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# DESCRIPTION

## TECHNICAL FIELD

**[0001]** The invention is directed to TREM-1 mAbs and to mutation of specific negatively charged and uncharged amino acids involved in either TREM-1 mAb self-interactions or TREM-1 mAb to TREM-1 interactions in order to lower the mAb solution viscosity and retain target affinity, and the invention relates to such antibodies for therapeutic use as recited in the claims.

## SEQUENCE LISTING

### [0002]

SEQ ID NO: 1 represents the amino acid sequence of wild type (wt) human TREM-1.

SEQ ID NO: 2 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0170 of WO2013/120553).

SEQ ID NO: 3 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0170 of WO2013/120553).

SEQ ID NO: 4 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0317, E27Q, E97S).

SEQ ID NO: 5 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0318, E27Q, E97Q).

SEQ ID NO: 6 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0319, E97S).

SEQ ID NO: 7 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0320, E97Q).

SEQ ID NO: 8 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0321, E27Q).

SEQ ID NO: 9 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0322, F32A).

SEQ ID NO: 10 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0323, F32S).

SEQ ID NO: 11 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0324, A59Y).

SEQ ID NO: 12 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0325, N57S).

SEQ ID NO: 13 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0326, A59Y, N57S).

SEQ ID NO: 14 represents the amino acid sequence of the light chain of a humanised TREM-1 antibody (mAb 0330, F32A, E27Q, E97Q).

SEQ ID NO: 15 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0332, A59Y; 0332 is a mAb combined of SEQ ID NO: 15 as the HC and SEQ ID NO 5 as the LC, Table 1).

SEQ ID NO: 16 represents the amino acid sequence of the heavy chain of a humanised TREM-1 antibody (mAb 0333, A59Y; 0333 is a mAb combined of SEQ ID NO: 16 as the HC and SEQ ID NO 14 as the LC, Table 1).

SEQ ID NO: 17 represents the amino acid sequence of full length cTREM-1.

SEQ ID NO 18 represents the heavy chain of the Fab region of mAb 0170.

SEQ ID NO 19 represents the light chain of the Fab region of mAb 0170.

## BACKGROUND

**[0003]** TREM-1 is an activating receptor expressed on monocytes, macrophages and neutrophils. These cells play a central role in chronic inflammatory diseases by releasing cytokines and other mediators that drive inflammation. TREM-1 mRNA and protein expression is up-regulated in patients with RA and IBD, and TREM-1-positive cells accumulate at sites of inflammation, correlating with disease severity. Peptidoglycan-recognition-protein 1 (PGLYRP1) expressed primarily by activated neutrophils is a ligand for TREM-1 and mediate TREM-1 signalling upon binding.

**[0004]** *In vitro*, engagement of TREM-1 triggers secretion of pro-inflammatory cytokines including TNF, IL-8, and monocyte chemotactic protein-1. In addition, TREM-1 signalling synergizes with multiple Toll-like Receptors (TLR) to further boost pro-inflammatory signals. In turn, this up-regulates expression of TREM-1, leading to a vicious cycle amplifying the inflammation. Increasing evidence indicates that TLRs contribute to the development and progression of chronic inflammatory diseases such as RA and IBD.

**[0005]** WO 2013/120553 discloses humanized anti-TREM-1 mAbs which inhibit both human and cynomolgus TREM-1 function. However, the viscosity profile of anti-TREM-1 mAbs may hamper the manufacturing process to produce a drug product at >50 mg/ml and could limit the optimal dose setting in the clinic. High dosage (several mg/kg) of protein therapeutics is often needed to achieve an adequate clinical effect and since the vast majority of these therapeutics are administered by subcutaneous delivery, the consequence is that patient self-administration of the therapeutic is limited to volumes of < 1.5 mL (Shire et al., J. Pharm. Sci. 2004, 93, 1390-1402). The development of high concentration protein formulations suitable for patient self-administration is a general obstacle for manufacturing and delivery when protein formulation results in high viscosity of the resultant solution.

**[0006]** Charge distribution of mAbs has been studied with regards to the effect on the viscosity behaviour of mAb solutions (Yadav et al., Mol. Pharmaceutics 2012, 9, 791-802). Also, weak non-specific charge interactions that persist in dilute solutions have been shown to influence the viscosity of concentrated mAb solutions (Connolly et al., Biophys. J., 2012, 103, 69-78.). The remedies to reduce the viscosity of mAb solutions have been to introduce site-directed charge swap mutations that disrupt direct charge-charge intermolecular interactions (Yadav et al., Mol. Pharmaceutics 2012, 9, 791-802) or the addition of salts or counter ions (Liu et al., J. Pharm. Sci. 2005, 94, 1928-1940; Yadav et al., J. Pharm. Sci. 2010, 99, 1152-1168; Yadav et al., J. Pharm. Sci. 2012, 101, 998-1011; Kanai et al., J. Pharm. Sci. 2008, 97, 4219-4227). The addition of salts and counter ions can, however, result in adverse effects for the patient in terms of hyper-osmolality of the administered solution.

**[0007]** WO 2013/120553 describes antibodies that bind and block triggering receptor expressed on myeloid cells-1 (TREM-1).

**[0008]** WO 2014/072876 describes platelet-derived growth factor b specific antibodies and compositions and uses thereof.

**[0009]** Ketchum Randal et al., American Chemical Society. Abstracts of papers (at the national meeting). American Chemical Society, US, Vol 243 (2012-03-25) describes mitigation of monoclonal antibody viscosity by modification of protein surface charge.

**[0010]** Anuj Chaudhri et al. The Journal of physical chemistry B, Vol 117, No.5 (2013-02-07), pages 1269-1279

describes the role of amino acid sequence in the self-association of therapeutic monoclonal antibodies: insights from coarse-grained modeling.

**[0011]** Sandeep Yadav et al., Pharmaceutical research, Vol 28, no. 7 (2011-04-06), pages 1750-1764 describes establishing a link between amino acid sequences and self-associating and viscoelastic behavior of two closely related monoclonal antibodies.

**[0012]** D. Bethea et al., Protein engineering design and selection, Vol. 25, no.10 (2012-08-22), pages 531-538 describes mechanisms of self-association of a human monoclonal antibody CNTO607.

**[0013]** Tim J Kamerzell et al., Advanced drug delivery reviews, Vol 63. No. 13, (2011-07-26), pages 1118-1159 describes protein excipient interactions: mechanisms and biophysical characterization applied to protein formulation development.

**[0014]** Jan Jezek et al., Advanced drug delivery reviews, Vol 63. No 13 (2011-09-02), pages 1107-1117 describes viscosity of concentrated therapeutic protein compositions.

**[0015]** Anja Drescher, Internet citation posted on 27 June 2011 describes characterization of biological interactions with Biacore.

**[0016]** Disclosed herein are TREM-1 antibodies generated by site-specific mutation of the CDR's of the WO 2013/120553 disclosed humanized anti-TREM-1 mAb 0170. The disclosed antibodies do not disrupt direct mAb intermolecular charge-charge self-interactions but do have a favourable viscosity profile and maintained target binding profile. The favourable viscosity profile allows drug product to be produced at high concentrations that could be essential for therapeutic and pharmaceutical use. Such antibodies may have a substantial impact upon the quality of life of individuals with sepsis or a chronic inflammatory disease such as rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease.

## SUMMARY

**[0017]** The present invention is defined in the independent claims and certain optional features thereof are defined in the dependent claims. The technical information set out below may in some respects go beyond the scope of the invention, which is defined exclusively by the appended claims. The additional technical information is provided to place the actual invention in a broader technical context, and to illustrate possible related technical developments. In so far as the terms "invention", "example" and "embodiment" are used herein, these terms will be interpreted in such a way that protection does not extend beyond the scope of the claims. Any references to methods for treatment of the human or animal body by surgery or therapy and diagnostic methods practices on the human or animal body are not to be construed as claiming protection for such methods as such but are instead to be construed as referring to products, in particular substances or compositions for use in any of these methods.

**[0018]** In one aspect, the present invention provides an antibody that is capable of binding to and blocking TREM-1, comprising:

1. (i) a heavy chain, comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO:4;
2. (ii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO:5;
3. (iii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO:6;
4. (iv) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO:7;
5. (v) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO:8;
6. (vi) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ

ID NO:9;

7. (vii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO: 10;
8. (viii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO: 14;
9. (ix) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 11 and a light chain comprising SEQ ID NO:3;
10. (x) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 12 and a light chain comprising SEQ ID NO:3;
11. (xi) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 13 and a light chain comprising SEQ ID NO:3;
12. (xii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 15 and a light chain comprising SEQ ID NO:5; or
13. (xiii) a heavy chain comprising the heavy chain variable region of SEQ ID NO: 16 and a light chain comprising SEQ ID NO: 14;

wherein the heavy chain comprises an IgG4 constant region.

**[0019]** In some embodiments, the antibody of the invention comprises a heavy chain comprising a heavy chain variable region of SEQ ID NO: 2 and a light chain comprising SEQ ID NO: 5.

**[0020]** In a further aspect, the present invention provides a pharmaceutical composition comprising the antibody of the invention (as defined in the claims) and a pharmaceutically acceptable carrier.

**[0021]** Further aspects of the present invention relate to a prokaryotic or eukaryotic cell that recombinantly expresses the antibody of the invention (as defined in the claims); and a method of producing the antibody of the invention, comprising recombinantly expressing the antibody in a prokaryotic or eukaryotic cell.

**[0022]** In a further aspect, the present invention also provides the antibody of the invention (as defined in the claims) or the pharmaceutical composition of the invention (as defined in the claims), for use as a medicament in a subject in need thereof.

**[0023]** In some embodiments, the treatment is of an inflammatory disease or an autoimmune disease in a subject in need thereof, such as an inflammatory disease or autoimmune disease selected from the group consisting of: inflammatory bowel disease, Crohn's disease, ulcerative colitis, irritable bowel syndrome, rheumatoid arthritis, psoriasis, psoriatic arthritis, systemic lupus erythematosus, lupus nephritis, type I diabetes, Grave's disease, multiple sclerosis, autoimmune myocarditis, Kawasaki disease, coronary artery disease, chronic obstructive pulmonary disease, interstitial lung disease, autoimmune thyroiditis, scleroderma, systemic sclerosis, osteoarthritis, atopic dermatitis, vitiligo, graft-versus-host disease, Sjögren's syndrome, autoimmune nephritis, Goodpasture's syndrome, chronic inflammatory demyelinating polyneuropathy, allergy, asthma and other autoimmune diseases that are a result of acute or chronic inflammation. In some embodiments, the antibody or pharmaceutical composition of the invention is administered parenterally. In some embodiments, the antibody or pharmaceutical composition of the invention is administered prophylactically. In some embodiments, the subject is a human.

**[0024]** As demonstrated herein, the anti-TREM-1 mAb of the invention (as defined in the claims) has a viscosity profile in the range expected for monomeric mAbs and blocks TREM-1 function as potently as mAb0170 of WO 2013/120553. Further to the advantageous viscosity profiles of the variant mAbs of the invention, said variants do not show agonism to the TREM-1 receptor.

#### **BRIEF Description of drawings**

**[0025]**

Figure 1 depicts the viscosity profile of anti-TREM-1 mAb variants and their exponential curve fits.

Figure 2 depicts the ability of mAb 0170 variants to inhibit human TREM-1 signalling in human TREM-1 reporter cell line (BVZ'36/hTREM-1) stimulated with PGN and (A) recombinant PGLYRP1 or (B) PGLYRP1 expressed by activated neutrophils (average of N donors).

Figure 3 depicts the ability of mAb 0170 variants to inhibit cynomolgus TREM-1 signalling in cynomolgus TREM-1 reporter cell line (TE426.27) stimulated with PGN and recombinant PGLYRP1.

Figure 4 depicts the ability of mAb 0170 variants to inhibit TNF $\alpha$  release from hypoxic M2 macrophages stimulated with PGN and recombinant PGLYRP1.

Figure 5 depicts the agonistic potential of plate-bound of mAb 0170 variants to induce TNF release from hypoxic M2 macrophages. MAb 1278 (R&D) was used as positive control.

Figure 6 depicts the hydrodynamic radius of the anti-TREM-1 mAb variants compared to mAb 0170.

## DESCRIPTION

**[0026]** TREM-1 is a transmembrane protein that consists of 234 amino acids, including a single extracellular immunoglobulin domain and a short cytoplasmic tail with no apparent signaling motif. When activated, TREM-1 associates with the ITAM-containing signaling adaptor protein, DAP12. Downstream signalling may include activation of the NFAT transcription factor, causing an up-regulation of pro-inflammatory cytokine production.

**[0027]** The present invention relates to antibodies that are capable of specifically binding and blocking the function of TREM-1, as defined in the claims. Antibodies of the invention may block TREM-1 function by reducing/blocking TREM-1 activation and downstream signalling.

**[0028]** Antibodies according to the invention may block TREM-1 by means of one or a combination of several different mechanisms, blocking TREM-1 directly or indirectly. For example, antibodies of the invention may prevent the natural ligand of TREM-1, peptidoglycan recognition protein 1 (PGLYRP1), from creating a functional complex with TREM-1 and/or antibodies of the invention may block TREM-1 by preventing individual TREM-1 molecules from forming dimers or multimers. TREM-1 dimerisation or multimerisation may be reduced or prevented by TREM-1 antibodies that are capable of binding to a portion of TREM-1 that would otherwise reside in the interface of a TREM-1 dimer, thus preventing individual TREM-1 molecules from associating with one another.

**[0029]** TREM-1 dimerisation or multimerisation may be reduced or prevented by TREM-1 antibodies that interfere with the interaction of TREM-1 with its ligand. Antibodies according to the current invention may block PGLYRP1-induced activation of TREM-1. PGLYRP1, a highly conserved, 196 amino acid long protein consisting of a signal peptide and a peptidoglycan binding domain, is expressed in neutrophils and released upon their activation. Antibodies according to the current invention may down-regulate pro-inflammatory cytokine release from myeloid cells. Antibodies according to the current invention may block the release of TNF, MIP-1 $\beta$ , MCP-1, IL-1 $\beta$ , GM-CSF, IL-6 and/or IL-8 from macrophages, neutrophils, synovial tissue cells and/or a reporter cell, as disclosed herein.

**[0030]** Antibodies of the invention may be capable of binding both human TREM-1 and TREM-1 from another species than a human being. The term "TREM-1", as used herein, thus encompasses any naturally occurring form of TREM-1 which may be derived from any suitable organism. For example, TREM-1 for use as described herein may be vertebrate TREM-1, such as mammalian TREM-1, such as TREM-1 from a primate (such as a human, a chimpanzee, a cynomolgus monkey or a rhesus monkey); a rodent (such as a mouse or a rat), a lagomorph (such as a rabbit), or an artiodactyl (such as a cow, sheep, pig or camel). Preferably, the TREM-1 is SEQ ID NO: 1 (human TREM-1). The TREM-1 may be a mature form of TREM-1 such as a TREM-1 protein that has undergone post-translational processing within a suitable cell. Such a mature TREM-1 protein may, for example, be glycosylated. The TREM-1 may be a full length TREM-1 protein.

**[0031]** Antibodies of the invention may be monoclonal antibodies, in the sense that they are directly or indirectly

derived from a single clone of a B lymphocyte. TREM-1 antibodies may be produced, screened and purified using, for example, the methods described in the Examples of WO2013/120553. In brief, a suitable mouse such as a TREM-1 or TREM-1/TREM-3 knock-out (KO) mouse may be immunised with TREM-1, a cell expressing TREM-1 or a combination of both.

**[0032]** Antibodies of the invention may be polyclonal in the sense of being a mixture of monoclonal antibodies according to the current invention.

**[0033]** Primary screening of hybridoma supernatants may be performed using direct ELISA or FMAT and secondary screening may be performed using flow cytometry. Positive hybridoma supernatants may then be screened in a reporter gene assay.

**[0034]** Antibodies may be recombinantly expressed in prokaryotic or eukaryotic cells. The prokaryotic cell may be *E. coli*. The eukaryotic cell may be a yeast, insect or mammalian cell, such as a cell derived from an organism that is a primate (such as a human, a chimpanzee, a cynomolgus monkey or a rhesus monkey), a rodent (such as a mouse or a rat), a lagomorph (such as a rabbit) or an artiodactyl (such a cow, sheep, pig or camel). Suitable mammalian cell lines include, but are not limited to, HEK293 cells, CHO cells and HELA cells. TREM-1 antibodies may also be produced by means of other methods known to the person skilled in the art, such as a phage display or a yeast display.

**[0035]** Once produced, antibodies may be screened for binding to, for example, full length TREM-1 or mutants thereof using the methods described in the Examples of WO2013/120553.

**[0036]** Functional TREM-1 antibodies of the current invention are antibodies that are capable of specifically binding TREM-1 and that have an effect upon TREM-1 activation and downstream signalling by blocking TREM-1 and they are herein referred to as "functional TREM-1 antibodies". The method of identifying a functional TREM-1 antibody comprises (a) culturing a first cell expressing TREM-1, a signalling protein and a reporter construct; (b) measuring the activity of the first cell when said cell is incubated with a TREM-1 modifying agent; (c) contacting the co-culture of (b) with a TREM-1 antibody; and (d) measuring that the activity of the first cell is less than or more than the activity measured in (b).

**[0037]** The "first cell" of (a) may be a cell of haematopoietic origin, such as a myeloid cell, such as a T-cell. The signalling protein of (a) may be any signalling protein that is capable of forming a complex with TREM-1. Suitable signalling proteins include DAP10, DAP12, TCR zeta, Fc gamma RIII and an Fc receptor, or part thereof. The reporter construct of (a) may be any construct that is capable of being activated via the signalling protein and generating a recognisable signal. Suitable reporter constructs comprise a transcription factor and a reporter gene. The signalling protein may signal via a transcription factor selected from the group consisting of the NFAT and NFkB. The reporter gene is a gene that is not natively expressed in said first cell and may be but is not limited to be a gene that encodes  $\beta$ -galactosidase, luciferase, green fluorescent protein (GFP) or chloramphenicol transferase. Said first cell may be transfected with a transcription factor and a reporter gene using methods that are well known in the art.

**[0038]** The "BWZ/hTREM-1 reporter cell" and "TE426.27 reporter cell" described in the Examples is one example of a "first cell".

**[0039]** The modifying agent of (b) may be a TREM-1 ligand or an activated neutrophil. The "TREM-1 antibody" of (c) may be a TREM-1 specific hybridoma supernatant or a purified antibody. The activity measured in (d) is the signal produced by the reporter construct. An example of such signalling is the luminescence caused by NFAT-driven LacZ ( $\beta$ -lactamase luciferase) production.

**[0040]** The method may be tailored to identify a blocking TREM-1 antibody. The method of identifying a blocking TREM-1 antibody comprises (a) culturing a first cell expressing TREM-1, a signalling protein and a reporter construct; (b) measuring the activity of the first cell when said cell is incubated with an activated neutrophil; (c) contacting the co-culture of the first cell and the activated neutrophil with a TREM-1 antibody; and (d) measuring that the activity of the first cell is less than the activity measured in (b).



**[0041]** The present invention relates to blocking TREM-1 antibodies which are defined in the claims, that may be identified by means of the method, herein disclosed, of identifying a blocking antibody. When tested using the method described above and in the Examples, an antibody according to the current invention may, at a concentration of less than 50 µg/ml, such as less than 40 µg/ml, such as less than 30 µg/ml, such as less than 20 µg/ml, such as less than 10 µg/ml, such as less than 5 µg/ml, such as less than 1 µg/ml - be capable of reducing the activity of said first cell by 50%, such as 60%, such as 70%, such as 80%, such as 90%, such as 95%, such as 100%. An antibody according to the invention may be capable of completely extinguishing the activity of the first cell. When tested using the method described above and in the Examples, an antibody according to the current invention may, at a concentration of less than 1 µg/ml - such as less than 0.9 µg/ml, such as less than 0.8 µg/ml, such as less than 0.7 µg/ml, such as less than 0.6 µg/ml, such as less than 0.5 µg/ml, such as less than 0.4 µg/ml, such as less than 0.3 µg/ml, such as less than 0.2 µg/ml - be capable of extinguishing the activity of the first cell.

**[0042]** Blocking TREM-1 antibodies may be identified by other means than the method herein disclosed.

**[0043]** The term "antibody" herein refers to a protein, derived from a germline immunoglobulin sequence, which is capable of specifically binding to an antigen (TREM-1) or a portion thereof. The term "antibody" described herein which does not relate to the antibody of the invention includes full length antibodies of any class or isotype (that is, IgA, IgE, IgG, IgM and/or IgY) and any single chain or fragment thereof. An antibody that specifically binds to an antigen, or portion thereof, may bind exclusively to that antigen, or portion thereof, or it may bind to a limited number of homologous antigens, or portions thereof. Full-length antibodies usually comprise at least four polypeptide chains: two heavy (H) chains and two light (L) chains that are interconnected by disulfide bonds. One immunoglobulin sub-class of particular pharmaceutical interest is the IgG family. In humans, the IgG class may be sub-divided into 4 sub-classes: IgG1, IgG2, IgG3 and IgG4, based on the sequence of their heavy chain constant regions. The light chains can be divided into two types, kappa and lambda, based on differences in their sequence composition. IgG molecules are composed of two heavy chains, interlinked by two or more disulfide bonds, and two light chains, each attached to a heavy chain by a disulfide bond. A heavy chain may comprise a heavy chain variable region (VH) and up to three heavy chain constant (CH) regions: CH1, CH2 and CH3. A light chain may comprise a light chain variable region (VL) and a light chain constant region (CL). VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). VH and VL regions are typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The hypervariable regions of the heavy and light chains form a [binding] domain that is capable of interacting with an antigen, whilst the constant region of an antibody may mediate binding of the immunoglobulin to host tissues or factors, including but not limited to various cells of the immune system (effector cells), Fc receptors and the first component (C1q) of the classical complement system.

**[0044]** Antibodies of the current invention may be isolated. The term "isolated antibody" refers to an antibody that has been separated and/or recovered from (an)other component(s) in the environment in which it was produced and/or that has been purified from a mixture of components present in the environment in which it was produced.

**[0045]** Certain antigen-binding fragments of antibodies are described herein but do not form part of the current invention. The term "antigen-binding fragment" of an antibody refers to one or more fragment(s) of an antibody that retain the ability to specifically bind to an antigen, such as TREM-1, as described herein. Examples of antigen-binding fragments include Fab, Fab', F(ab)2, F(ab')2, F(ab)S, Fv (typically the VL and VH domains of a single arm of an antibody), single-chain Fv (scFv; see e.g., Bird et al., Science 1988; 242:42S-426; and Huston et al. PNAS 1988; 85:5879-5883), dsFv, Fd (typically the VH and CH1 domain), and dAb (typically a VH domain) fragments; VH, VL, VhH, and V-NAR domains; monovalent molecules comprising a single VH and a single VL chain; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., Protein Eng 1997;10:949-57); camel IgG; IgNAR; as well as one or more isolated CDRs or a functional paratope, where the isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, Nat Biotechnol 2005;2S:1126-1136; WO2005040219, and published U.S. Patent Applications 20050238646 and 20020161201. 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.

**[0046]** 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 at least a portion of a framework region and/or at least a portion of a CDR region are derived from human germline immunoglobulin sequences. (For example, a human antibody may have 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 is also 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*).

**[0047]** Such a human antibody may be a human monoclonal antibody. Such a human monoclonal antibody may be produced by a hybridoma, which 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.

**[0048]** Human antibodies may be isolated from sequence libraries built on selections of human germline sequences, further diversified with natural and synthetic sequence diversity.

**[0049]** Human antibodies may be prepared by *in vitro* immunisation of human lymphocytes followed by transformation of the lymphocytes with Epstein-Barr virus.

**[0050]** The term "human antibody derivative" refers to any modified form of the human antibody, such as a conjugate of the antibody and another agent or antibody.

**[0051]** The term "humanised antibody", as used herein, refers to a human/non-human chimeric antibody that contains one or more sequences (CDR regions or parts thereof) that are derived from a non-human immunoglobulin. A humanised antibody is, thus, a human immunoglobulin (recipient antibody) in which at least residues from a hyper-variable region of the recipient are replaced by residues from a hyper-variable region of an antibody from a non-human species (donor antibody) such as from a mouse, rat, rabbit or non-human primate, which have the desired specificity, affinity, sequence composition and functionality. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. An example of such a modification is the introduction of one or more so-called back-mutations, which are typically amino acid residues derived from the donor antibody. Humanisation of an antibody may be carried out using recombinant techniques known to the person skilled in the art (see, e.g., Antibody Engineering, Methods in Molecular Biology, vol. 248, edited by Benny K. C. Lo). A suitable human recipient framework for both the light and heavy chain variable domain may be identified by, for example, sequence or structural homology. Alternatively, fixed recipient frameworks may be used, e.g., based on knowledge of structure, biophysical and biochemical properties. The recipient frameworks can be germline derived or derived from a mature antibody sequence. CDR regions from the donor antibody can be transferred by CDR grafting. The CDR grafted humanised antibody can be further optimised for e.g. affinity, functionality and biophysical properties by identification of critical framework positions where re-introduction (backmutation) of the amino acid residue from the donor antibody has beneficial impact on the properties of the humanised antibody. In addition to donor antibody derived backmutations, the humanised antibody can be engineered by introduction of germline residues in the CDR or framework regions, elimination of immunogenic epitopes, site-directed mutagenesis, affinity maturation, etc.

**[0052]** Furthermore, humanised antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, a humanised antibody will comprise at least one - typically two - variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and in which all or substantially all of the FR residues are those of a human immunoglobulin sequence. The humanised antibody can, optionally, also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

**[0053]** The term "humanised antibody derivative" refers to any modified form of the humanised antibody, such as a conjugate of the antibody and another agent or antibody.

**[0054]** The term "chimeric antibody", as used herein, refers to an antibody whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes that

originate from different species. For example, the variable segments of genes from a mouse monoclonal antibody may be joined to human constant segments.

**[0055]** The fragment crystallisable region ("Fc region"/"Fc domain") of an antibody is the N-terminal region of an antibody, which comprises the constant CH2 and CH3 domains. The Fc domain may interact with cell surface receptors called Fc receptors, as well as some proteins of the complement system. The Fc region enables antibodies to interact with the immune system. In one aspect of the invention, antibodies may be engineered to include modifications within the Fc region, typically to alter one or more of its functional properties, such as serum half-life, complement fixation, Fc-receptor binding, protein stability and/or antigen-dependent cellular cytotoxicity, or lack thereof, among others. Furthermore, an antibody of the invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. An IgG1 antibody (which does not form part of the claimed invention) may carry a modified Fc domain comprises one or more, and perhaps all of the following mutations that will result in decreased affinity to certain Fc receptors (L234A, L235E, and G237A) and in reduced C1q-mediated complement fixation (A330S and P331S), respectively (residue numbering according to the EU index).

**[0056]** As recited in the claims, the isotype of an antibody of the invention is IgG4.

**[0057]** In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further for instance in U.S. Patent No. 5,677,425 by Bodmer et al.

**[0058]** The constant region may be modified to stabilize the antibody, e.g., to reduce the risk of a bivalent antibody separating into two monovalent VH-VL fragments. For example, in an IgG4 constant region, residue S228 (residue numbering according to the EU index) may be mutated to a proline (P) residue to stabilise inter heavy chain disulphide bridge formation at the hinge (see, e.g., Angal et al., Mol Immunol. 1995; 30: 105-8).

**[0059]** Antibodies or fragments thereof may also be defined in terms of their complementarity-determining regions (CDRs). The term "complementarity-determining region" or "hypervariable region", when used herein, refers to the regions of an antibody in which amino acid residues involved in antigen binding are situated. The region of hypervariability or CDRs can be identified as the regions with the highest variability in amino acid alignments of antibody variable domains. Databases can be used for CDR identification such as the Kabat database, the CDRs e.g. being defined as comprising amino acid residues 24-34 (L1), 50-59 (L2) and 89-97 (L3) of the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; (Kabat et al. 1991; Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) Alternatively CDRs can be defined as those residues from a "hypervariable loop" (residues 26-33 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, J. Mol. Biol 1987; 196: 901-917). Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat *et al.*, *supra*. Phrases such as "Kabat position", "Kabat residue", and "according to Kabat" herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a framework (FR) or CDR of the variable domain. For example, a heavy chain variable domain may include amino acid insertions (residue 52a, 52b and 52c according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

**[0060]** The term "framework region" or "FR" residues refer to those VH or VL amino acid residues that are not within the CDRs, as defined herein.

**[0061]** The mAb 0170 antibody has a variable heavy chain sequence as shown in SEQ ID NO: 2 and a variable light chain sequence as shown in SEQ ID NO: 3. An antibody of the invention may comprise this variable heavy chain sequence and/or this variable light chain sequence, as recited in the pending claims. The mAb 0170 antibody has the CDR sequences shown at amino acids 31 to 35, 50 to 68 and 101 to 110 of SEQ ID NO: 2 and amino acids 24 to 38,

54 to 60 and 93 to 101 of SEQ ID NO: 3.

**[0062]** The heavy chain of an antibody described herein (which does not form part of the invention) may comprise a CDR1 sequence of amino acids 31 to 35 (TYAMH) of SEQ ID NO: 2, wherein one of these amino acids may be substituted by a different amino acid.

**[0063]** The heavy chain of an antibody described herein (which does not form part of the invention) may comprise a CDR2 sequence of amino acids 50 to 68 (RIRTKSSNYATYYADSVKD) of SEQ ID NO: 2, wherein one, two or three of these amino acids may be substituted by a different amino acid.

**[0064]** The heavy chain of an antibody described herein (which does not form part of the invention) may comprise a CDR3 sequence of amino acids 101 to 110 (DMGQRRQFAY) of SEQ ID NO: 2, wherein one, two or three of these amino acids may be substituted by a different amino acid.

**[0065]** The light chain of an antibody described herein (which does not form part of the invention) may comprise a CDR1 sequence of amino acids 24 to 38 (RASESVDTFDYSFLH) of SEQ ID NO: 3, wherein one, two or three of these amino acids may be substituted with a different amino acid.

**[0066]** The light chain of an antibody described herein (which does not form part of the invention) may comprise a CDR2 sequence of amino acids 54 to 60 (RASNLES) of SEQ ID NO: 3, wherein one or two of these amino acids may be substituted with a different amino acid.

**[0067]** The light chain of an antibody described herein (which does not form part of the invention) may comprise a CDR3 sequence of amino acids 93 to 101 (QQSNEDPYT) of SEQ ID NO: 3, wherein one or two of these amino acids may be substituted with a different amino acid.

**[0068]** The mAb 0170 antibody has a heavy chain sequence as shown in SEQ ID NO: 2 and a light chain sequence as shown in SEQ ID NO: 3. An antibody of the invention as defined in the claims may comprise this heavy chain sequence or this light chain sequence. Either the heavy or light chain of the antibody of the invention (which is as defined in the claims), or both, may be a variant of mAb 0170. The mAb 0170 antibody has the CDR sequences shown at amino acids 31 to 35, 50 to 68 and 101 to 110 of SEQ ID NO: 2 and amino acids 24 to 38, 54 to 60 and 93 to 101 of SEQ ID NO: 3. An antibody described herein (which does not form part of the invention) may comprise 1, 2, 3, 4, 5 or all 6 of these CDR sequences.

**[0069]** The heavy chain of an antibody described herein (which does not form part of the invention) may comprise a CDRH3 sequence of amino acids 101 to 110 (DMGIRRQFAY) of SEQ ID NO: 2, wherein one, two or three of these amino acids may be substituted by a different amino acid.

**[0070]** The term "antigen" (Ag) refers to the molecular entity used for immunization of an immunocompetent vertebrate to produce the antibody (Ab) that recognizes the Ag. Herein, Ag is termed more broadly and is generally intended to include target molecules that are specifically recognized by the Ab, thus including fragments or mimics of the molecule used in the immunization process, or other process, e.g. phage display, used for generating the Ab.

**[0071]** The term "epitope", as used herein, is defined in the context of a molecular interaction between an "antigen binding polypeptide", such as an antibody (Ab), and its corresponding antigen (Ag). Generally, "epitope" refers to the area or region on an Ag to which an Ab specifically binds, i.e. the area or region in physical contact with the Ab. Physical contact may be defined through various criteria (e.g. a distance cut-off of 2-6Å, such as 3Å, such as 4 Å, such as 5Å; or solvent accessibility) for atoms in the Ab and Ag molecules. A protein epitope may comprise amino acid residues in the Ag that are directly involved in binding to a Ab (also called the immunodominant component of the epitope) and other amino acid residues, which are not directly involved in binding, such as amino acid residues of the Ag which are effectively blocked by the Ab, i.e. amino acid residues within the "solvent-excluded surface" and/or the "footprint" of the Ab.

**[0072]** The term epitope herein comprises both types of binding region in any particular region of TREM-1 that specifically binds to a TREM-1 antibody. TREM-1 may comprise a number of different epitopes, which may include,

without limitation, conformational epitopes which consist of one or more non-contiguous amino acids located near each other in the mature TREM-1 conformation and post-translational epitopes which consist, either in whole or part, of molecular structures covalently attached to TREM-1, such as carbohydrate groups.

**[0073]** The epitope for a given antibody (Ab)/antigen (Ag) pair can be described and characterized at different levels of detail using a variety of experimental and computational epitope mapping methods. The experimental methods include mutagenesis, X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy, Hydrogen deuterium eXchange Mass Spectrometry (HX-MS) and various competition binding methods; methods that are known in the art. As each method relies on a unique principle, the description of an epitope is intimately linked to the method by which it has been determined. Thus, depending on the epitope mapping method employed, the epitope for a given Ab/Ag pair may be described differently.

**[0074]** At its most detailed level, the epitope for the interaction between the Ag and the Ab can be described by the spatial coordinates defining the atomic contacts present in the Ag-Ab interaction, as well as information about their relative contributions to the binding thermodynamics. At a less detailed level, the epitope can be characterized by the spatial coordinates defining the atomic contacts between the Ag and Ab. At an even less detailed level the epitope can be characterized by the amino acid residues that it comprises as defined by a specific criteria such as the distance between or solvent accessibility of atoms in the Ab:Ag complex. At a further less detailed level the epitope can be characterized through function, e.g. by competition binding with other Abs. The epitope can also be defined more generically as comprising amino acid residues for which substitution by another amino acid will alter the characteristics of the interaction between the Ab and Ag.

**[0075]** In the context of an X-ray derived crystal structure defined by spatial coordinates of a complex between an Ab, e.g. a Fab fragment, and its Ag, the term epitope is herein, unless otherwise specified or contradicted by context, specifically defined as TREM-1 residues characterized by having a heavy atom (i.e. a non-hydrogen atom) within a distance of, eg., 4 Å from a heavy atom in the Ab.

**[0076]** From the fact that descriptions and definitions of epitopes, dependant on the epitope mapping method used, are obtained at different levels of detail, it follows that comparison of epitopes for different Abs on the same Ag can similarly be conducted at different levels of detail.

**[0077]** Epitopes described on the amino acid level, e.g. determined from an X-ray structure, are said to be identical if they contain the same set of amino acid residues. Epitopes are said to overlap if at least one amino acid is shared by the epitopes. Epitopes are said to be separate (unique) if no amino acid residue are shared by the epitopes.

**[0078]** Epitopes may also be defined indirectly, by means of comparing the binding kinetics of antibodies to wild type human TREM-1 with those of human TREM-1 variants that have alanine mutations in anticipated epitopes. Decreased affinity or abrogated binding of an antibody to variants of human TREM-1 in which an amino acid residue has been replaced with an alanine residue indicates that the mutated amino acid contributes to the interaction between said antibody and wild type human TREM-1. This approach provides a negative identification of the epitope. The method is compromised in effectively defining the epitope by the fact that protein misfolding or unfolding would give similar results as abrogation of interaction. The analysis can be complemented by comparative gain of function mutational analyses of an orthologous target protein (eg., cynomolgus monkey TREM-1), if a cross-reactive antibody exists. The comparison will define the epitope differences between the antibody that does not cross-react with, eg., cynomolgus monkey TREM-1 and the cross-reactive antibody.

**[0079]** Indirect identification of the epitope can also be provided by means of measuring antibody (or antibody fragment) binding to variants of the wild type antigen (TREM-1). If an antibody or fragment thereof binds, eg., human but not cynomolgus monkey TREM-1 and if said antibody or fragment thereof is capable of binding a partly humanised variant of cynomolgus monkey TREM-1 then this regained binding indicates that the substituted amino acid residue(s) is/are important for the interaction of the antibody with the antigen. In the same way, increased affinity for humanized variants of cynomolgus monkey TREM-1, of an anti-human TREM-1 antibody (or its Fab fragment) that has a weaker binding to cynomolgus monkey TREM-1 compared to human TREM-1, can provide information on the identity of residues composing the binding epitope.

**[0080]** The effect of the same mutations on any given cross-reactive antibody makes it possible to discriminate between possible protein misfolding (abrogated binding to both antibodies) and loss of interaction in human TREM-1 (binding to one of the antibodies and abrogated binding to the other antibody), whilst unambiguously providing information on the epitope differences between the antibody that does not cross-react and the cross reactive antibody on an amino acid level.

**[0081]** Antibodies of the current invention may be capable of binding variants of human TREM-1 as determined using, eg., surface plasmon resonance.

**[0082]** Antibodies of the current invention may be capable of binding variants of cynomolgus monkey TREM-1 as determined using, eg., surface plasmon resonance.

**[0083]** An antibody of the invention may be capable of specifically binding TREM-1, wherein said antibody is capable of specifically binding (i) at least one amino acid residue selected from the group consisting of the A21, T22, K23, L24, T25, E26, and (ii) at least one amino acid residue selected from the group consisting of the A49, S50, S51, Q52, K53, A54, W55, Q56, I57, I58, R59, D60, G61, E62, M63, P64, K65, T66, L67, A68, C69, T70, E71, R72, P73, S74, K75, N76, S77, H78, P79, V80, Q81, V82, G83, R84, I85 and (iii) at least one amino acid residue selected from the group consisting of the C113, V114, I115, Y116, Q117, P118 and P119 of human TREM-1.

**[0084]** An antibody of the invention may be capable of specifically binding a polypeptide comprising amino acids D38 to F48 of SEQ ID NO: 1 (human TREM-1), as determined using, eg., HX-MS or X-ray diffraction.

**[0085]** An antibody of the invention may have an epitope comprising one, two, three, four, five, six, seven or all of the amino acid residues D38, V39, K40, C41, D42, Y43, T44 and L45 of SEQ ID NO: 1 (human TREM-1) and one, two or all of the amino acid residues selected from the group consisting of the E46, K47 and F48 of SEQ ID NO: 1 (human TREM-1), as determined using, eg., HX-MS or X-ray diffraction.

**[0086]** An antibody of the invention may have an epitope comprising one, two, three or all of the amino acid residues selected from the group consisting of the D42, E46, D92 and H93 of SEQ ID NO: 1 (human TREM-1), as determined using variants of TREM-1 and surface plasmon resonance.

**[0087]** An antibody of the invention may have an epitope comprising at least the amino acid residues E46 and/or D92 of SEQ ID NO: 1 (human TREM-1), as determined using variants of TREM-1 and surface plasmon resonance.

**[0088]** An antibody of the invention may further comprise one, two or all of the amino acid residues selected from the group consisting of L31, I86 and V101 of SEQ ID NO: 1 (human TREM-1).

**[0089]** An antibody of the invention may be capable of specifically binding a polypeptide comprising amino acid residues E19 to L26 of cynomolgus monkey TREM-1 (SEQ ID NO: 17), as determined using, eg. HX-MS or X-ray diffraction.

**[0090]** An antibody of the invention may be capable of specifically binding human TREM-1, wherein the epitope of said antibody comprises one, two, three, four, five, six, seven, eight, nine or all of the amino acid residues selected from the group consisting of the V39, K40, C41, D42, Y43, L45, E46, K47, F48 and A49 of SEQ ID NO: 1.

**[0091]** An antibody of the invention may be capable of specifically binding human TREM-1, wherein the epitope of said antibody comprises the D42 of SEQ ID NO: 1. An antibody of the invention may be capable of specifically binding human TREM-1, wherein the epitope of said antibody comprises the E46 of SEQ ID NO: 1. The epitope of said antibody may comprise the V39, C41, D42, Y43, L45 of SEQ ID NO: 1. The epitope of said antibody may comprise the E46, K47 and A49 of SEQ ID NO: 1. The epitope of said antibody may further comprise the F48 of SEQ ID NO: 1.

**[0092]** The definition of the term "paratope" is derived from the above definition of "epitope" by reversing the perspective. Thus, the term "paratope" refers to the area or region on the antibody to which an antigen specifically binds, i.e. with which it makes physical contact to the antigen.

**[0093]** In the context of an X-ray derived crystal structure, defined by spatial coordinates of a complex between an antibody, such as a Fab fragment, and its antigen, the term paratope is herein, unless otherwise specified or contradicted by context, specifically defined as antigen residues characterized by having a heavy atom (i.e. a non-hydrogen atom) within a distance of 4 Å from a heavy atom in TREM-1.

**[0094]** The epitope and paratope for a given antibody (Ab)/antigen (Ag) pair may be identified by routine methods. For example, the general location of an epitope may be determined by assessing the ability of an antibody to bind to different fragments or variant TREM-1 polypeptides. The specific amino acids within TREM-1 that make contact with an antibody (epitope) and the specific amino acids in an antibody that make contact with TREM-1 (paratope) may also be determined using routine methods. For example, the antibody and target molecule may be combined and the Ab:Ag complex may be crystallised. The crystal structure of the complex may be determined and used to identify specific sites of interaction between the antibody and its target.

**[0095]** Antibodies that bind to the same antigen can be characterised with respect to their ability to bind to their common antigen simultaneously and may be subjected to "competition binding"/"binning". In the present context, the term "binning" refers to a method of grouping antibodies that bind to the same antigen. "Binning" of antibodies may be based on competition binding of two antibodies to their common antigen in assays based on standard techniques such as surface plasmon resonance (SPR), ELISA or flow cytometry.

**[0096]** An antibody's "bin" is defined using a reference antibody. If a second antibody is unable to bind to an antigen at the same time as the reference antibody, the second antibody is said to belong to the same "bin" as the reference antibody. In this case, the reference and the second antibody competitively bind the same part of an antigen and are coined "competing antibodies". If a second antibody is capable of binding to an antigen at the same time as the reference antibody, the second antibody is said to belong to a separate "bin". In this case, the reference and the second antibody do not competitively bind the same part of an antigen and are coined "non-competing antibodies".

**[0097]** Antibody "binning" does not provide direct information about the epitope. Competing antibodies, i.e. antibodies belonging to the same "bin" may have identical epitopes, overlapping epitopes or even separate epitopes. The latter is the case if the reference antibody bound to its epitope on the antigen takes up the space required for the second antibody to contact its epitope on the antigen ("steric hindrance"). Non-competing antibodies generally have separate epitopes.

**[0098]** An antibody of the invention may compete with mAb 0170 for binding to human TREM-1. An antibody of the invention may compete with mAb 0170 for binding to cynomolgus monkey TREM-1. In other words, an antibody of the invention may belong to the same "bin" as mAb 0170.

**[0099]** The term "binding affinity" herein refers to a measurement of the strength of a noncovalent interaction between two molecules, e.g. an antibody, or fragment thereof, and an antigen. The term "binding affinity" is used to describe monovalent interactions (intrinsic activity).

**[0100]** Binding affinity between two molecules, e.g. an antibody, or fragment thereof, and an antigen, through a monovalent interaction may be quantified by determination of the equilibrium dissociation constant ( $K_D$ ). In turn,  $K_D$  can be determined by measurement of the kinetics of complex formation and dissociation, e.g. by the SPR method. The rate constants corresponding to the association and the dissociation of a monovalent complex are referred to as the association rate constant  $k_a$  (or  $k_{on}$ ) and dissociation rate constant  $k_d$  (or  $k_{off}$ ), respectively.  $K_D$  is related to  $k_a$  and  $k_d$  through the equation  $K_D = k_d / k_a$ .

**[0101]** Following the above definition, binding affinities associated with different molecular interactions, such as comparison of the binding affinity of different antibodies for a given antigen, may be compared by comparison of the  $K_D$  values for the individual antibody/antigen complexes.

**[0102]** An antibody of the invention may bind human TREM-1 with an affinity ( $K_D$ ) that is  $1 \times 10^{-7}$  M or less,  $1 \times 10^{-8}$  M or less, or  $1 \times 10^{-9}$  M or less, or  $1 \times 10^{-10}$  M or less,  $1 \times 10^{-11}$  M or less,  $1 \times 10^{-12}$  M or less or  $1 \times 10^{-13}$  M or less, as determined using surface plasmon resonance. An antibody of the invention may bind cynomolgus monkey TREM-1

with an affinity ( $K_D$ ) that is  $1 \times 10^{-7}M$  or less,  $1 \times 10^{-8}M$  or less, or  $1 \times 10^{-9}M$  or less, or  $1 \times 10^{-10}M$  or less,  $1 \times 10^{-11}M$  or less,  $1 \times 10^{-12}M$  or less or  $1 \times 10^{-13}M$  or less, as determined using surface plasmon resonance.

**[0103]** The term "binding specificity" herein refers to the interaction of a molecule such as an antibody, or fragment thereof, with a single exclusive antigen, or with a limited number of highly homologous antigens (or epitopes). In contrast, antibodies that are capable of specifically binding to TREM-1 are not capable of binding dissimilar molecules. Antibodies according to the invention may not be capable of binding Nkp44, the Natural killer cell p44-related protein.

**[0104]** The specificity of an interaction and the value of an equilibrium binding constant can be determined directly by well-known methods. Standard assays to evaluate the ability of ligands (such as antibodies) to bind their targets are known in the art and include, for example, ELISAs, Western blots, RIAs, and flow cytometry analysis. The binding kinetics and binding affinity of the antibody also can be assessed by standard assays known in the art, such as SPR.

**[0105]** A competitive binding assay can be conducted in which the binding of the antibody to the target is compared to the binding of the target by another ligand of that target, such as another antibody.

**[0106]** In another aspect, the present disclosure provides compositions and formulations (which do not form part of the present invention) comprising molecules of the invention, such as the TREM-1 antibodies, polynucleotides, vectors and cells described herein. As recited in the claims, the invention provides a pharmaceutical composition that comprises one or more TREM-1 antibodies of the invention, formulated together with a pharmaceutically acceptable carrier.

**[0107]** The pharmaceutical formulation may comprise such a TREM-1 antibody which is present in a concentration from 0.25 mg/ml to 250 mg/ml, such as a concentration of from 10 to 200 mg/ml, and wherein said formulation has a pH from 2.0 to 10.0, such as a pH of from 4.0 to 8.0. The formulation may further comprise one or more of a buffer system, a preservative, a tonicity agent, a chelating agent, a stabilizer and/or a surfactant, as well as various combinations thereof. The use of preservatives, isotonic agents, chelating agents, stabilizers and surfactants in pharmaceutical compositions is well-known to the skilled person. Reference may be made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

**[0108]** In one embodiment, the pharmaceutical formulation is an aqueous formulation. Such a formulation is typically a solution or a suspension, but may also include colloids, dispersions, emulsions, and multi-phase materials. The term "aqueous formulation" is defined as a formulation comprising at least 50% w/w water. Likewise, the term "aqueous solution" is defined as a solution comprising at least 50 % w/w water, and the term "aqueous suspension" is defined as a suspension comprising at least 50 % w/w water.

**[0109]** In another embodiment, the pharmaceutical formulation is a freeze-dried formulation, to which the physician or the patient adds solvents and/or diluents prior to use.

**[0110]** The pharmaceutical formulation may comprise an aqueous solution of such an antibody, and a buffer, wherein the antibody is present in a concentration from 1 mg/ml or above, and wherein said formulation has a pH from about 2.0 to about 10.0, such as a pH of from 4.0 to 8.0.

**[0111]** In another aspect, the TREM-1 antibodies of the present invention and pharmaceutical compositions comprising such antibodies (as defined in the claims) are for use in the treatment of inflammatory diseases such as the following: inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome, rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, systemic lupus erythematosus (SLE), lupus nephritis, type I diabetes, Grave's disease, multiple sclerosis (MS), autoimmune myocarditis, Kawasaki disease, coronary artery disease, chronic obstructive pulmonary disease, interstitial lung disease, autoimmune thyroiditis, scleroderma, systemic sclerosis, osteoarthritis, atopic dermatitis, vitiligo, graft versus host disease, Sjogren's syndrome, autoimmune nephritis, Goodpasture's syndrome, chronic inflammatory demyelinating polyneuropathy, allergy, asthma and other autoimmune diseases that are a result of either acute or chronic inflammation.

**[0112]** TREM-1 antibodies of the invention may be suitable for use in the treatment of individuals with inflammatory bowel disease. Inflammatory Bowel Disease (IBD) is a disease that may affect any part of the gastrointestinal tract from mouth to anus, causing a wide variety of symptoms. IBD primarily causes abdominal pain, diarrhoea (which may



be bloody), vomiting or weight loss, but may also cause complications outside of the gastrointestinal tract such as skin rashes, arthritis, inflammation of the eye, fatigue and lack of concentration. Patients with IBD can be divided into two major classes, those with ulcerative colitis (UC) and those with Crohn's disease (CD). CD generally involves the ileum and colon, it can affect any region of the intestine but is often discontinuous (focused areas of disease spread throughout the intestine). UC always involves the rectum (colonic) and is more continuous. In CD, the inflammation is transmural, resulting in abscesses, fistulas and strictures, whereas in UC, the inflammation is typically confined to the mucosa. There is no known pharmaceutical or surgical cure for Crohn's disease, whereas some patients with UC can be cured by surgical removal of the colon. Treatment options are restricted to controlling symptoms, maintaining remission and preventing relapse. Efficacy in inflammatory bowel disease in the clinic may be measured as a reduction in the Crohn's Disease Activity Index (CDAI) score for CD which is scoring scale based on laboratory tests and a quality of life questionnaire. In animal models, efficacy is mostly measured by increase in weight and also a disease activity index (DAI), which is a combination of stool consistency, weight and blood in stool.

**[0113]** TREM-1 antibodies of the invention may be suitable for use in the treatment of individuals with rheumatoid arthritis. Rheumatoid arthritis (RA) is a systemic disease that affects nearly if not all of the body and is one of the most common forms of arthritis. It is characterized by inflammation of the joint, which causes pain, stiffness, warmth, redness and swelling. This inflammation is a consequence of inflammatory cells invading the joints, and these inflammatory cells release enzymes that may digest bone and cartilage. As a result, this inflammation can lead to severe bone and cartilage damage and to joint deterioration and severe pain, among other physiologic effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement.

**[0114]** There are several animal models for rheumatoid arthritis known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop an inflammatory arthritis that resembles human rheumatoid arthritis. Since CIA shares similar immunological and pathological features with RA, this makes it a suitable model for screening potential human anti-inflammatory compounds. Efficacy in this model is measured by decrease in joint swelling. Efficacy in RA in the clinic is measured by the ability to reduce symptoms in patients which is measured as a combination of joint swelling, erythrocyte sedimentation rate, C-reactive protein levels and levels of serum factors, such as anti-citrullinated protein antibodies.

**[0115]** TREM-1 antibodies of the invention may be suitable for use in the treatment of individuals with psoriasis. Psoriasis is a T-cell mediated inflammatory disorder of the skin that can cause considerable discomfort. It is a disease for which there is currently no cure and it affects people of all ages. Although individuals with mild psoriasis can often control their disease with topical agents, more than one million patients worldwide require ultraviolet light treatments or systemic immunosuppressive therapy. Unfortunately, the inconvenience and risks of ultraviolet radiation and the toxicities of many therapies limit their long-term use. Moreover, patients usually have recurrence of psoriasis, and in some cases rebound shortly after stopping immunosuppressive therapy. A recently developed model of psoriasis based on the transfer of CD4+ T cells mimics many aspects of human psoriasis and therefore can be used to identify compounds suitable for use in treatment of psoriasis (Davenport et al., *Internat. Immunopharmacol* 2:653-672, 2002). Efficacy in this model is measured by reduction in skin pathology using a scoring system. Similarly, efficacy in patients is measured by a decrease in skin pathology.

**[0116]** TREM-1 antibodies of the invention may be suitable for use in the treatment of individuals with psoriatic arthritis. Psoriatic arthritis (PA) is a type of inflammatory arthritis that occurs in a subset of patients with psoriasis. In these patients, the skin pathology/symptoms are accompanied by a joint swelling similar to that seen in rheumatoid arthritis. It features patchy, raised, red areas of skin inflammation with scaling. Psoriasis often affects the tips of the elbows and knees, the scalp, the navel and around the genital areas or anus. Approximately 10% of patients who have psoriasis also develop an associated inflammation of their joints.

**[0117]** The term "treatment", as used herein, refers to the medical therapy of any human or other animal subject in need thereof. Said subject is expected to have undergone physical examination by a medical or veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said treatment is beneficial to the health of said human or other animal subject. The timing and purpose of said treatment may vary from one individual to another, according to many factors, such as the status quo of the subject's health. Thus, said treatment may be prophylactic, palliative, symptomatic and/or curative.

**[0118]** In terms of the present invention, prophylactic, palliative, symptomatic and/or curative treatments may represent separate aspects of the invention.

**[0119]** An antibody of the invention may be for administration parenterally, such as intravenously, such as intramuscularly, such as subcutaneously. Alternatively, an antibody of the invention may be for administration via a non-parenteral route, such as perorally or topically. An antibody of the invention may be for administration prophylactically. An antibody of the invention may be for administration therapeutically (on demand).

**[0120]** As demonstrated herein, the substitution of negatively charged residues of the CDR1 and CDR3 regions of SEQ ID NO: 3 (the variable light chain of mAb 0170) was observed to influence the viscosity of the mAb. The mAb described herein (which does not form part of the invention) is a variant of mAb 0170 having a heavy chain and a light chain, wherein the light chain of mAb 0170, namely SEQ ID NO: 3, comprises mutations wherein negatively charged residues in CDR1 and CDR3 region of SEQ ID NO: 3 are substituted with uncharged residues. Accordingly, the disclosure provides a variant of mAb 0170 (which does not form part of the invention) comprising substituting any one or any combination of residues D1, D30, D33, D74, D98, E27, E97 of SEQ ID NO: 3 with an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, and tyrosine. Otherwise stated, an interesting embodiment described herein (which does not form part of the invention) is directed to an antibody or fragment thereof comprising SEQ ID NO: 2 (or a variant thereof) as a heavy chain and as light chain comprising a variant of SEQ ID NO: 3 wherein any one or any combination of residues D1, D30, D33, D74, D98, E27, E97 of SEQ ID NO: 3 is mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, and tyrosine. These mutations will be referred to as "charge patch" mutations. In a preferred embodiment of the disclosure (which does not form part of the invention), at least one or both of E27 and E97 of the CDR1 and CDR3 regions of SEQ ID NO: 3 are substituted with uncharged amino acid residues, such as an amino acid selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, and tyrosine. In the antibody of the invention as defined in the claims, E27 of the CDR1 and CDR3 regions of SEQ ID NO: 3 may be mutated to glutamine and E97 may be substituted with an amino acid selected from the group consisting of serine and glutamine. Alternatively, in the antibody of the invention defined in the claims, E27 may remain unmutated and E97 may be mutated with an amino acid selected from the group consisting of serine and glutamine. In the antibody of the invention defined in the claims, residue E97 may remain unmutated and E27 may be substituted with glutamine.

**[0121]** Another aspect of the invention is based on the observation that Fab-Fab dimers were formed due to interactions in the paratope area. Since mAbs comprise two Fabs, it was envisioned that mAbs would be able to multimerize, which could have an impact on the viscosity properties. Thus, the present disclosure further describes mutating residues in the Fab-Fab interaction area of SEQ ID NO: 2 (namely in the variable heavy chain area of mAb 0170) to reduce Fab-Fab dimerization. These mutations are referred to as "Fab-Fab interaction" mutations. An aspect of the disclosure (which does not form part of the invention) is directed to substituting any one of residues Y32, R52, S55, S56, N57, A59, M102, I104 and R106 of SEQ ID NO: 2 or F32, D33, Y34, Y53, R54, D98 of SEQ. ID NO 3 with an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, tryptophan, histidine and tyrosine. Otherwise stated, an interesting embodiment described herein (which does not form part of the invention) is directed to an antibody or fragment thereof comprising a variant of SEQ ID NO: 2 wherein any one of residues Y32, R52, S55, S56, N57, A59, M102, I104 and R106 of SEQ ID NO: 2 or F32, D33, Y34, Y53, R54, D98 of SEQ. ID NO 3 ("Fab-Fab interaction" mutations) is substituted with another amino acid, such as natural amino acid, preferably an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, tryptophan, histidine and tyrosine. Preferably in the present disclosure (which does not form part of the invention), at least one of residues A59 and N57 SEQ ID NO: 2 is mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, tryptophan, histidine and tyrosine, more preferably serine or tyrosine. In the antibody of the invention defined in the claims, A59 may remain unmutated and N57 may be mutated to serine. In the antibody of the invention defined in the claims, N57 may remain unmutated and A59 may be mutated to tyrosine. In at least one embodiment of the disclosure (which does not form part of the invention), both A59 and N57 are mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, tryptophan, histidine and tyrosine, more preferably serine or tyrosine.

**[0122]** In an interesting embodiment of the disclosure (which does not form part of the invention), i) at least one negatively charged residues of the CDR1 and CDR3 regions of SEQ ID NO: 3 is mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, and tyrosine and ii) at least one residue of residues Y32, R52, S55, S56, N57, A59, M102, I104 and R106 of SEQ ID NO: 2 or F32, D33, Y34, Y53, R54, D98 of SEQ. ID NO 3 ("Fab-Fab interaction" mutations) is mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, and tryptophan, histidine and tyrosine.

**[0123]** In a suitable embodiment of the disclosure (which does not form part of the invention), one or both of E27 and E97 of SEQ ID NO: 3 is/are mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, and tyrosine and one or both of A59 and N57 of SEQ ID NO: 2 is/are mutated to an amino acid residue selected from the group consisting of glycine, alanine, serine, asparagine, glutamine, threonine, cysteine, lysine, arginine, tryptophan, histidine and tyrosine, more preferably serine or tyrosine.

**[0124]** A further aspect of the disclosure (which does not form part of the invention) is based on the observation that an Ala substitution in position Y90 of SEQ. ID NO 1 of TREM-1 improved the affinity of SEQ. ID NO 3 to TREM-1. The Y90 was found to interact with a phenylalanine residue of SEQ ID NO: 3. Mutation of SEQ ID NO: 3 in order to improve the Fab-TREM-1 interaction are referred to as Fab-TREM-1 interaction mutations. In the antibody of the invention defined in the claims, phenylalanine at position 32 of SEQ ID NO: 3 may be mutated to alanine or serine.

**[0125]** Charge patch mutations in the CDR1 and CDR3 of SEQ ID NO: 3 reduced the hydrodynamic radius (Rh) while mutations in the Fab-Fab interaction region increased Rh (Figure 6). Mutations in the "Fab-TREM-1 interaction" site did not influence Rh and had very little effect on viscosity (Figures 6 and 1, Tables 3C and 3E).

Table 1: Overview of SEQ ID No (0170) mAb variants generated. L = light chain, H = heavy chain.

	mAb ID	Comprise Sequence ID No.	Mutation(s) to SEQ ID NO: 2 or 3	Chain
<b>Charge patch mutations</b>	0317	SEQ ID NO: 4 and 2	E27Q, E97S	L
	0318	SEQ ID NO: 5 and 2	E27Q, E97Q	L
	0319	SEQ ID NO: 6 and 2	E97S	L
	0320	SEQ ID NO: 7 and 2	E97Q	L
	0321	SEQ ID NO: 8 and 2	E27Q	L
<b>Fab-Fab interaction mutations</b>	0324	SEQ ID NO: 11 and 3	A59Y	H
	0325	SEQ ID NO: 12 and 3	N57S	H
	0326	SEQ ID NO: 13 and 3	A59Y,N57S	H
<b>Fab-TREM-1 interaction mutations</b>	0322	SEQ ID NO: 9 and 2	F32A	L
	0323	SEQ ID NO: 10 and 2	F32S	L
<b>Charge patch and Fab-TREM-1 interaction mutations</b>	0330	SEQ ID NO: 14 and 2	F32A, E27Q, E97Q	L
<b>Charge patch and Fab-Fab interaction mutations</b>	0332	SEQ ID NO: 15 and 5	A59Y/E27Q, E97Q	H/L
<b>Charge patch, Fab-TREM-1 interaction and Fab-Fab interaction</b>	0333	SEQ ID NO: 14 and 16	A59Y/F32A, E27Q, E97Q	H/L

**[0126]** The viscosity was determined for the mAb 0170 variants by DLS or micro rheology and showed that both "charge patch" mutations and "Fab-Fab interaction" mutations reduced the viscosity. The mAb variant 0318, which comprise the charge patch mutations E27Q and E97Q was found to have the lowest viscosity.

**[0127]** The binding kinetics of the mAb 0170 variants towards human TREM-1-Fc and cynomolgus TREM-1-Fc were

determined, respectively. The mAb 0170 variants were all found to have similar affinity towards human TREM-1-Fc. Interestingly, the mAb variant, SEQ ID NO: 9 comprising a "Fab-TREM-1 interaction" mutation was found to have increased affinity towards cynomolgus TREM-1-Fc (Table 2).

Table 2: Affinity of anti-TREM-1 mAb variants towards human TREM-1-Fc and cynomolgus TREM-1-Fc respectively.

Human TREM-1-Fc				Cynomolgus TREM-1-Fc		
mAb	ka (1/Ms)	kd (1/s)	KD (nM)	ka (1/Ms)	kd (1/s)	KD (nM)
0170	3E+06	5E-04	0.1	0.3E+06	4E-04	2
0317	4E+06	6E-04	0.2	0.3E+06	7E-04	3
0318	3E+06	7E-04	0.2	0.3E+06	7E-04	2
0319	4E+06	6E-04	0.2	0.3E+06	6E-04	2
0320	4E+06	7E-04	0.2	0.2E+06	6E-04	3
0321	3E+06	6E-04	0.2	0.3E+06	6E-04	2
0322	3E+06	3E-04	0.1	0.6E+06	1E-04	0.1
0323	2E+06	6E-04	0.3	0.4E+06	2E-04	0.6
0324	3E+06	8E-04	0.3	0.2E+06	6E-04	4
0325	4E+06	3E-04	0.08	0.2E+06	6E-04	1
0326	3E+06	7E-04	0.2	0.2E+06	3E-04	5
0330	2E+06	3E-04	0.1	0.6E+06	7E-05	0.1
0332	3E+06	1E-03	0.4	0.2E+06	8E-04	4
0333	2E+06	5E-04	0.3	0.4E+06	1E-04	0.3

**[0128]** In an embodiment with both improved affinity towards TREM-1 and having low viscosity, the mutations from SEQ ID NO: 9 were combined with the mutations in SEQ ID NO: 5 to generate the light chain from SEQ ID NO: 14 and the mutations from the light chain from SEQ ID NO: 14 were further combined with the heavy chain mutations from SEQ ID NO: 16. The increased affinity towards cynomolgus TREM-1-Fc was retained for the mAb variants comprising the combined mutations of SEQ ID NO: 14 and the mutations did not negatively affect the affinity towards human TREM-1-Fc. Accordingly, embodiments comprising both "charge patch" mutations and "Fab-TREM-1 interaction" mutations are envisaged by the present invention, as well as embodiments comprising both "charge patch" mutations and "Fab-Fab interaction" mutations as well as embodiments comprising both "Fab-Fab interaction" mutations and "Fab-TREM-1 interaction" mutations and embodiments comprising "Fab-Fab interaction" mutations, "Fab-TREM-1 interaction" mutations and "charge patch" mutations.

Table 3A: Viscosity versus protein concentrations of mAb 0170, 0317 and 0318

mAb					
0170		0317		0318	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
100.0	26.0	85.0	2.9	85.0	1.9
80.0	14.5	63.8	2.2	63.8	1.9
60.0	6.0	42.5	1.9	42.5	2.0
40.0	3.1	17.0	1.3	17.0	1.2
20.0	1.6	10.2	1.2	10.2	1.2
12.0	1.3	3.4	1.1	3.4	1.2
4.0	1.1	0.0	1.0	0.0	1.0
0.0	0.9				

Table 3B: Viscosity versus protein concentrations of mAb 0319 and 0320 and 321.

mAb					
0319		0320		0321	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
85.0	2.2	107.0	7.8	106.0	5.9
63.8	2.3	80.3	3.7	79.5	3.0
42.5	2.0	53.5	2.5	53.0	2.0
17.0	1.2	26.8	1.6	26.5	1.6
10.2	1.1	13.4	1.3	13.3	1.2
3.4	1.1	6.7	1.2	6.6	1.1
0.0	1.0	3.3	1.1	3.3	1.1
		0.0	1.0	0.0	0.9

Table 3C: Viscosity versus protein concentrations of mAb 0322, 0323 and 324.

mAb					
0322		0323		0324	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
100.0	16.4	101.0	21.3	103.0	5.4
75.0	4.9	75.8	11.3	77.3	3.1
50.0	3.3	50.5	3.7	51.5	2.2
25.0	2.0	25.3	2.6	25.8	1.6
12.5	1.6	12.6	2.0	12.9	1.4
6.3	1.4	6.3	1.4	6.4	1.3
3.1	1.3	3.2	1.2	3.2	1.2
0.0	1.0	0.0	1.0	0.0	0.9

Table 3D: Viscosity versus protein concentrations of mAb 0325, 0326 and 0330.

mAb					
0325		0326		0330	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
95.0	7.8	109.0	7.5	104.5	3.8
71.3	3.7	81.8	3.1	50.5	2.1
47.5	2.3	54.5	2.2	23.9	1.4
23.8	1.6	27.3	1.6	9.4	1.3
11.9	1.3	6.8	1.5	4.7	1.1
5.9	1.3	3.4	1.1	1.9	1.1
3.0	1.2	0.0	0.9	1.0	1.3
0.0	1.0				

Table 3E Viscosity versus protein concentrations of mAb 0332 and 0333.

mAb			
0332		0333	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
114.1	5.9	113.2	5.2
53.6	2.1	47.1	2.1
22.2	2.2	24.9	2.0
9.0	2.2	9.0	1.8

mAb			
0332		0333	
Protein concentration (mg/mL)	Viscosity (cP)	Protein concentration (mg/mL)	Viscosity (cP)
4.6	1.8	4.5	1.5
1.9	1.2	1.8	1.6
1.1	1.1	0.9	1.4

**[0129]** Further embodiments of the present invention are described below:

The antibody of the invention that is capable of binding to and blocking TREM-1 of SEQ ID NO: 1 (as defined in the claims), may be characterized in that the antibody has a viscosity of less than 5 cP at a concentration of 50 mg/mL, such as less than 4 cP, preferably less than 3 cP (wherein the method of determining viscosity versus protein concentration conditions are conventional or as described herein.)

**[0130]** The antibody of the invention that is capable of binding to and blocking TREM-1 (as defined in the claims), may be characterized in that the antibody has a viscosity of less than 5 cP at a concentration of 80 mg/mL, preferably less than 4 cP (wherein the method of determining viscosity versus protein concentration conditions are conventional or as described herein.)

**[0131]** The antibody of the invention (as defined in the claims) may block TREM-1 function with a  $K_D$  to SEQ ID NO: 1 of less than 0.5 nM, such as less than 0.4 nM, preferably of 0.3 nM or less.

**[0132]** The antibody of the invention (as defined in the claims) may competitively bind with mAb 0170 for binding to SEQ ID NO: 1 and may have a viscosity profile such that:

1. (a) the antibody has a viscosity of less than 5 cP at a concentration of 50 mg/mL, such as less than 4 cP, preferably less than 3 cP ; or
2. (b) the antibody has a viscosity of less than 5 cP at a concentration of 80 mg/mL, preferably less than 4 cP.

**[0133]** The antibody of the invention (as defined in the claims) that is capable of specifically binding to and blocking TREM-1 may have "Fab-Fab interaction" mutations of SEQ ID NO: 2 described herein.

**[0134]** The antibody of the invention (as defined in the claims) that is capable of specifically binding to and blocking TREM-1 of SEQ ID NO: 1 may have "charge patch" mutations as described herein.

**[0135]** The antibody of the invention (as defined in the claims) may have a lowered viscosity compared to mAb 0170, but still has a  $K_D$  to SEQ ID NO: 1 of less than 0.5 nM.

**[0136]** The antibody of the invention (as defined in the claims) may have a lowered viscosity compared to mAb 0170, a  $K_D$  to SEQ ID NO: 1 of less than 0.5 nM and a  $K_D$  to SEQ ID NO: 17 of less than 0.6 nM, by substituting uncharged amino acids of SEQ ID NO: 2 and/or 3, involved in specific "Fab-Fab interactions" with amino acids with altered size or hydrogen bonding potential.

**[0137]** The antibody of the invention (defined in the claims) may compete for binding with SEQ ID NO: 3, and comprise a LC having one or both glutamate (E) residues of positions 27 and 97 of SEQ ID NO: 3 mutated to serine (S) or glutamine (Q), wherein E27 is mutated to glutamine and E97 is mutated to serine (E27Q and E97S); wherein both E27 and E97 are mutated to glutamine (E27Q and E97Q); or wherein E27 remains un-mutated and E97 is mutated to S (E27, E97S), or wherein E27 remains un-mutated and E97 is mutated to Q (E27, E97Q), or wherein E97 remains un-mutated and E27 is mutated to Q.

**[0138]** The antibody of the invention (as defined in the claims) may comprise SEQ ID NO: 3, wherein the phenylalanine at position 32 (F32) is mutated to A (mAb 0322) or to S (mAb 0323).

**[0139]** The antibody of the invention (as defined in the claims), may comprise SEQ ID NO: 2 wherein residue A59 has been mutated to a tyrosine, or wherein residue N57 has been mutated to a serine, or wherein residue N57 has been mutated to a serine and residue A59 has been mutated to a tyrosine.

**[0140]** The antibody of the invention (as defined in the claims) may comprise a variant of SEQ ID NO: 3 which competes for binding with SEQ ID NO: 3 wherein both E27 and E97 are mutated to glutamine (318), or wherein E27 remains un-mutated and E97 is mutated to S (E27, E97S), or wherein E27 remains un-mutated and E97 is mutated to Q (E27, E97Q), or wherein E97 remains un-mutated and E27 is mutated to Q.

**[0141]** The antibody of the invention (as defined in the claims) may comprise a variant of SEQ ID NO: 2 which competes for binding with SEQ ID NO: 2, wherein residue A59 has been mutated to a tyrosine, or wherein residue N57 has been mutated to a serine, or wherein both residue N57 has been mutated to a serine and residue A59 has been mutated to a tyrosine.

**[0142]** In some embodiments, the antibody of the invention (as defined in the claims) comprises the sequence of SEQ ID NO: 4.

**[0143]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 5.

**[0144]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 6.

**[0145]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 7.

**[0146]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 8.

**[0147]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 9.

**[0148]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 10.

**[0149]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 11.

**[0150]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 12.

**[0151]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 13.

**[0152]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 14.

**[0153]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 15.

**[0154]** In some embodiments, the antibody of the invention (as defined in the claims) comprises SEQ ID NO: 16.

**[0155]** The present invention provides an antibody of the invention (as defined in the claims), for use as a medicament, as recited in the claims.

**[0156]** In some embodiments, the invention provides an antibody of the invention, for use in the treatment of a disease selected from an inflammatory disease or an autoimmune disease, as recited in the claims.

**[0157]** In some embodiments, the disease is selected from the group consisting of rheumatoid arthritis and inflammatory bowel disease.

## **EXAMPLES**

### **Example 1: Hydrodynamic radius and viscosity of mAb0170 variants**

**[0158]** The hydrodynamic radius of mAb0170 of WO2013/120553 was found to be higher (9-10 nm) than desired (5-6 nm). The hydrodynamic radius was determined by dynamic light scattering analysis using a 96-well plate setup with a DynaPro plate reader (Wyatt Inc.). The plates used were Corning 3540 assay plates (Corning). Total sample volume was approximately 20  $\mu$ L and the temperature was kept at 25 °C for the mAb0170 variants (Figure 6).

**[0159]** Charge patch mutations in the CDR1 and CDR3 of the variable light chain reduced the Rh to the level expected for monomeric mAbs while mutations in the Fab-Fab interaction region seemed to increase Rh. Mutations in the Fab-TREM-1 interaction site did not influence Rh.

**[0160]** The viscosity was determined for the mAb0170 variants 0317, 0318, 0319, 0320, 0321, 0322, 0323, 0324, 0325, 0326 and 0330 by DLS. Hydrodynamic radius was measured on a Wyatt DynaPro Platereader using Corning 3540 clear bottom, black not-treated polystyrene microplates and plain polystyrene nanospheres from Phosphorex Inc. with a mean diameter of 206.5 nm (Cat. no. 106). The protein samples were transferred to a Corning 3540 plate and covered with a top seal. The samples were centrifuged and the hydrodynamic radii of the protein samples were measured in the Wyatt DynaPro DLS plate reader. Each well was added 0.5  $\mu$ L polystyrene beads and mixed gently by pipetting. The plate was centrifuged again and the hydrodynamic radii of the beads were measured in the Wyatt DynaPro DLS plate reader.

**[0161]** The viscosities of the protein samples were calculated as  

$$\text{viscosity}(\text{protein}) = (\text{hydrodynamic radius}(\text{beads, meas}) \times \text{viscosity}(\text{buffer})) / \text{hydrodynamic radius}(\text{beads, real})$$

where viscosity(protein) is the calculated viscosity of the protein solution, hydrodynamic radius(beads, meas) is the measured hydrodynamic radius of the polystyrene beads in the protein solution, viscosity(buffer) is the viscosity of the buffer and hydrodynamic radius(beads, real) is the real mean diameter of the beads [He et al., Anal Biochem, 399, 141-143, 2010].

**[0162]** The viscosity was determined by micro-rheology for the mAb0170 variants 0332 and 0333. For the rheological analysis, a 250  $\mu$ L Hamilton LT syringe (Hamilton-Bonaduz Inc) with a 30G Novofine needle attached was used. The syringe was placed in a custom made aluminium holder fastened to a platform. The plunger of the syringe was driven by a TAXTPlus Texture Analyzer, which measured the resulting force on the plunger with a pre-determined injection speed. Each sample was tested with three different injection speeds. The cross-head speed and the measured force were used to calculate the shear rate and the shear stress respectively. Viscosity can be expressed in relation to shear rate as:

$$\eta_{\text{protein}} = \tau_w / \dot{\gamma} = (\Delta P D / 4 L) / ((32 Q) / (\pi D^3))$$

where  $\eta_{\text{protein}}$  is the viscosity of the protein solution,  $\dot{\gamma}$  is the apparent shear rate,  $\tau_w$  is the shear stress, P is the pressure resulting from driving the plunger, Q is the volumetric flow rate of the fluid passing through the capillary needle and D and L are the internal diameter and length, respectively of the capillary (Allahham et al., 2004; Intl J Pharm 270, 139-148). The viscosity,  $\eta_{\text{protein}}$  was calculated from the know values of D, L and Q and the measured value of P.

**[0163]** The analysis showed that both "charge patch" mutations and "Fab-Fab interaction" mutations reduced the viscosity (Figure 1). The mAb variant SEQ ID NO: 5, which comprises the charge patch mutations E27Q and E97Q was found to have the lowest viscosity.

#### **Example 2: Kinetics of mAb0170 variants**

**[0164]** The binding kinetics of the mAb 0170 variants towards human TREM-1-Fc and cynomolgus TREM-1-Fc were determined, respectively. Binding studies were performed on a ProteOn Analyzer (BioRad) that measures molecular interactions in real time through surface plasmon resonance. Experiments were run at 25°C and the samples were stored at 15 °C in the sample compartment. The signal (RU, response units) reported by the ProteOn is directly correlated to the mass on the individual sensor chip surfaces in the six parallel flow cells. Anti-human Fc monoclonal or anti-murine Fc polyclonal antibody from Biacore human or mouse Fc capture kits were immobilized in horizontal



direction onto flow cells of a GLM sensor chip according to the manufacturer's instructions. The final immobilization level of capture antibody was approximately 2600-6000 RU in each experiment. The capture of purified monoclonal mouse or recombinantly expressed anti-hTREM-1 antibodies was conducted by diluting the antibodies to 5-10 nM into running buffer (10 mM Hepes 0,15 M NaCl, 5 mM EDTA, 0.05% surfactant P20, pH 7.4) followed by injection in vertical direction at 30  $\mu$ L/min for 60 sec, creating reference interspots adjacent to all flow cells with only anti-Fc antibody immobilized. This typically resulted in final capture levels of test antibodies of approximately 100-300 RU and  $R_{max}$  values of analyte of 30-90 RU. Binding of hTREM-1 or cTREM-1 proteins was conducted by injecting analyte (antigen) over all flow cells in horizontal direction to allow for comparative analyses of binding to different captured anti-TREM-1 antibodies relative to binding to the reference interspot. hTREM-1 or cTREM-1 proteins were diluted serially 1:3 to 1.2-100 nM or into running buffer, injected at 100  $\mu$ L/min for 250 s and allowed to dissociate for 600s. The GLM surface was regenerated after each injection cycle of analyte via two 18 s injections of 10 mM Glycine, pH 1.7 and 50 mM NaOH at 100  $\mu$ L/min. This regeneration step removed the anti-TREM-1 antibody and any bound TREM-1 protein from the immobilized capture antibody surface and allowed for the subsequent binding of the next interaction sample pair. The regeneration procedure did not remove the directly immobilized anti-Fc capture antibody from the chip surface.

**[0165]** Binding affinity between antibodies and the antigen was quantified by determination of the equilibrium dissociation constant ( $K_D$ ) determined by measurement of the kinetics of complex formation and dissociation. The rate constants corresponding to the association and the dissociation of a monovalent complex such as  $k_a$  (association rate) and  $k_d$  (dissociation rate) were retrieved by fitting data to 1:1 Langmuir model using the ProteOn evaluation software for data analysis.  $K_D$  is related to  $k_a$  and  $k_d$  through the equation  $K_D = k_d / k_a$ .

**[0166]** Binding curves were processed by double referencing (subtraction of reference surface signals as well as blank buffer injections over captured anti-TREM-1 antibodies) prior to data analysis. This allowed correction for instrument noise, bulk shift and drift during sample injections.

**[0167]** The mAb 0170 variants were all found to have similar affinity towards human TREM-1-Fc as mAb 0170. Interestingly, mAb variants comprising a "Fab-TREM-1 interaction" mutation, such as mAb 0322, had increased affinity towards cynomolgus TREM-1-Fc (Table 2).

#### **Example 3: Kinetics and viscosity of mAb 0330 and mAb 0333**

**[0168]** In the attempt to generate a mAb with both improved affinity towards cynomolgus TREM-1 and having low viscosity, the mutations from SEQ ID NO 9 was combined with the mutations in SEQ ID NO 5, which was found to have the lowest viscosity among the mAb0170 variants and with SEQ ID NO: 11, which was found to have a modest effect on the viscosity. The increased affinity towards cynomolgus TREM-1-Fc was retained for the mAb variant comprising the combined mutations (mAb 0330 and mAb 0333) and the mutations did not affect the affinity towards human TREM-1-Fc. The viscosity of mAb0330 and mAb 0333 were markedly reduced compared to mAb 0170 of WO2013/120553 (Figure 1).

#### **Example 4: Cultivation of a BWZ'36/hTREM-1 stable cell line**

**[0169]** BWZ/hTREM-1 reporter cell were cultured in RPMI 1640 w/o phenol red (Cat# 11835, Gibco, Carlsbad CA, USA), supplemented with 10% FCS (Cat# 16140-071, Gibco, New York, USA), 1% Pen/Strep (Cat# 15070-06, Gibco), 1 mM Sodium Pyruvate (Cat #11360, Gibco), 5  $\mu$ M -2ME (Cat# 31350-010, Gibco) and 2 mM L-Glutamine (Cat # 25030, Gibco). No special plates or coating was required. 10 ml Versene (Cat # 15040, Gibco) was added to detach the cells which then were transferred to tubes, centrifuged 1200 rpm 5 min and washed in fresh RPMI 1640 w/o phenol red. These cells were then ready to use in an assay or re-culture for further propagation.

#### **Example 5: Functional characterization mAb 0170 variants**

**[0170]** The ability of the anti-TREM-1 mAb 0170 variants to inhibit human TREM-1 signalling was determined using a

reporter cell line (BWZ'36/hTREM-1) provided by Bioxell. The reporter cell line was stimulated with PGN and TREM-1 ligand PGLYRP1 either as recombinant protein or expressed by activated neutrophils prior to incubation with mAbs. The potency of the variants to inhibit TREM-1 signalling was found to be clinically relevant and on par with that of the mAb 0170 of WO2013/120553 (Figure 2A and B).

**[0171]** Likewise, the ability to inhibit cynomolgus TREM-1 signalling was determined for the three mAb0170 variants with the lowest viscosity and two variants observed to have increased affinity towards cynomolgus TREM-1. In this assay using an established reporter cell line TE 426.27, similar potencies of the mAb variants and mAb0170 of WO2013/120553 was observed (Figure 3).

**[0172]** Also, the potency of the mAb variants to block TNF $\alpha$  release from primary cells from healthy donors was assessed. In this assay monocytes were differentiated to M2 macrophages under hypoxic conditions in order to increase TREM-1 expression and activated with PGN and recombinant PGLYRP1 prior to incubation with mAb. The mAb variants were found to be as potent as 0170 at inhibiting TREM-1-mediated TNF $\alpha$  release from hypoxic M2 macrophages (Figure 4).

#### **Example 6 Crystal structure of a Fab 0170-Fab 0170 complex**

**[0173]** The potential of mAb 0170 molecules to interact by specific self-interactions were evaluated from by crystallographic analysis of a Fab 0170-Fab 0170 crystal structure. *Materials:* The Fab region of mAb 0170 (SEQ ID NO: 18 and SEQ ID NO: 19) in a buffer consisting of 10 mM phosphate, 2.68 mM KCl, 140 mM NaCl, pH 7.4 at a protein concentration of 8.5 mg/mL.

**[0174]** *Methods:* The Fab region of mAb 0170 was crystallized in a hanging drop vapour diffusion experiment by equilibration of a droplet consisting of 2  $\mu$ L protein solution mixed with 2  $\mu$ L reservoir solution against a 0.5 mL reservoir composed of 20% (w/v) PEG 8000, 200 mM K<sub>2</sub>HPO<sub>4</sub>. The crystal was transferred to a drop consisting of 2  $\mu$ L 35% (w/v) PEG3350, 200 mM K<sub>2</sub>HPO<sub>4</sub> and mounted in a 0.2 mm diameter litholoop (Molecular Dimensions Limited) and flash-cooled in liquid nitrogen.

**[0175]** X-ray diffraction data was collected at MAXLAB 911-2, Lund University, Sweden using a Cryo-stream operated at 100 K. The raw data images were indexed, integrated and scaled using the XDS program package (Kabsch, Acta Crystallogr. D66, 133-144 (2010)). The space group of the crystal was P2(1)2(1)2(1), with unit cell parameters, a = 62.4 Å, b = 110.9 Å, c = 158.4 Å. Data were collected to a resolution of 2.40 Å. The structure was solved by molecular replacement using the Phenix software (Adams et al., Acta Crystallogr. D66, 213-221 (2010)) as implemented in the CCP4i program suite (Pottornton et al., Acta Crystallogr. D59, 1131-1137 (2003)). The search models were structures of the heavy chain from the pdb entry 1AD0.pdb (85% identity) and the light chain from the pdb entry 2QRG (94% identity). Structure refinement was carried out using Refmac5 (Murshudov et al., Acta Crystallogr. D53, 240-255 (1997)) from the CCP4i program suite. Coot version 7 (Emsley et al., Acta Crystallogr. D66, 486-501 (2010)) was used for manual structure rebuilding and validation.

#### **Results and discussion**

**[0176]** The crystal structure of the mAb 170 Fab region contained four Fab molecules in the asymmetric unit (heavy chains were labelled A and C, light chains were labelled B and D). The Fab molecules were packed as dimers with the antigen binding region of one Fab molecule interacting with the antigen binding region of another Fab molecule. It was not possible to include and refine the last cysteine from the SEQ ID NO: 19 in the crystal structure although it would be expected to be in disulphide bond with a Cys from SEQ ID NO: 18 from the heavy chain. There was some indication of excess 2Fo-Fc and Fo-Fc electron density in the area, and it is possible that the disulphide bond is present in a subset of the Fab molecules. There was not significant sigmaa weighted 2Fo-Fc electron density for residues G26, K78-N79, I104-R105 and S138-E141 from SEQ ID NO: 18 in chain A in the asymmetric unit and for E1, G26-F 27, M102-R105, S136-E141, S195-K200 from SEQ ID NO: 18 chain C in the other Fab molecule in the asymmetric unit. Significant sigmaa weighted 2Fo-Fc electron density was also missing for residues D30-Y34 from SEQ ID NO: 19 in chain D.

These residues were not included in the crystal structure but were included in molecular interaction analysis of the Fab-Fab interface by superposition with the mAb 0170 fragment crystal structure from the WO2013/120553 disclosed humanized anti-TREM-1 mAb 0170 crystal structure.

**[0177]** The quality parameters of the mAb 0170 Fab fragment structure showed an overall R-factor of the structure = 24% and the Free R-factor = 30%. The overall correlation coefficient was 0.93 and the diffraction-component precision index, DPI = 0.3 Å (Cruickshank, Acta Crystallogr. D55, 583-601 (1999)). The root-mean-square deviation of the bond lengths in the structure from ideal bond lengths = 0.025 Å and the root-mean-square deviation from ideal bond angles = 2.326° (Engh and Huber, Acta Crystallogr. A47, 392-400 (1991)).

**[0178]** Analysis of intermolecular distances was carried out using the program NCONT in the CCP4 program suite (Potterton et al., Acta Crystallogr. D59, 1131-1137 (2003)) with a cutoff of 4Å for intermolecular distances (Table 4). The analysis showed amino acid residues N57 and A59 from SEQ ID NO: 2 to belong to the group of amino acids involved in Fab-Fab interactions in the crystal structure.

Table 4. Predicted Fab-Fab interactions based on superposition of the WO2013/120553 disclosed humanized anti-TREM-1 mAb 0170 crystal structure with the present observed mAb 0170 Fab region crystal structure.

Source amino acids, Fab 1 (Chain A and B)		Target amino acids, Fab 2 (Chain C and D)	
F32	SEQ ID NO: 3	Y32	SEQ ID NO: 2
F32	SEQ ID NO: 3	M102	SEQ ID NO: 2
D33	SEQ ID NO: 3	Y53	SEQ ID NO: 3
Y34	SEQ ID NO: 3	Y34	SEQ ID NO: 3
Y34	SEQ ID NO: 3	R54	SEQ ID NO: 3
D98	SEQ ID NO: 3	S55	SEQ ID NO: 2
R52	SEQ ID NO: 2	S55	SEQ ID NO: 2
R52	SEQ ID NO: 2	S56	SEQ ID NO: 2
S55	SEQ ID NO: 2	A59	SEQ ID NO: 2
S55	SEQ ID NO: 2	R52	SEQ ID NO: 2
S56	SEQ ID NO: 2	S56	SEQ ID NO: 2
N57	SEQ ID NO: 2	S56	SEQ ID NO: 2
N57	SEQ ID NO: 2	N57	SEQ ID NO: 2
N57	SEQ ID NO: 2	A59	SEQ ID NO: 2
A59	SEQ ID NO: 2	S55	SEQ ID NO: 2
A59	SEQ ID NO: 2	S56	SEQ ID NO: 2
A59	SEQ ID NO: 2	N57	SEQ ID NO: 2
I104	SEQ ID NO: 2	I104	SEQ ID NO: 2
I104	SEQ ID NO: 2	R106	SEQ ID NO: 2
R106	SEQ ID NO: 2	F32	SEQ ID NO: 3

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50 55 60

Lys Thr Leu Ala Cys Thr Glu Arg Pro Ser Lys Asn Ser His Pro Val  
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Gln Val Gly Arg Ile Ile Leu Glu Asp Tyr His Asp His Gly Leu Leu  
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Arg Val Arg Met Val Asn Leu Gln Val Glu Asp Ser Gly Leu Tyr Gln  
100 105 110

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115

120

125

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Thr Asn Val Thr Asp Ile Ile Arg Val Pro Val Phe Asn Ile Val Ile  
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 35 40 45

Gly Arg Ile Arg Thr Lys Ser Ser Asn Tyr Ala Thr Tyr Tyr Ala Ala  
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
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Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Ser Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
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Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
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Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
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Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
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Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
                     85                    90                    95

Gln Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
                     100                    105                    110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
                     115                    120                    125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
                     130                    135                    140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
                     145                    150                    155                    160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
                     165                    170                    175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
                     180                    185                    190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
                     195                    200                    205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
                     210                    215

<210> 6

<211> 218

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain of a humanised TREM-1 antibody

<400> 6

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
   1                    5                    10                    15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Thr Phe  
                     20                    25                    30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
           35                          40                          45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
       50                          55                          60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
   65                          70                          75                          80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
                           85                          90                          95

Ser Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
                   100                          105                          110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
           115                          120                          125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
       130                          135                          140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
   145                          150                          155                          160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
                           165                          170                          175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
                   180                          185                          190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
           195                          200                          205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
       210                          215

<210> 7

<211> 218

<212> PRT

<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; light chain of a humanised TREM-1 antibody

&lt;400&gt; 7

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Thr Phe  
 20 25 30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Gln Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 8

<211> 218

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain of a humanised TREM-1 antibody

<400> 8

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Gln Ser Val Asp Thr Phe  
 20 25 30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 9  
 <211> 218  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> light chain of a humanised TREM-1 antibody

<400> 9

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Thr Ala  
 20 25 30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95



Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 10

<211> 218

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain of a humanised TREM-1 antibody

<400> 10

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Thr Ser  
 20 25 30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro

35

40

45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

&lt;210&gt; 11

&lt;211&gt; 448

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; heavy chain of a humanised TREM-1 antibody

&lt;400&gt; 11

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30  
 Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Gly Arg Ile Arg Thr Lys Ser Ser Asn Tyr Tyr Thr Tyr Tyr Ala Ala  
 50 55 60  
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80  
 Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95  
 Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125  
 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
 130 135 140  
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160  
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175  
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190  
 Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly  
 210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser  
 225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
 245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro  
 260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
 275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val  
 290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr  
 325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
 355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser  
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
                   420                  425                  430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
           435                  440                  445

<210> 12

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain of a humanised TREM-1 antibody

<400> 12

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
   1                  5                  10                  15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
           20                  25                  30

Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
           35                  40                  45

Gly Arg Ile Arg Thr Lys Ser Ser Ser Tyr Ala Thr Tyr Tyr Ala Ala  
   50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
   65                  70                  75                  80

Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
           85                  90                  95

Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
          100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
          115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
      130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly  
 210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser  
 225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
 245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro  
 260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
 275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val  
 290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr  
 325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys

355

360

365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser  
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 435 440 445

&lt;210&gt; 13

&lt;211&gt; 448

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; heavy chain of a humanised TREM-1 antibody

&lt;400&gt; 13

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30

Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Gly Arg Ile Arg Thr Lys Ser Ser Ser Tyr Tyr Thr Tyr Tyr Ala Ala  
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly  
210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser  
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro  
260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val



290

295

300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr  
 325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
 355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser  
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 435 440 445

&lt;210&gt; 14

&lt;211&gt; 218

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; light chain of a humanised TREM-1 antibody

&lt;400&gt; 14

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Gln Ser Val Asp Thr Ala

20

25

30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Gln Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 15  
 <211> 448  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> heavy chain of a humanised TREM-1 antibody

<400> 15

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30

Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Gly Arg Ile Arg Thr Lys Ser Ser Asn Tyr Tyr Thr Tyr Tyr Ala Ala  
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95

Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly  
 210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser  
 225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
 245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro  
 260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
 275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val  
 290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr  
 325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
 355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser  
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 435 440 445

<210> 16  
 <211> 448  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> heavy chain of a humanised TREM-1 antibody

<400> 16

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30

Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Gly Arg Ile Arg Thr Lys Ser Ser Asn Tyr Tyr Thr Tyr Tyr Ala Ala  
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95

Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly  
 210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser  
 225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
 245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro  
 260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala  
 275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val  
 290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr  
 305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr  
 325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
 355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
 370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser  
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 435 440 445

<210> 17  
 <211> 233  
 <212> PRT  
 <213> Macaca fascicularis

<400> 17

Met Arg Lys Thr Arg Leu Trp Gly Leu Leu Trp Met Leu Phe Val Ser  
 1 5 10 15

Glu Leu Arg Ala Thr Thr Glu Leu Thr Glu Glu Lys Tyr Glu Tyr Lys  
 20 25 30

Glu Gly Gln Thr Leu Glu Val Lys Cys Asp Tyr Ala Leu Glu Lys Tyr  
 35 40 45

Ala Asn Ser Arg Lys Ala Trp Gln Lys Met Glu Gly Lys Met Pro Lys  
 50 55 60

Ile Leu Ala Lys Thr Glu Arg Pro Ser Glu Asn Ser His Pro Val Gln

65		70		75		80
Val Gly Arg Ile Thr Leu Glu Asp Tyr Pro Asp His Gly Leu Leu Gln						
		85		90		95
Val Gln Met Thr Asn Leu Gln Val Glu Asp Ser Gly Leu Tyr Gln Cys						
		100		105		110
Val Ile Tyr Gln His Pro Lys Glu Ser His Val Leu Phe Asn Pro Ile						
		115		120		125
Cys Leu Val Val Thr Lys Gly Ser Ser Gly Thr Pro Gly Ser Ser Glu						
		130		135		140
Asn Ser Thr Gln Asn Val Tyr Arg Thr Pro Ser Thr Thr Ala Lys Ala						
		145		150		155
Leu Gly Pro Arg Tyr Thr Ser Pro Arg Thr Val Thr Gln Ala Pro Pro						
		165		170		175
Glu Ser Thr Val Val Val Ser Thr Pro Gly Ser Glu Ile Asn Leu Thr						
		180		185		190
Asn Val Thr Asp Ile Ile Arg Val Pro Val Phe Asn Ile Val Ile Ile						
		195		200		205
Val Ala Gly Gly Phe Leu Ser Lys Ser Leu Val Phe Ser Val Leu Phe						
		210		215		220
Ala Val Thr Leu Arg Ser Phe Gly Pro						
		225		230		

&lt;210&gt; 18

&lt;211&gt; 219

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; Expression construct for crystallization

&lt;400&gt; 18



Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30  
 Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Gly Arg Ile Arg Thr Lys Ser Ser Asn Tyr Ala Thr Tyr Tyr Ala Ala  
 50 55 60  
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80  
 Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95  
 Tyr Cys Thr Arg Asp Met Gly Ile Arg Arg Gln Phe Ala Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125  
 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
 130 135 140  
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160  
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175  
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190  
 Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
 195 200 205  
 Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val  
 210 215

<210> 19  
 <211> 218  
 <212> PRT  
 <213> artificial sequence

<220>  
 <223> Expression construct for crystallization

<400> 19

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Asp Thr Phe  
 20 25 30

Asp Tyr Ser Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro  
 35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Asp  
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
 65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn  
 85 90 95

Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

## PATENTKRAV

1. Antistof, der er i stand til at binde til og blokere TREM-1, som omfatter:

- (i) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 4;
- (ii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 5;
- (iii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 6;
- (iv) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 7;
- (v) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 8;
- (vi) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 9;
- (vii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 10;
- (viii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 2, og en let kæde, der omfatter SEQ ID NO: 14;
- (ix) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 11, og en let kæde, der omfatter SEQ ID NO: 3;
- (x) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 12, og en let kæde, der omfatter SEQ ID NO: 3;
- (xi) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 13, og en let kæde, der omfatter SEQ ID NO: 3;
- (xii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 15, og en let kæde, der omfatter SEQ ID NO: 5; eller
- (xiii) en tung kæde, der omfatter den variable region af tung kæde ifølge SEQ ID NO: 16, og en let kæde, der omfatter SEQ ID NO: 14;

hvor den tunge kæde omfatter en IgG4-konstant region.

2. Antistof ifølge krav 1, hvor den tunge kæde omfatter en variabel region af tung kæde ifølge SEQ ID NO: 2, og hvor den lette kæde omfatter SEQ ID NO: 5.

3. Farmaceutisk sammensætning, som omfatter antistoffet ifølge krav 1 eller krav 2 og et farmaceutisk acceptabelt bæremateriale.

4. Prokaryot eller eukaryot celle, som har rekombinant ekspresion af antistoffet ifølge krav 1 eller krav 2.

5. Fremgangsmåde til fremstilling af et antistof ifølge krav 1 eller krav 2, som omfatter rekombinant ekspression af antistoffet i en prokaryot eller eukaryot celle.
6. Antistof ifølge krav 1 eller krav 2 eller farmaceutisk sammensætning ifølge krav 3 til anvendelse som et medikament hos et individ, der har brug for det.
7. Antistof ifølge krav 1 eller krav 2 eller farmaceutisk sammensætning ifølge krav 3 til anvendelse til behandling af en inflammationssygdom eller en autoimmunsygdom hos et individ, der har brug for det.
8. Antistof eller farmaceutisk sammensætning til anvendelse ifølge krav 7, hvor inflammationssygdommen eller autoimmunsygdommen er valgt fra gruppen, der består af: inflammatorisk tarmsygdom, Crohns sygdom, ulcerativ colitis, irritabel tarmsyndrom, reumatoid arthritis, psoriasis, psoriasis arthritis, systemisk lupus erythematosus, lupus nefritis, type 1-diabetes, Graves sygdom, multipel sklerose, autoimmun myokarditis, Kawasaki sygdom, koronararteriesygdom, kronisk obstruktiv lungesygdom, interstitiel lungesygdom, autoimmun thyroiditis, sklerodermi, systemisk sklerose, osteoartrose, atopisk dermatitis, vitiligo, graft-versus-host-sygdom, Sjögrens syndrom, autoimmun nefritis, Goodpastures syndrom, kronisk inflammatorisk demyeliniserende polyneuropati, allergi, astma og andre autoimmunsygdomme, der skyldes akut eller kronisk inflammation.
9. Antistof eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 6 til 8, hvor antistoffet eller den farmaceutiske sammensætning administreres parenteralt.
10. Antistof eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 6 til 8, hvor antistoffet eller den farmaceutiske sammensætning administreres profylaktisk.
11. Antistof eller farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 6 til 10, hvor individet er et menneske.

## DRAWINGS

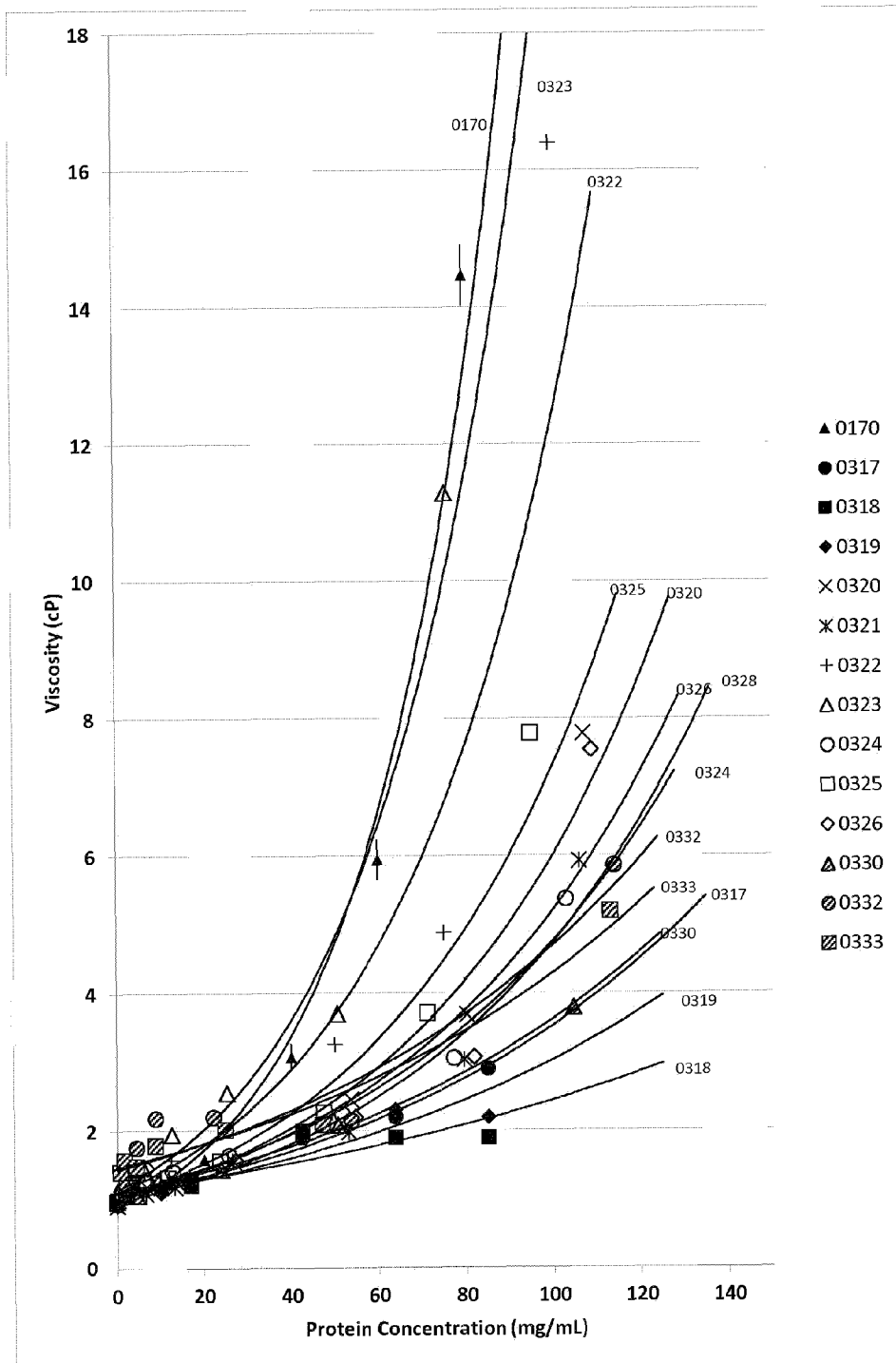


Figure 1

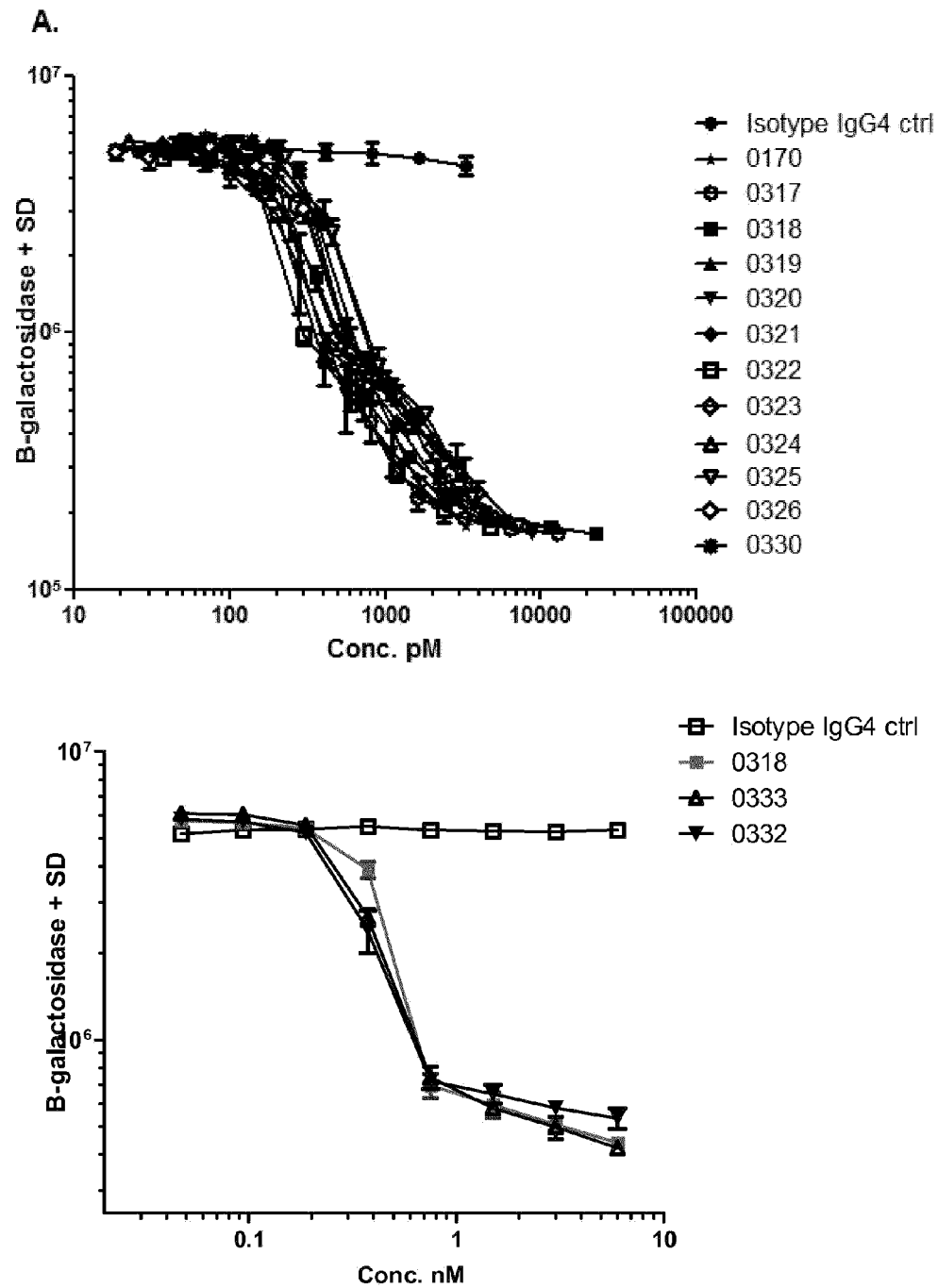
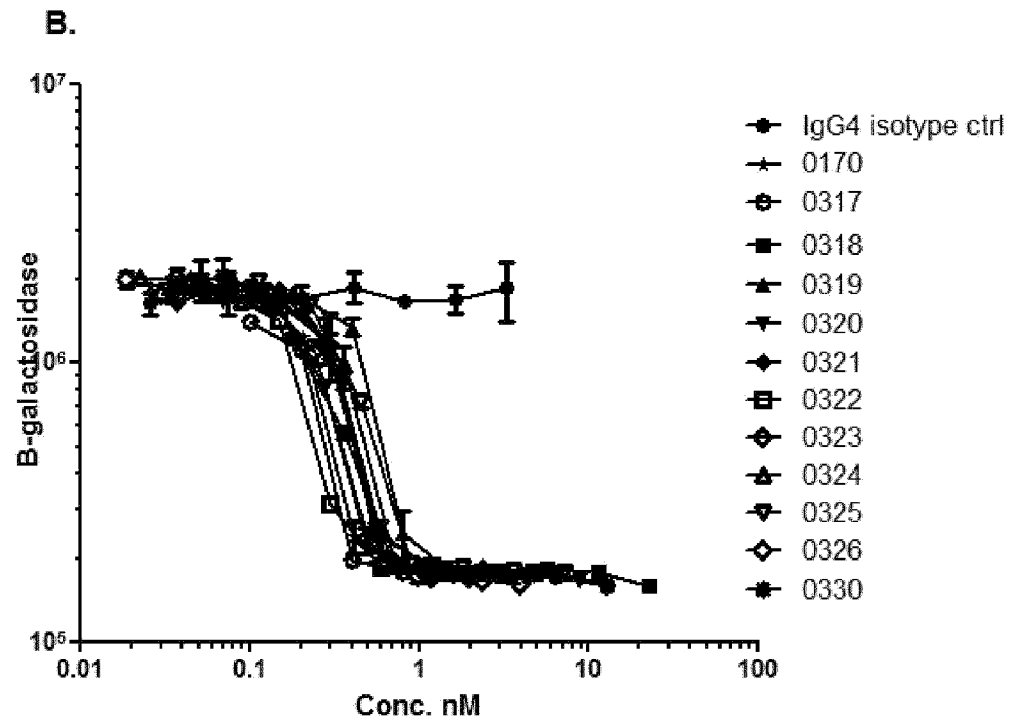


Figure 2

mAb ID	IC50 (nM)
0170	0.24
0317	0.21
0318	0.47
0319	0.23
0320	0.39
0321	0.20
0322	0.26
0323	0.35
0324	0.41
0325	0.31
0326	0.36
0330	0.24
0332	0.32
0333	0.33

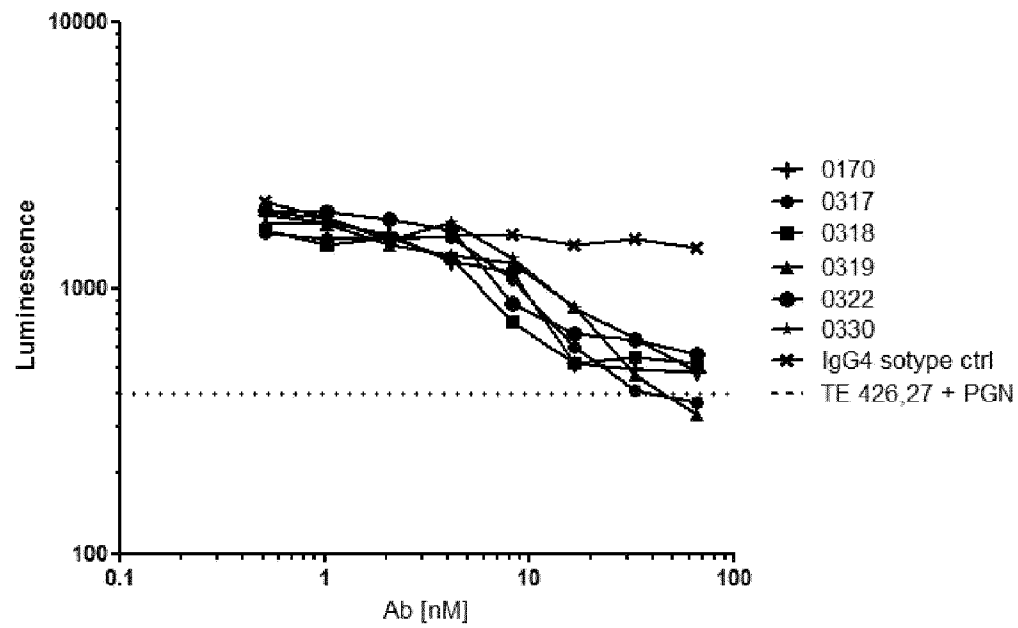
Figure 2A





SEQ. ID. NO	IC50 (nM)
0170	0.24
0317	0.33
0318	0.49
0319	0.27
0320	0.32
0321	0.19
0322	0.26
0323	0.34
0324	0.37
0325	0.31
0326	0.31
0330	0.22

Figure 2B



SEQ. ID. NO	IC50 (nM)
0170	6.0
0317	9.5
0318	5.6
0319	17.7
0322	5.9
0330	10.7

Figure 3

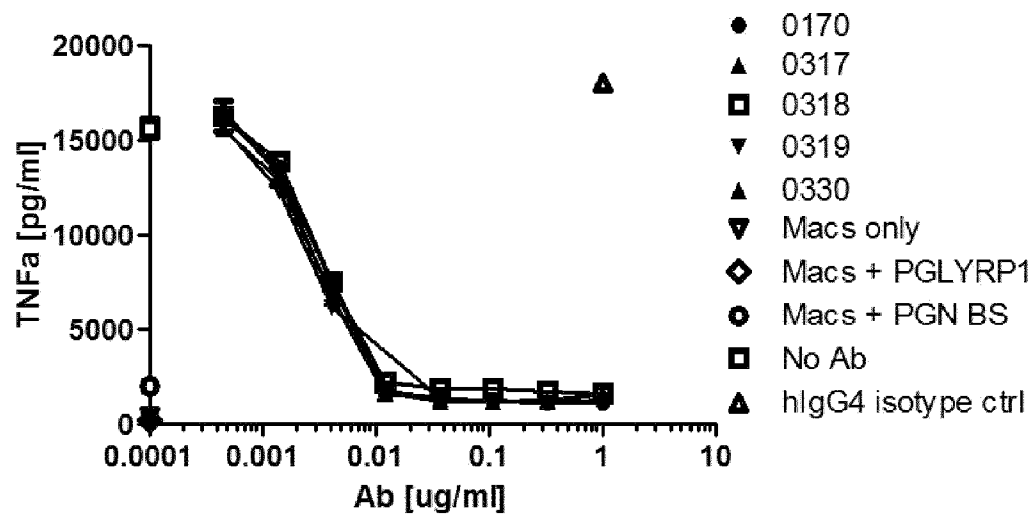


Figure 4

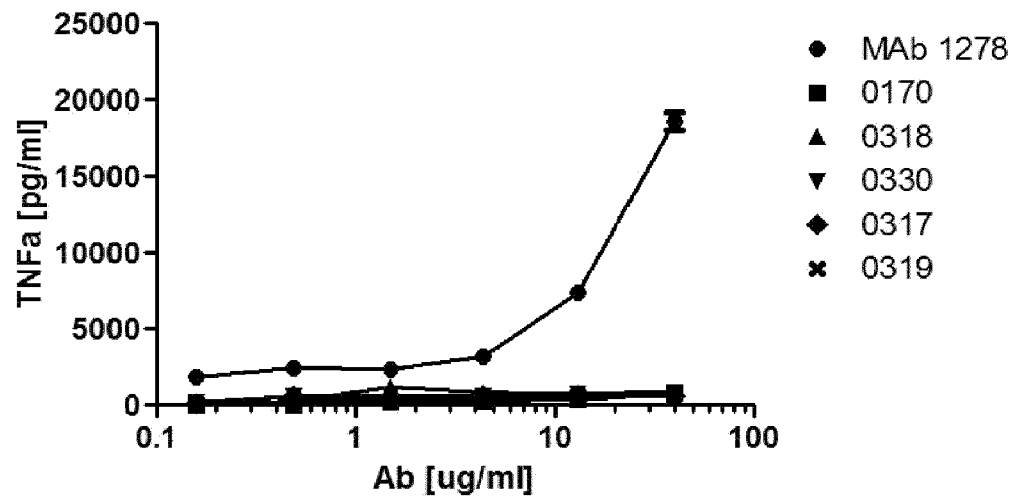


Figure 5

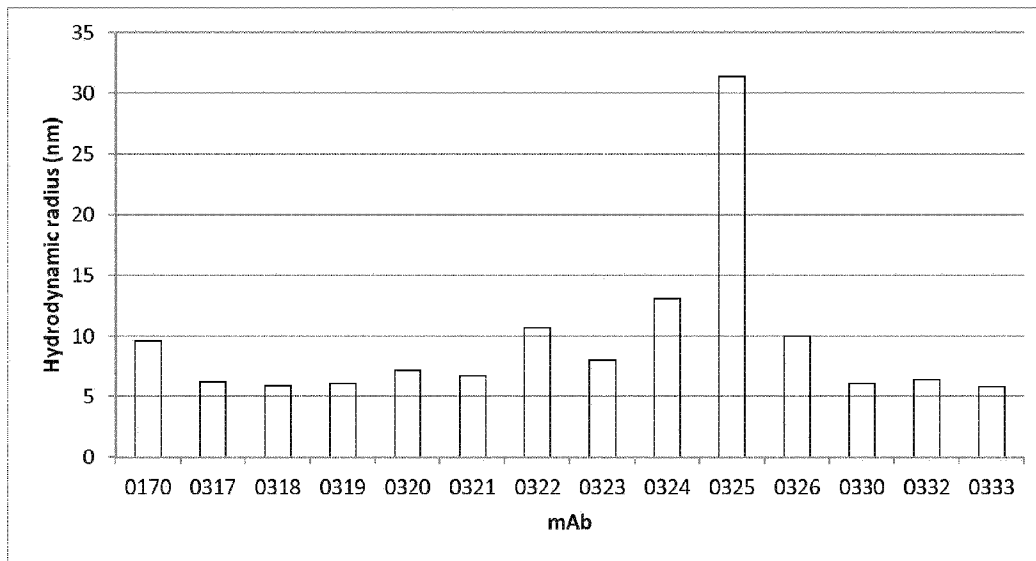


Figure 6

## SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

