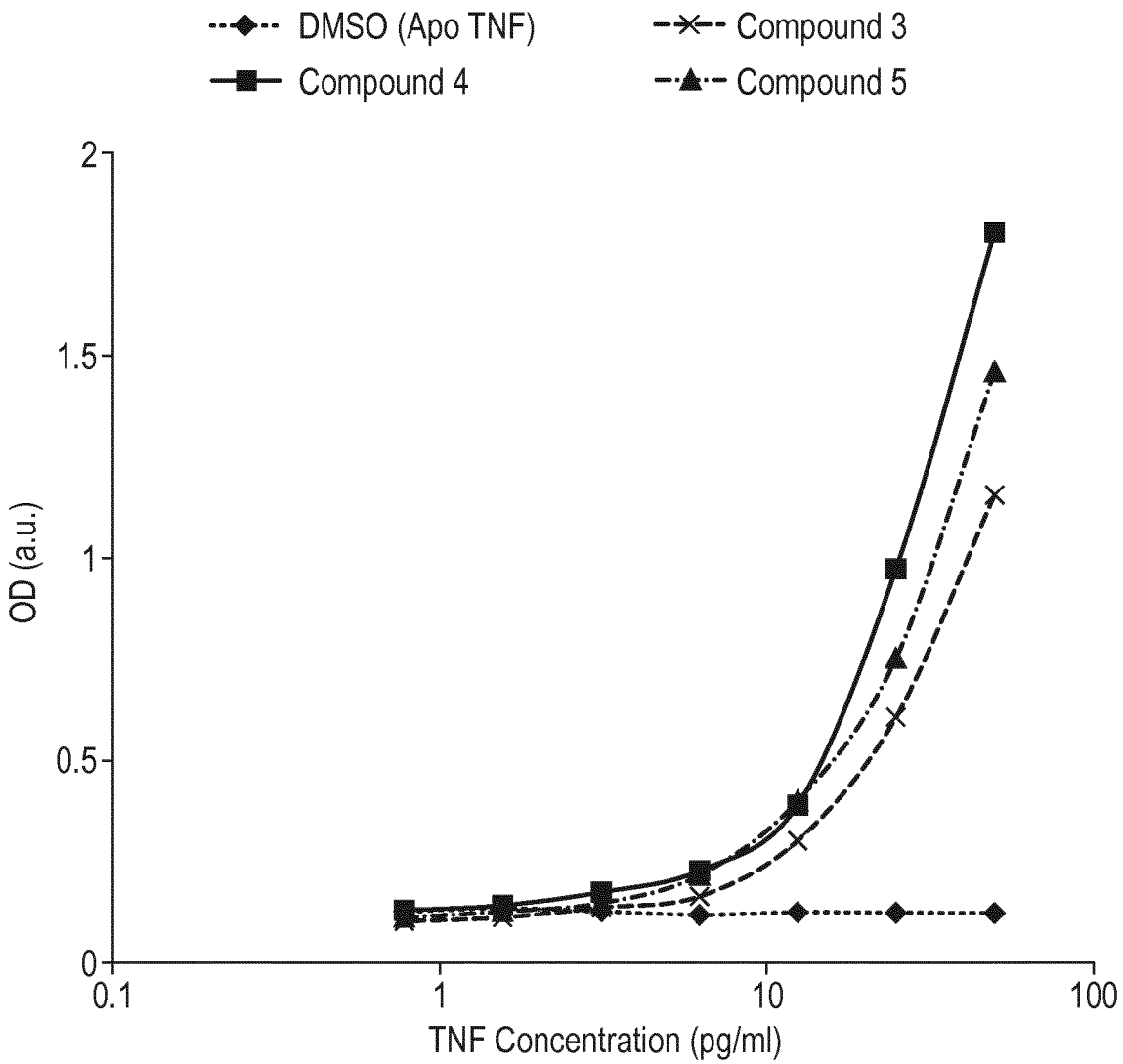

DMSO (Apo TNF)
Compound 3
Compound 4
Compound 5
OD (a.u.)
2
1.5
1
0.5
0
0.1
1
10
100
TNF Concentration (pg/ml)

Fig. 6

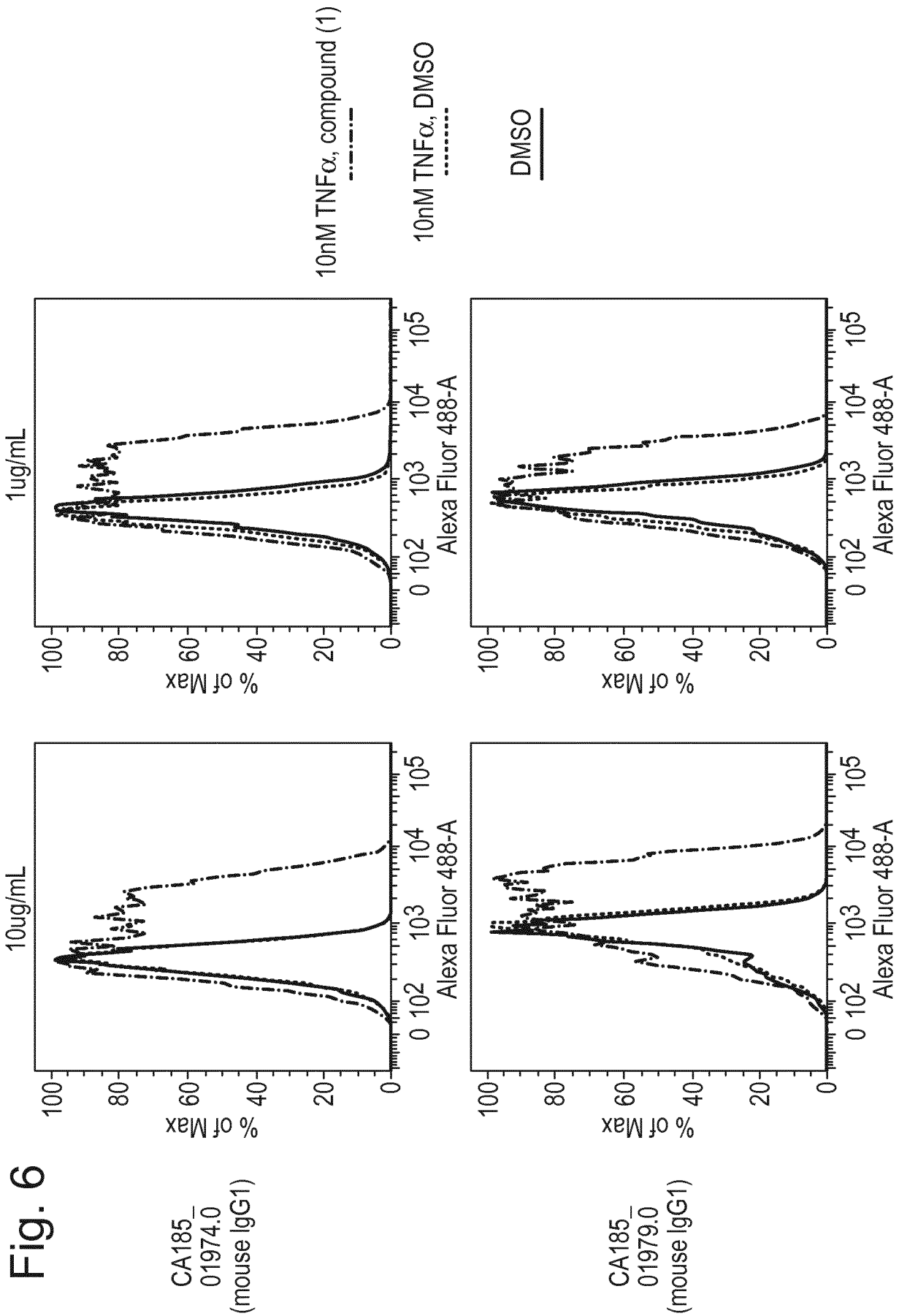
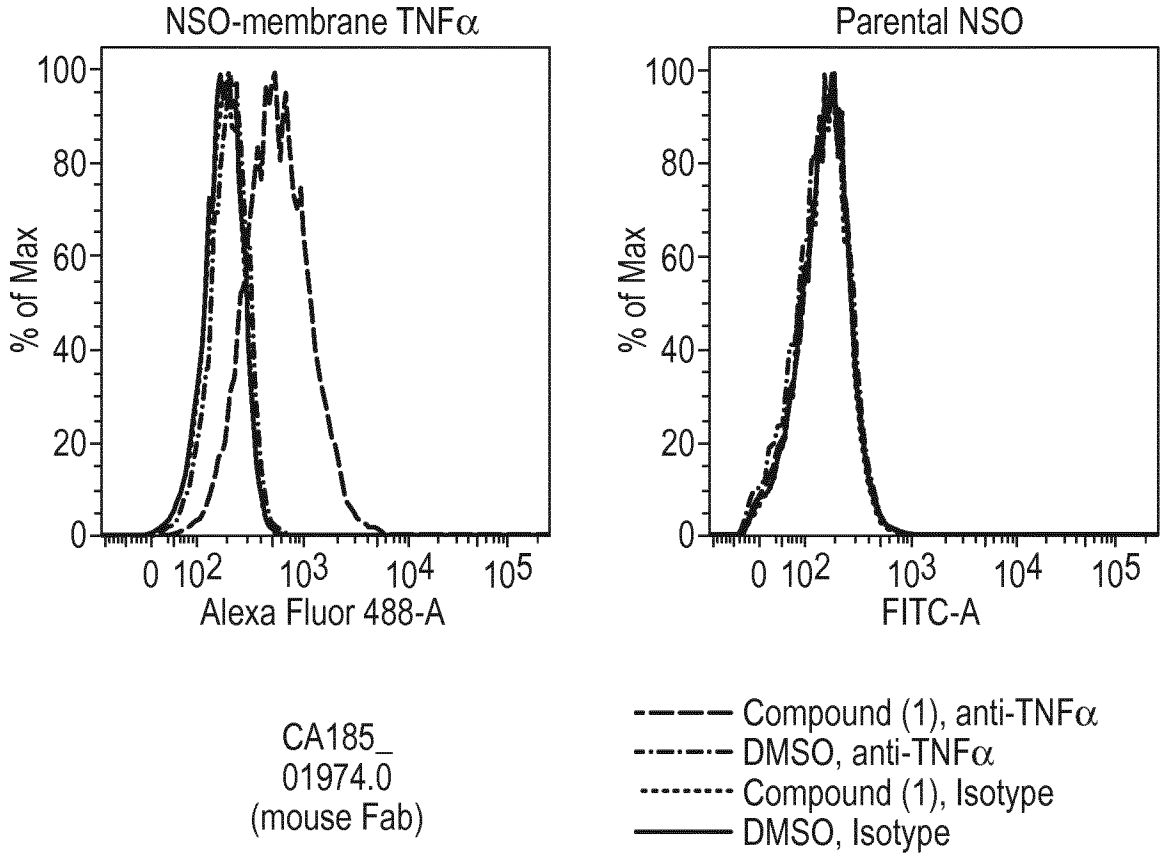
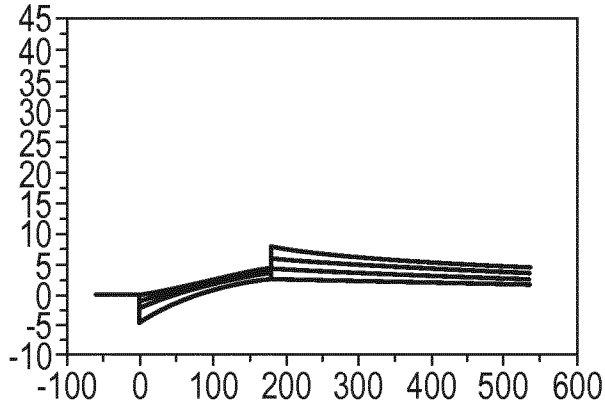

10nM TNFα, compound (1)
10nM TNFα, DMSO
DMSO
1ug/mL
10ug/mL
CA185_01974.0 (mouse IgG1)
CA185_01979.0 (mouse IgG1)
% of Max
Alexa Fluor 488-A
0
20
40
60
80
100
0
10^2
10^3
10^4
10^5

Fig. 7


NSO-membrane TNFα
Parental NSO
100
80
60
40
20
0
% of Max
0 10^2 10^3 10^4 10^5
Alexa Fluor 488-A
FITC-A
CA185_
01974.0
(mouse Fab)
Compound (1), anti-TNFα
DMSO, anti-TNFα
Compound (1), Isotype
DMSO, Isotype

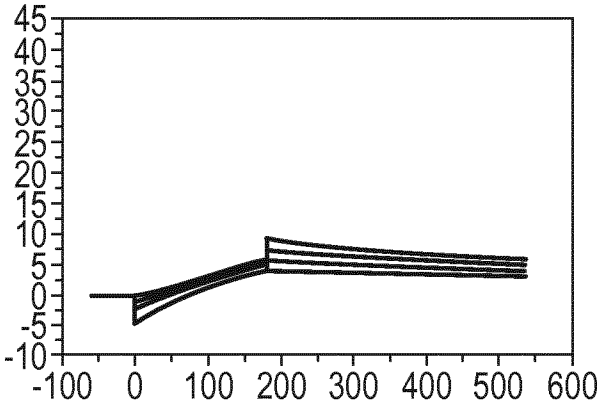
# Fig. 8

**File:** 13090601T
**Item:** 1974 cyno TNF control 1
**Ligand:** CA185_1974
**Curve:** Fe=2-1
**Sample:** cyno
**Temperature** 25 °C



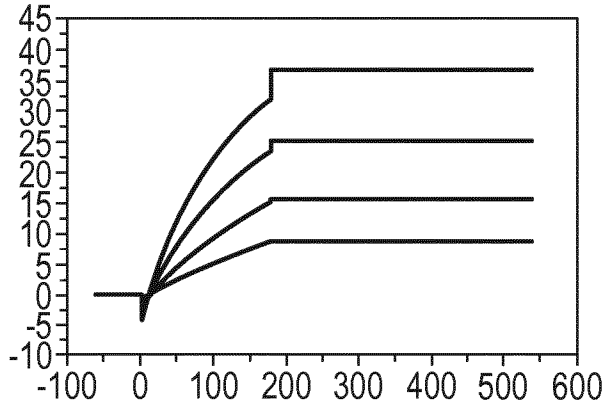

**Fit:** 1:1: Binding
**Ka(1/Ms):** 1.025E+5
**Kd (1/s):** 0.001872

**File:** 13090601T
**Item:** 1974 cyno TNF control 2
**Ligand:** CA185_1974
**Curve:** Fe=2-1
**Sample:** cyno
**Temperature** 25 °C



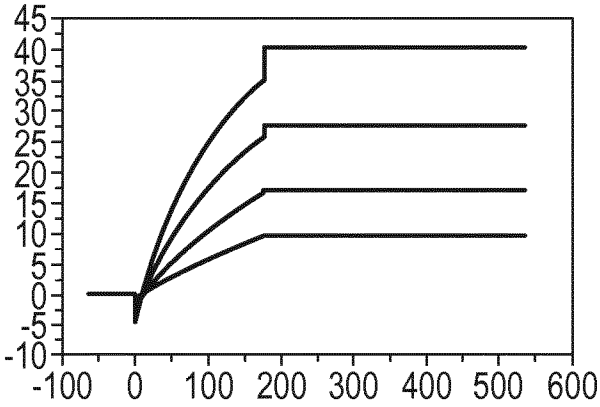

**Fit:** 1:1: Binding
**Ka(1/Ms):** 1.253E+5
**Kd (1/s):** 0.001923

**File:** 13090601T
**Item:** 1974 cyno TNF+2080 1
**Ligand:** CA185_1974
**Curve:** Fe=2-1
**Sample:** cyno+NCE
**Temperature** 25 °C




**Fit:** 1:1: Binding
**Ka(1/Ms):** 1.842E+5
**Kd (1/s):** 1.457E-5

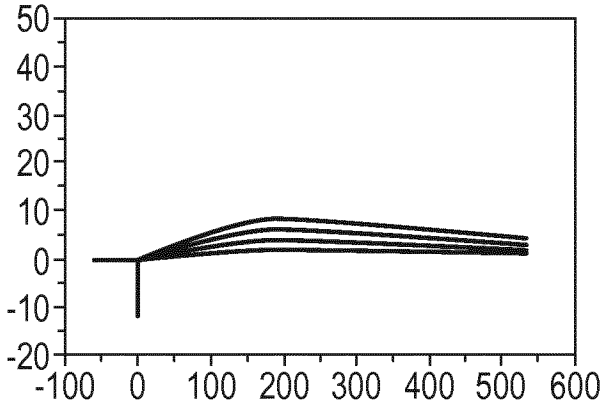
**File:** 13090601T
**Item:** 1974 cyno TNF+2080 2
**Ligand:** CA185_1974
**Curve:** Fe=2-1
**Sample:** cyno+NCE
**Temperature** 25 °C




**Fit:** 1:1: Binding
**Ka(1/Ms):** 2.008E+5
**Kd (1/s):** 2.053E-5

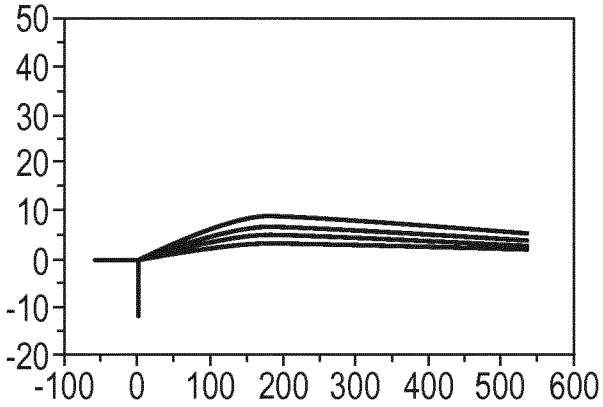
# Fig. 9

**File:** 13090601T
**Item:** 1974 human TNF control 1
**Ligand:** CA185_1974
**Curve:** Fe=4-3
**Sample:** human
**Temperature** 25 °C



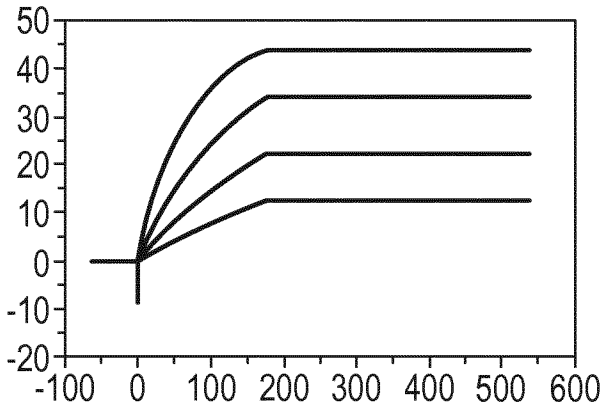

**Fit:** 1:1: Binding
**Ka(1/Ms):** 8.023E+4
**Kd (1/s):** 0.001773

**File:** 13090601T
**Item:** 1974 human TNF control 2
**Ligand:** CA185_1974
**Curve:** Fe=4-3
**Sample:** human
**Temperature** 25 °C



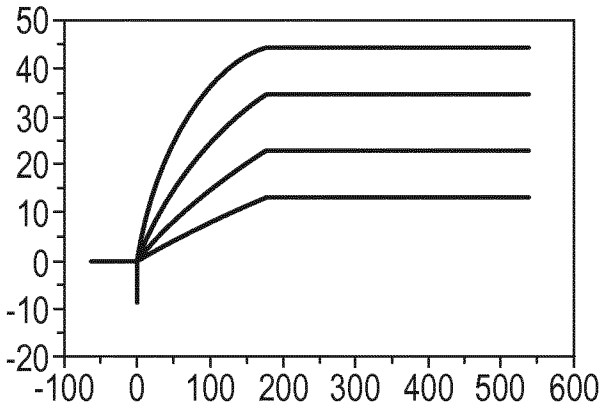

**Fit:** 1:1: Binding
**Ka(1/Ms):** 1.051E+5
**Kd (1/s):** 0.001671

**File:** 13090601T
**Item:** 1974 human TNF+2080 1
**Ligand:** CA185_1974
**Curve:** Fe=4-3
**Sample:** human+NCE
**Temperature** 25 °C



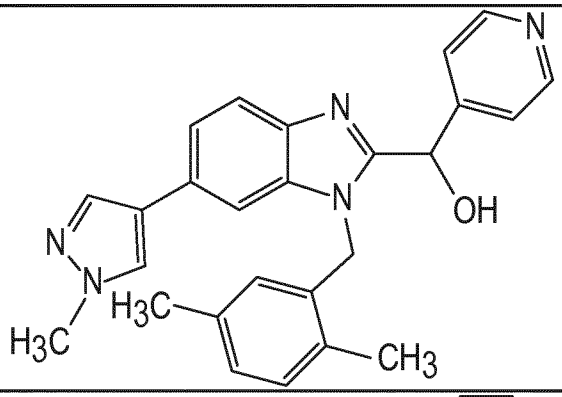
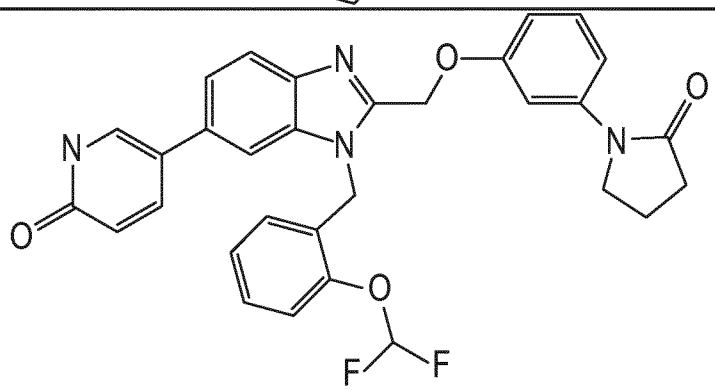
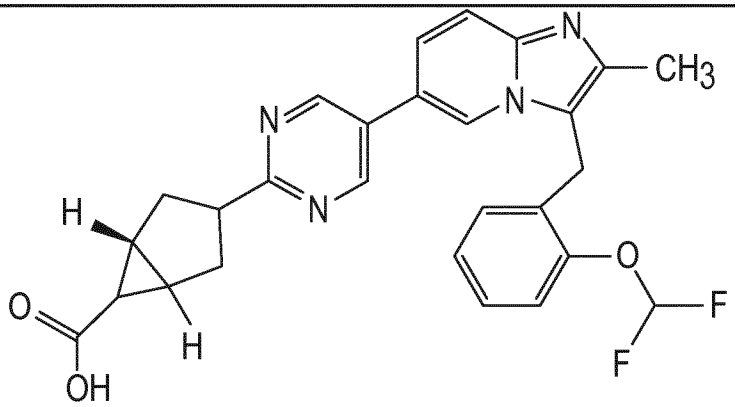
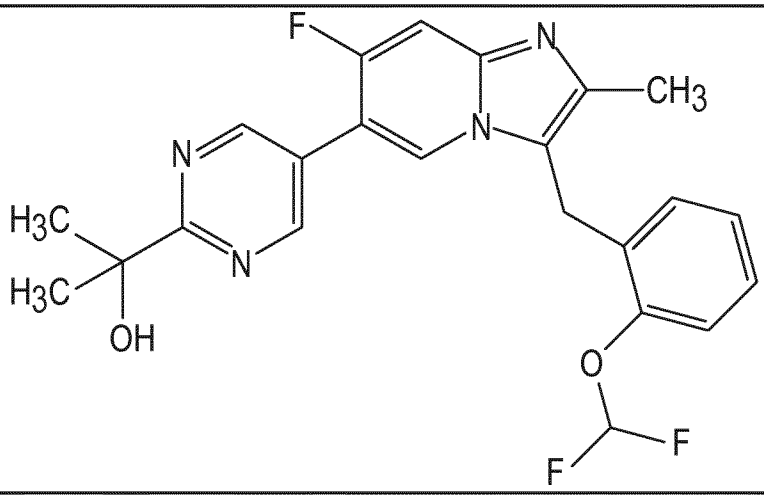

**Fit:** 1:1: Binding
**Ka(1/Ms):** 3.053E+5
**Kd (1/s):** 1.477E-7

**File:** 13090601T
**Item:** 1974 human TNF+2080 2
**Ligand:** CA185_1974
**Curve:** Fe=4-3
**Sample:** human+NCE
**Temperature** 25 °C

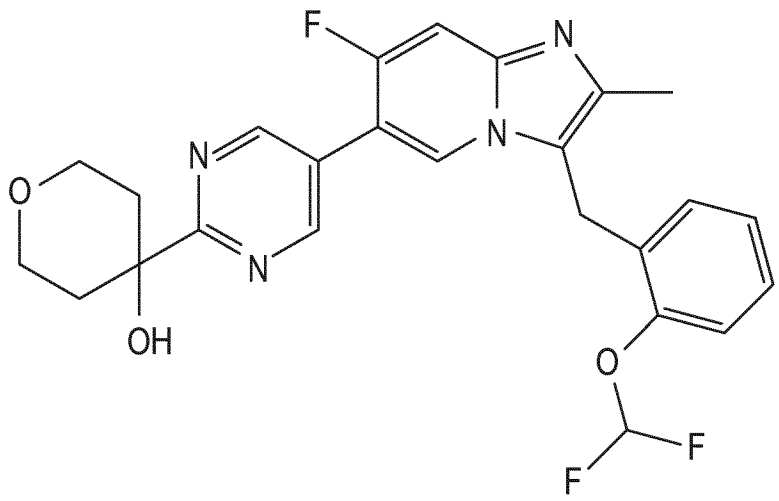
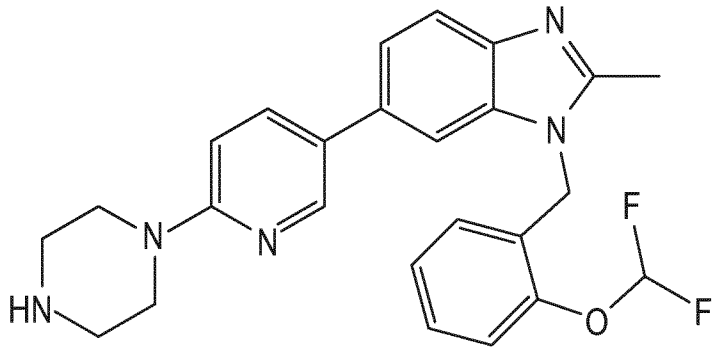



**Fit:** 1:1: Binding
**Ka(1/Ms):** 3.069E+5
**Kd (1/s):** 2.726E-5

# Fig. 10

| Compound | Stucture |
|---|---|
| 1 |  |
| 2 |  |
| 3 |  |
| 4 |  |

# Fig. 10 (Cont.)

| Compound | Stucture |
|---|---|
| 5 |  |
| 6 |  |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/074527

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 C07K14/705 C07K14/525 C07D401/14 C07D471/00 C07K16/24
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07D C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SHI HU ET AL: "COMPARISON OF THE INHIBITION MECHANISMS OF ADALIMUMAB AND INFLIXIMAB IN TREATING TUMOR NECROSIS FACTOR ALFA-ASSOCIATED DISEASES FROM A MOLECULAR VIEW", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US , vol. 288, no. 38 20 September 2013 (2013-09-20), pages 27059-27067, XP003033999, ISSN: 0021-9258, DOI: 10.1074/JBC.M113.491530 Retrieved from the Internet: URL:http://www.jbc.org/content/288/38/27059 [retrieved on 2013-08-13] | 1-28, 38-43, 49,50 |
| Y | the whole document | 44-46 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 11 May 2016 | 20/05/2016 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | R. von Eggelkraut-G. |

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | | 29-37 |
| | ----- | |
| X | TRACEY ET AL: "Tumor necrosis factor antagonist mechanisms of action: A comprehensive review", PHARMACOLOGY AND THERAPEUTICS, ELSEVIER, GB, vol. 117, no. 2, 26 October 2007 (2007-10-26), pages 244-279, XP022432143, ISSN: 0163-7258, DOI: 10.1016/J.PHARMTHERA.2007.10.001 | 1-28, 38-43, 49,50 |
| Y | the whole document | 44-46 |
| A | | 29-37 |
| | ----- | |
| X | US 2009/022659 A1 (OLSON KATIE [US] ET AL) 22 January 2009 (2009-01-22) | 1-28, 38-43, 49,50 |
| Y | p. 1, paragraph [0011] - p. 2, paragraph | 44-46 |
| A | [0016], p. 7, paragraph [0077] - p. 45, paragraph [0484], p. 55, paragraph [0625] - p. 57, paragraph [0646], claims 1-132 | 29-37 |
| | ----- | |
| X | WO 2014/009295 A1 (UCB PHARMA SA [BE]) 16 January 2014 (2014-01-16) cited in the application p. 1, line 1 - p. 103, line 14; Ex. 4, Ex. 326, claims 1-19 | 47-58 |
| | ----- | |
| X | WO 2013/186229 A1 (UCB PHARMA SA [BE]) 19 December 2013 (2013-12-19) p. 1, line 1 - p. 89, line 21, Ex. 44, 29, 89, 156, 490, claims 1-17 | 47-58 |
| | ----- | |
| Y | WO 2014/001557 A1 (UCB PHARMA SA [BE]) 3 January 2014 (2014-01-03) p. 1, line 1 - p. 16, line 15, claims 1-25 | 44-46 |
| | ----- | |

2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/074527

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-58

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-58

An antibody binding to a complex comprising a trimeric protein that is a TNF superfamily member and a compound that is capable of binding to the trimeric TNF superfamily member, thereby changing the conformation of the trimeric TNF superfamily member and modulating the signalling of its receptor, as well as its uses

---

2. claims: 59-77

Screening methods for identifying a compound that is capable of binding to a trimeric TNF superfamily member and modulating the signalling of its receptor

---

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/074527

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2009022659 A1 | 22-01-2009 | TW 201531484 A | 16-08-2015 |
| | | US 2009022659 A1 | 22-01-2009 |
| | | US 2015337035 A1 | 26-11-2015 |
| | | WO 2008144753 A2 | 27-11-2008 |
| WO 2014009295 A1 | 16-01-2014 | CA 2877550 A1 | 16-01-2014 |
| | | CN 104619709 A | 13-05-2015 |
| | | EA 201500130 A1 | 30-07-2015 |
| | | EP 2872508 A1 | 20-05-2015 |
| | | JP 2015522051 A | 03-08-2015 |
| | | US 2015203486 A1 | 23-07-2015 |
| | | WO 2014009295 A1 | 16-01-2014 |
| WO 2013186229 A1 | 19-12-2013 | CA 2874303 A1 | 19-12-2013 |
| | | CN 104428293 A | 18-03-2015 |
| | | EA 201401350 A1 | 29-05-2015 |
| | | EP 2858983 A1 | 15-04-2015 |
| | | JP 2015519381 A | 09-07-2015 |
| | | US 2015152065 A1 | 04-06-2015 |
| | | WO 2013186229 A1 | 19-12-2013 |
| WO 2014001557 A1 | 03-01-2014 | EP 2867674 A1 | 06-05-2015 |
| | | US 2015219635 A1 | 06-08-2015 |
| | | WO 2014001557 A1 | 03-01-2014 |