ANTIBODIES AND USES THEREOF

Abstract:
The disclosure relates to antibodies specific to FcRn, formulations comprising the same, use of each in therapy, processes for expressing and optionally formulating said antibody, DNA encoding the antibodies and hosts comprising said DNA.

Figure 18  Change in plasma IgG levels in cynomolgus monkeys treated with 30 mg/Kg 1515; g67 IgG4 on day 0 followed by 5mg/Kg 1515; g57 IgG4 daily for 41 days

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(54) Title: ANTI-FcRn ANTIBODIES
Anti-FcRn Antibodies

The disclosure relates to antibodies specific to FcRn, formulations comprising the same, use of each in therapy, processes for expressing and optionally formulating said antibody, DNA encoding the antibodies and hosts comprising said DNA.

FcRn is a non-covalent complex of membrane protein FcRn a chain and β2 microglobulin (β2M). In adult mammals FcRn plays a key role in maintaining serum antibody levels by acting as a receptor that binds and salvages antibodies of the IgG isotype. IgG molecules are endocytosed by endothelial cells, and if they bind to FcRn, are recycled transcytosed out into, for example circulation. In contrast, IgG molecules that do not bind to FcRn enter the cells and are targeted to the lysosomal pathway where they are degraded. A variant IgGl in which His435 is mutated to alanine results in the selective loss of FcRn binding and a significantly reduced serum half-life (Firan et al. 2001, International Immunology 13:993).

It is hypothesised that FcRn is a potential therapeutic target for certain autoimmune disorders caused at least in part by autoantibodies. The current treatment for certain such disorders includes plasmapheresis. Sometimes the plasmapheresis is employed along with immunosuppressive therapy for long-term management of the disease. Plasma exchange offers the quickest short-term answer to removing harmful autoantibodies. However, it may also be desirable to suppress the production of autoantibodies by the immune system for example by the use of medications such as prednisone, cyclophosphamide, cyclosporine, mycophenolate mofetil, rituximab or a mixture of these.

Examples of diseases that can be treated with plasmapheresis include: Guillain-Barre syndrome; Chronic inflammatory demyelinating polyneuropathy; Goodpasture's syndrome; hyperviscosity syndromes; cryoglobulinemia; paraproteinemia; Waldenstrom macroglobulinemia; myasthenia gravis; thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome; Wegener's granulomatosis; Lambert-Eaton Syndrome; antiphospholipid antibody syndrome (APS or APLS); microscopic polyangiitis; recurrent focal and segmental glomerulosclerosis in the transplanted kidney; HELLP syndrome; PANDAS syndrome; Refsum disease; Behcet syndrome; HIV-related neuropathy; Graves' disease in infants and neonates; pemphigus vulgaris; multiple sclerosis, rhabdomyolysis and alloimmune diseases.

Plasmapheresis is sometimes used as a rescue therapy for removal of Fc containing therapeutics, for example in emergencies to reduced serious side effects.

Though plasmapheresis is helpful in certain medical conditions there are potential risks and complications associated with the therapy. Insertion of a rather large intravenous catheter can lead to bleeding, lung puncture (depending on the site of catheter insertion), and, if the catheter is left in too long, it can lead to infection and/or damage to the veins giving limited opportunity to repeat the procedure.

The procedure has further complications associated with it, for example when a patient's blood is outside of the body passing through the plasmapheresis instrument, the blood has a tendency to
clot. To reduce this tendency, in one common protocol, citrate is infused while the blood is running through the circuit. Citrate binds to calcium in the blood, calcium being essential for blood to clot. Citrate is very effective in preventing blood from clotting; however, its use can lead to life-threateningly low calcium levels. This can be detected using the Chvostek's sign or Trousseau's sign. To prevent this complication, calcium is infused intravenously while the patient is undergoing the plasmapheresis; in addition, calcium supplementation by mouth may also be given.

Other complications of the procedure include: hypotension; potential exposure to blood products, with risk of transfusion reactions or transfusion transmitted diseases, suppression of the patient's immune system and bleeding or hematoma from needle placement.

Additionally facilities that provide plasmapheresis are limited and the procedure is very expensive.

An alternative to plasmapheresis is intravenous immunoglobulin (IVIG), which is a blood product containing pooled polyclonal IgG extracted from the plasma of over one thousand blood donors. The therapy is administered intravenously and lasts in the region of 2 weeks to 3 months.

Complications of the IVIG treatment include headaches, dermatitis, viral infection from contamination of the therapeutic product, for example HIV or hepatitis, pulmonary edema, allergic reactions, acute renal failure, venous thrombosis and aseptic meningitis.

Thus there is a significant unmet need for therapies for autoimmune disorders which are less invasive and which expose the patients to less medical complications.

Thus there is a significant unmet need for therapies for immunological disorders and/or autoimmune disorders which are less invasive and which expose the patients to less medical complications.

Accordingly agents that block or reduce the binding of IgG to FcRn may be useful in the treatment or prevention of such autoimmune and inflammatory diseases. Anti-FcRn antibodies have been described previously in WO2009/131702, WO2007/087289 and WO2006/1 18772.

However, there remains a need for improved anti-FcRn antibodies.

**Summary of the Disclosure**

Thus in one aspect there is provided an anti-FcRn antibody or binding fragment thereof comprising a heavy chain or heavy chain fragment having a variable region, wherein said variable region comprises one, two or three CDRs independently selected from SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, for example wherein CDR H1 is SEQ ID NO: 1, CDR H2 is SEQ ID NO: 2 and CDR H3 is SEQ ID NO: 3.

In another aspect there is provided an antibody or fragment comprising a sequence or combinations of sequences as defined herein, for example a cognate pair variable region.
The antibodies of the disclosure block binding of IgG to FcRn and are thought to be useful in reducing one or more biological functions of FcRn, including reducing half-life of circulating antibodies. This may be beneficial in that it allows the patient to more rapidly clear antibodies, such as autoantibodies.

Importantly the antibodies of the present invention are able to bind human FcRn at both pH6 and pH7.4 with comparable and high binding affinity. Advantageously therefore the antibodies are able to continue to bind FcRn even within the endosome, thereby maximising the blocking of FcRn binding to IgG, see Figure 10 for an illustration of the mechanism.

In one embodiment the antibodies or binding fragments according to the present disclosure comprise a light chain or light chain fragment having a variable region, for example comprising one, two or three CDRs independently selected from SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, in particular wherein CDR L1 is SEQ ID NO: 4, CDR L2 is SEQ ID NO: 5 and CDR L3 is SEQ ID NO: 6.

In one embodiment the antibodies or binding fragments according to the present disclosure comprise CDR sequences of SEQ ID NOs: 1 to 6, for example wherein CDR H1 is SEQ ID NO: 1, CDR H2 is SEQ ID NO: 2, CDR H3 is SEQ ID NO: 3, CDR L1 is SEQ ID NO: 4, CDR L2 is SEQ ID NO: 5 and CDR L3 is SEQ ID NO: 6.

The disclosure also extends to a polynucleotide, such as DNA, encoding an antibody or fragment as described herein.

Also provided is a host cell comprising said polynucleotide.

Methods of expressing an antibody or fragment are provided herein as are methods of conjugating an antibody or fragment to a polymer, such as PEG.

The present disclosure also relates to pharmaceutical compositions comprising said antibodies and fragments.

In one embodiment there is provided a method of treatment comprising administering a therapeutically effective amount of an antibody, fragment or composition as described herein.

The present disclosure also extends to an antibody, fragment or composition according to the present disclosure for use in treatment, particularly in the treatment of an immunological and/or autoimmune disorder.

Thus the present disclosure provides antibodies, fragments thereof and methods for removal of pathogenic IgG, which is achieved by accelerating the body's natural mechanism for catabolising IgG.

In essence the antibodies and fragments according to the disclosure block the system that recycles IgG in the body.
The present therapy is likely to provide a replacement or supplement for certain diseases where plasmapheresis is a therapy or IVIg therapy, which is advantageous for patients.

**Brief Description of the Figures**

**Figure 1** shows certain amino acid and polynucleotide sequences.

**Figure 2** shows alignments of certain sequences.

**Figure 3** shows a comparison of binding on human MDCK II for a Fab' fragment according to the present disclosure and a PEGylated version thereof.

**Figure 4** shows a Fab' fragment according to the present disclosure and a PEGylated version thereof inhibiting IgG recycling on MDCK II cells.

**Figure 5** shows a PEGylated Fab' fragment according to the present disclosure inhibits apical to basolateral IgG transcytosis in MDCK II cells.

**Figure 6** shows a comparison of binding of cyno monkey MDCK II for a Fab' fragment according to the present disclosure and a PEGylated version thereof.

**Figure 7** shows a PEGylated Fab' fragment according to the present inhibiting IgG recycling on MDCK II cells for human and cyno monkey versions thereof.

**Figure 8** shows the effect of a single dose of a PEGylated Fab' molecule according to the disclosure on plasma IgG levels in cynomolgus monkeys.

**Figure 9** shows the effect of four weekly doses of a PEGylated Fab' molecule according to the disclosure on plasma IgG levels.

**Figure 10** shows a diagrammatic representation of antibody recycling function of FcRn inhibited by a blocking protein.

**Figure 11** shows flow cytometry based human IgG blocking assay using purified gamma 1 IgG antibodies.

**Figure 12** shows Fab'PEG single/intermittent IV doses in normal cyno 20mg/Kg days 1 and 25 67 IgG pharmacodynamics.

**Figure 13** shows Fab'PEG: repeat IV doses in normal cyno- 4x 20 or 100 mg/Kg per week IgG pharmacodynamics.

**Figure 14** shows Fab'PEG single/intermittent IV doses in normal cyno -20mg/Kg and 100 mg/Kg days 1 and 67 IgG Pharmacodynamics.

**Figure 15** shows plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 20mg/Kg 15 19.g57 Fab'PEG.

**Figure 16** shows plasma IgG levels in 4 cynomolgus monkeys receiving 10 IV doses of 20mg/Kg 1519.g57 Fab'PEG, one every 3 days.

**Figure 17** shows the effect of two 30mg/Kg IV doses of 15 19.g57 IgG4P on the endogenous plasma IgG in cynomolgus monkeys.

**Figure 18** shows the effect of 30 mg/Kg if followed by 41 daily doses of 5mg/Kg 15 19.g57 IgG4P on plasma IgG in cynomolgus monkeys.

**Figure 19** shows the result of daily dosing with vehicle on the plasma IgG in cynomolgus monkeys.

**Figure 20** shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'PEG or PBS IV.

**Figure 21** shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 IgGl or IgG4 or PBS IV.

**Figure 22** shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'-human serum albumin or PBS IV.
Figure 23 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 FabFv or PBS IV

Figure 24 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab or Fab’PEG or PBS IV

Figure 25 shows a bispecific antibody fusion protein of the present invention, referred to as a Fab-dsFv.

Details of the Disclosure

FcRn as employed herein refers to the non-covalent complex between the human IgG receptor alpha chain, also known as the neonatal Fc receptor, the amino acid sequence of which is in UniProt under number P55899 together with β2 microglobulin (β2M), the amino acid sequence of which is in UniProt under number P61769.

Antibody molecule as employed herein refers to an antibody or binding fragment thereof.

The term 'antibody' as used herein generally relates to intact (whole) antibodies i.e. comprising the elements of two heavy chains and two light chains. The antibody may comprise further additional binding domains for example as per the molecule DVD-Ig as disclosed in WO 2007/024715, or the so-called (FabFv)2Fc described in WO2011/030107. Thus antibody as employed herein includes bi, tri or tetra-valent full length antibodies.

Binding fragments of antibodies include single chain antibodies (i.e. a full length heavy chain and light chain); Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, tribodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9): 1126-1 136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al, 1998, Journal of Immunological Methods, 216, 165-181). The Fab-Fv format was first disclosed in WO2009/040562 and the disulphide stabilised versions thereof, the Fab-dsFv was first disclosed in WO2010/035012, see also Figure 25 herein. Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003 169, WO2005/003 170 and WO2005/003 171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22583 and WO05/1 13605). One such example of the latter is a Tri-Fab (or TFM) as described in W092/22583.

A typical Fab' molecule comprises a heavy and a light chain pair in which the heavy chain comprises a variable region V_H, a constant domain C_H1 and a natural or modified hinge region and the light chain comprises a variable region V_L and a constant domain C_L.
In one embodiment there is provided a dimer of a Fab' according to the present disclosure to create a F(ab')₂ for example dimerisation may be through the hinge.

In one embodiment the antibody or binding fragment thereof comprises a binding domain. A binding domain will generally comprises 6 CDRs, three from a heavy chain and three from a light chain. In one embodiment the CDRs are in a framework and together form a variable region. Thus in one embodiment an antibody or binding fragment comprises a binding domain specific for antigen comprising a light chain variable region and a heavy chain variable region.

It will be appreciated that one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the CDRs or other sequences (e.g. variable domains) provided by the present invention without significantly altering the ability of the antibody to bind to FcRn. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described herein, in particular in the Examples, to determine FcRn.

In one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the framework region employed in the antibody or fragment provided by the present invention and wherein binding affinity to FcRn is retained or increased.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al. (supra)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1 ' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.
Antibodies and fragments of the present disclosure block FcRn and may thereby prevent it functioning in the recycling of IgG. Blocking as employed herein refers to physically blocking such as occluding the receptor but will also include where the antibody or fragments binds an epitope that causes, for example a conformational change which means that the natural ligand to the receptor no longer binds. Antibody molecules of the present invention bind to FcRn and thereby decrease or prevent (e.g. inhibit) FcRn binding to an IgG constant region.

In one embodiment the antibody or fragment thereof binds FcRn competitively with respect to IgG.

In one example the antibody or binding fragment thereof functions as a competitive inhibitor of human FcRn binding to human IgG. In one example the antibody or binding fragment thereof binds to the IgG binding site on FcRn. In one example the antibody or binding fragment thereof does not bind β2M.

Antibodies for use in the present disclosure may be obtained using any suitable method known in the art. The FcRn polypeptide/protein including fusion proteins, cells (recombinantly or naturally) expressing the polypeptide (such as activated T cells) can be used to produce antibodies which specifically recognise FcRn. The polypeptide may be the 'mature' polypeptide or a biologically active fragment or derivative thereof. The human protein is registered in Swiss-Prot under the number P55899. The extracellular domain of human FcRn alpha chain is provided in SEQ ID NO:94. The sequence of β2M is provided in SEQ ID NO:95.

In one embodiment the antigen is a mutant form of FcRn which is engineered to present FcRn on the surface of a cell, such that there is little or no dynamic processing where the FcRn is internalised in the cell, for example this can be achieved by making a mutation in the cytoplasmic tail of the FcRn alpha chain, wherein di-leucine is mutated to di-alanine as described in Ober et al 2001 Int. Immunol. 13, 1551-1559.

Polypeptides, for use to immunize a host, may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The FcRn polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag or similar.

Antibodies generated against the FcRn polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986).

Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

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

Antibodies for use in the invention may also be generated using single lymphocyte antibody
methods by cloning and expressing immunoglobulin variable region cDNAs generated from
single lymphocytes selected for the production of specific antibodies by, for example, the

Screening for antibodies can be performed using assays to measure binding to human FcRn
and/or assays to measure the ability to block IgG binding to the receptor. An example of a
binding assay is an ELISA, in particular, using a fusion protein of human FcRn and human Fc,
which is immobilized on plates, and employing a secondary antibody to detect anti-FcRn
antibody bound to the fusion protein. Examples of suitable antagonistic and blocking assays are
described in the Examples herein.

Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having
one or more complementarity determining regions (CDRs) from a non-human species and a
framework region from a human immunoglobulin molecule (see, e.g. US 5,585,089; WO9 1/09967). It will be appreciated that it may only be necessary to transfer the specificity
determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri et al.,
2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more
framework residues derived from the non-human species from which the CDRs were derived.
The latter are often referred to as donor residues.

Specific as employed herein is intended to refer to an antibody that only recognises the antigen
to which it is specific or an antibody that has significantly higher binding affinity to the antigen
to which it is specific compared to binding to antigens to which it is non-specific, for example at
least 5, 6, 7, 8, 9, 10 times higher binding affinity. Binding affinity may be measured by
techniques such as BIAcore as described herein below. In one example the antibody of the
present invention does not bind β2 microglobulin (β2M). In one example the antibody of the
present invention binds cynomolgus FcRn. In one example the antibody of the present invention
does not bind rat or mouse FcRn.

The amino acid sequences and the polynucleotide sequences of certain antibodies according to
the present disclosure are provided in the Figures.

In one embodiment the antibody or fragments according to the disclosure are humanised.

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

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions.

Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided specifically herein. Thus, provided in one embodiment is blocking humanised antibody which binds human FcRn wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat et al, supra). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/

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

One such suitable framework region for the heavy chain of the humanised antibody of the present invention is derived from the human sub-group VH3 sequence 1-3 3-07 together with JH4 (SEQ ID NO: 56).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2 and the sequence given in SEQ ID NO: 3 for CDRH3, wherein the heavy chain framework region is derived from the human subgroup VH3 sequence 1-3 3-07 together with JH4.

The sequence of human JH4 is as follows: (YFDY)WGQGTTLVTVS (Seq ID No: 70). The YFDY motif is part of CDR-H3 and is not part of framework 4 (Ravetch, JV. et al., 1981, Cell, 27, 583-591).

In one example the heavy chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 29.
A suitable framework region for the light chain of the humanised antibody of the present invention is derived from the human germline sub-group VK1 sequence 2-l-(l) A30 together with JK2 (SEQ ID NO: 54).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDRL3, wherein the light chain framework region is derived from the human subgroup VK1 sequence 2-l-(l) A30 together with JK2.

The JK2 sequence is as follows: (YT)FGQGTKLEIK (Seq ID No: 71). The YT motif is part of CDR-L3 and is not part of framework 4 (Hieter, PA., et al., 1982, J. Biol. Chem., 257, 1516-1522).

In one example the light chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 15.

In a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type.

Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann et al., 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in W09 1/09967.

Thus in one embodiment 1, 2, 3, 4, or 5 residues in the framework are replaced with an alternative amino acid residue.

Accordingly, in one example there is provided a humanised antibody, wherein at least the residues at each of positions 3, 24, 76, 93 and 94 of the variable domain of the heavy chain (Kabat numbering) are donor residues, see for example the sequence given in SEQ ID NO: 29.

In one embodiment residue 3 of the heavy chain variable domain is replaced with an alternative amino acid, for example glutamine.

In one embodiment residue 24 of the heavy chain variable domain is replaced with an alternative amino acid, for example alanine.

In one embodiment residue 76 of the heavy chain variable domain is replaced with an alternative amino acid, for example asparagine.

In one embodiment residue 93 of the heavy chain is replaced with an alternative amino acid, for example alanine.

In one embodiment residue 94 of the heavy chain is replaced with an alternative amino acid, for example arginine.
In one embodiment residue 3 is glutamine, residue 24 is alanine, residue 76 is asparagine, residue 93 is alanine and residue 94 is arginine in the humanised heavy chain variable region according to the present disclosure.

Accordingly, in one example there is provided a humanised antibody, wherein at least the residues at each of positions 36, 37 and 58 of the variable domain of the light chain (Kabat numbering) are donor residues, see for example the sequence given in SEQ ID NO: 15

In one embodiment residue 36 of the light chain variable domain is replaced with an alternative amino acid, for example tyrosine.

In one embodiment residue 37 of the light chain variable domain is replaced with an alternative amino acid, for example glutamine.

In one embodiment residue 58 of the light chain variable domain is replaced with an alternative amino acid, for example valine.

In one embodiment residue 36 is tyrosine, residue 37 is glutamine and residue 58 is valine, in the humanised heavy chain variable region according to the present disclosure.

In one embodiment the disclosure provides an antibody sequence which is 80% similar or identical to a sequence disclosed herein, for example 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% over part or whole of the relevant sequence, for example a variable domain sequence, a CDR sequence or a variable domain sequence, excluding the CDRs. In one embodiment the relevant sequence is SEQ ID NO: 15. In one embodiment the relevant sequence is SEQ ID NO: 29.

In one embodiment, the present invention provides an antibody molecule which binds human FcRn comprising a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity or similarity to the sequence given in SEQ ID NO:29.

In one embodiment, the present invention provides an antibody molecule which binds human FcRn comprising a light chain, wherein the variable domain of the light chain comprises a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity or similarity to the sequence given in SEQ ID NO: 15.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a heavy chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to the sequence given in SEQ ID NO:29 but wherein the antibody molecule has the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2 and the sequence given in SEQ ID NO: 3 for CDR-H3.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a light chain variable domain which is at least 90%, 91%, 92%,
93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to the sequence given in SEQ ID NO:15 but wherein the antibody molecule has the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a heavy chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to the sequence given in SEQ ID NO:29 and a light chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to the sequence given in SEQ ID NO:15 but wherein the antibody molecule has the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2, the sequence given in SEQ ID NO: 3 for CDR-H3, the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDR-L3.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and

The antibody molecules of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', modified Fab', F(ab') 2, Fv, single domain antibodies (e.g. VH or VL or VH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005,
The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003 169, WO2005/003 170 and WO2005/003 171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22853, WO05/1 13605, WO2009/040562 and WO2010/035012).

In one embodiment the antibody molecule of the present disclosure is an antibody Fab' fragment comprising the variable regions shown in SEQ ID NOs: 15 and 29 for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO:22 and a heavy chain comprising the sequence given in SEQ ID NO:36.

In one embodiment the antibody molecule of the present disclosure is a full length IgGl antibody comprising the variable regions shown in SEQ ID NOs: 15 and 29 for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO:22 and a heavy chain comprising the sequence given in SEQ ID NO:72.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4 format comprising the variable regions shown in SEQ ID NOs: 15 and 29 for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO:22 and a heavy chain comprising the sequence given in SEQ ID NO:87.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4P format comprising the variable regions shown in SEQ ID NOs: 15 and 29 for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO:22 and a heavy chain comprising the sequence given in SEQ ID NO:43.

IgG4P as employed herein is a mutation of the wild-type IgG4 isotype where amino acid 241 is replaced by proline see for example where serine at position 241 has been changed to proline as described in Angal et al., Molecular Immunology, 1993, 30 (1), 105-108.

In one embodiment the antibody according to the present disclosure is provided as FcRn binding antibody fusion protein which comprises an immunoglobulin moiety, for example a Fab or Fab' fragment, and one or two single domain antibodies (dAb) linked directly or indirectly thereto, for example as described in WO2009/040562, WO2010035012, WO201 1/030107, WO201 1/061492 and WO201 1/086091 all incorporated herein by reference.

In one embodiment the fusion protein comprises two domain antibodies, for example as a variable heavy (VH) and variable light (VL) pairing, optionally linked by a disulphide bond.
In one embodiment the Fab or Fab' element of the fusion protein has the same or similar
specificity to the single domain antibody or antibodies. In one embodiment the Fab or Fab' has a
different specificity to the single domain antibody or antibodies, that is to say the fusion protein
is multivalent. In one embodiment a multivalent fusion protein according to the present
invention has an albumin binding site, for example a VH/VL pair therein provides an albumin
binding site. In one such embodiment the heavy chain comprises the sequence given in SEQ ID NO:50 and the light chain comprises the sequence given in SEQ ID NO:46 or SEQ ID NO:78.
This Fab-dsFv format is illustrated in Figure 25 herein.

In one embodiment the Fab or Fab' according to the present disclosure is conjugated to a PEG
molecule or human serum albumin.

CA170_01519g57 and 1519 and 1519.g57 are employed inchangeably herein and are used to
refer to a specific pair of antibody variable regions which may be used in a number of different
formats. These variable regions are the heavy chain sequence given in SEQ ID NO:29 and the
light chain sequence given in SEQ ID NO:15 (Figure 1).

The constant region domains of the antibody molecule of the present invention, if present, may
be selected having regard to the proposed function of the antibody molecule, and in particular the
effector functions which may be required. For example, the constant region domains may be
human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains
may be used, especially of the IgGl and IgG3 isotypes when the antibody molecule is intended
for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4
isotypes may be used when the antibody molecule is intended for therapeutic purposes and
antibody effector functions are not required. It will be appreciated that sequence variants of
these constant region domains may also be used. For example IgG4 molecules in which the
serine at position 241 has been changed to proline as described in Angal et al., Molecular
Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by one skilled in
the art that antibodies may undergo a variety of posttranslational modifications. The type and
extent of these modifications often depends on the host cell line used to express the antibody as
well as the culture conditions. Such modifications may include variations in glycosylation,
methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine
deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as
lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of
Chromatography 705:129-134, 1995). Accordingly, the C-terminal lysine of the antibody heavy
chain may be absent.

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

In one embodiment the light chain has the sequence given in SEQ ID NO:22 and the heavy chain
has the sequence given in SEQ ID NO:43.

In one embodiment the light chain has the sequence given in SEQ ID NO:22 and the heavy chain
has the sequence given in SEQ ID NO:72.
In one embodiment a C-terminal amino acid from the antibody molecule is cleaved during post-
translation modifications.

In one embodiment an N-terminal amino acid from the antibody molecule is cleaved during post-
translation modifications.

Also provided by the present invention is a specific region or epitope of human FcRn which is
bound by an antibody provided by the present invention, in particular an antibody comprising the
heavy chain sequence gH20 (SEQ ID NO:29) and/or the light chain sequence gL20 (SEQ ID
NO:15).

This specific region or epitope of the human FcRn polypeptide can be identified by any suitable
epitope mapping method known in the art in combination with any one of the antibodies provided
by the present invention. Examples of such methods include screening peptides of varying lengths
derived from FcRn for binding to the antibody of the present invention with the smallest fragment
that can specifically bind to the antibody containing the sequence of the epitope recognised by the
antibody. The FcRn peptides may be produced synthetically or by proteolytic digestion of the
FcRn polypeptide. Peptides that bind the antibody can be identified by, for example, mass
spectrometric analysis. In another example, NMR spectroscopy or X-ray crystallography can be
used to identify the epitope bound by an antibody of the present invention. Once identified, the
epitopic fragment which binds an antibody of the present invention can be used, if required, as an
immunogen to obtain additional antibodies which bind the same epitope.

In one embodiment the antibody of the present disclosure binds the human FcRn alpha chain
extracellular sequence as shown below:

AESHLSLLYHLTAVSSPAPGTPAFWVSGLWL GPOQYLSYNSLRGEAEPCGA WVWENQWSWY WEKETTDLRI
KEKLFLFLEAFK ALGOKGKGPYTL QGLLGCELGP DNTSVPtAKF ALOGEEFMNF DLKQGTWWGD WPEA LAISQR
WQQQDKAANK ELTFLFSCP HRLEH LERG RGN LEWKEPP SM RLKARPSS PGFSVLTCSA FSFYPPEQL
RFLRNLGAAGTGQDFGPNS DGSFHASSSLTVKSGDEH HYCCIVHQAGLA QPLRVELESPAKSS (SEQ ID NO:
94).

The residues underlined are those known to be critical for the interaction of human FcRn with the
Fc region of human IgG and those residues highlighted in bold are those involved in the interaction
of FcRn with the 1519 antibody of the present disclosure comprising the heavy chain sequence
gH20 (SEQ ID NO:29) and the light chain sequence gL20 (SEQ ID NO:15).

In one example, the present invention provides an anti-FcRn antibody molecule which binds an
epitope of human FcRn which comprises at least one amino acid selected from the group
consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one
residue, for example at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues selected from the group consisting
of P100, E115, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, G129, D130,
W131, P132 and E133 of SEQ ID NO:94.
In one example the epitope of the antibody molecule is determined by X-ray crystallography using the FcRn alpha chain extracellular sequence (SEQ ID NO:94) in complex with β2M.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises at least one amino acid selected from the group consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue, for example at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues, selected from the group consisting of E115, E116, F117, M118, N119, F120, D121, L122, K123 and Q124 of SEQ ID NO:94.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises at least two, three, four or five amino acids selected from the group consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of E115, E116, F117, M118, N119, F120, D121, L122, K123 and Q124 of SEQ ID NO:94.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises at least one amino acid selected from the group consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of P100, E115, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, G129, D130, W131, P132 and E133 of SEQ ID NO:94.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises at least one amino acid selected from the group consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of P100, M118, N119, F120, D121, L122, K123, Q124 and G128 of SEQ ID NO:94.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of P100, M118, N119, F120, D121, L122, K123, Q124 and G128 of SEQ ID NO:94.

In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of P100, E115, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, G129, D130, W131, P132 and E133 of SEQ ID NO:94.
In one example, the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises residues P100, V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of E115, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, G129, D130, W131, P132 and E133 of SEQ ID NO:94.

In one example 'at least one residue' may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 residues.

In one example the present invention provides an anti-FcRn antibody molecule which binds an epitope of human FcRn which comprises or consists of residues 100, 105 to 109, 115 to 124 and 129 to 133 of SEQ ID NO: 94.

Antibodies which cross-block the binding of an antibody molecule according to the present invention in particular, an antibody molecule comprising the heavy chain sequence given in SEQ ID NO:29 and the light chain sequence given in SEQ ID NO:15 may be similarly useful in blocking FcRn activity. Accordingly, the present invention also provides an anti-FcRn antibody molecule, which cross-blocks the binding of any one of the antibody molecules described herein above to human FcRn and/or is cross-blocked from binding human FcRn by any one of those antibodies. In one embodiment, such an antibody binds to the same epitope as an antibody described herein above. In another embodiment the cross-blocking neutralising antibody binds to an epitope which borders and/or overlaps with the epitope bound by an antibody described herein above.

Cross-blocking antibodies can be identified using any suitable method in the art, for example by using competition ELISA or BIAcore assays where binding of the cross blocking antibody to human FcRn prevents the binding of an antibody of the present invention or *vice versa*. Such cross blocking assays may use isolated natural or recombinant FcRn or a suitable fusion protein/polypeptide. In one example binding and cross-blocking is measured using recombinant human FcRn extracellular domain (SEQ ID NO:94). In one example the recombinant human FcRn alpha chain extracellular domain is used in a complex with β2 microglobulin (β2M) (SEQ ID NO:95).

In one embodiment there is provided an anti-FcRn antibody molecule which blocks FcRn binding to IgG and which cross-blocks the binding of an antibody whose heavy chain comprises the sequence given in SEQ ID NO:29 and whose light chain comprises the sequence given in SEQ ID NO:15 to human FcRn. In one embodiment the cross-blocking antibodies provided by the present invention inhibit the binding of an antibody comprising the heavy chain sequence given in SEQ ID NO:29 and the light chain sequence given in SEQ ID NO:15 by greater than 80%, for example by greater than 85%, such as by greater than 90%, in particular by greater than 95%.
Alternatively or in addition, anti-FcRn antibodies according to this aspect of the invention may be cross-blocked from binding to human FcRn by an antibody comprising the heavy chain sequence given in SEQ ID NO: 29 and the light chain sequence given in SEQ ID NO: 15. Also provided therefore is an anti-FcRn antibody molecule which blocks FcRn binding to IgG and which is cross-blocked from binding human FcRn by an antibody comprising the heavy chain sequence given in SEQ ID NO: 29 and the light chain sequence given in SEQ ID NO: 15. In one embodiment the anti-FcRn antibodies provided by this aspect of the invention are inhibited from binding human FcRn by an antibody comprising the heavy chain sequence given in SEQ ID NO: 29 and the light chain sequence given in SEQ ID NO: 15 by greater than 80%, for example by greater than 85%, such as by greater than 90%, in particular by greater than 95%.

In one embodiment the cross-blocking antibodies provided by the present invention are fully human. In one embodiment the cross-blocking antibodies provided by the present invention are humanised. In one embodiment the cross-blocking antibodies provided by the present invention have an affinity for human FcRn of 100pM or less. In one embodiment the cross-blocking antibodies provided by the present invention have an affinity for human FcRn of 50pM or less. Affinity can be measured using the methods described herein below.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pi) is the pH at which a particular molecule or solvent accessible surface thereof carries no net electrical charge. In one example, the FcRn antibody and fragments of the invention may be engineered to have an appropriate isoelectric point. This may lead to antibodies and/or fragments with more robust properties, in particular suitable solubility and/or stability profiles and/or improved purification characteristics.

Thus in one aspect the invention provides a humanised FcRn antibody engineered to have an isoelectric point different to that of the originally identified antibody. The antibody may, for example be engineered by replacing an amino acid residue such as replacing an acidic amino acid residue with one or more basic amino acid residues. Alternatively, basic amino acid residues may be introduced or acidic amino acid residues can be removed. Alternatively, if the molecule has an unacceptably high pi value acidic residues may be introduced to lower the pi, as required. It is important that when manipulating the pi care must be taken to retain the desirable activity of the antibody or fragment. Thus in one embodiment the engineered antibody or fragment has the same or substantially the same activity as the "unmodified" antibody or fragment.

Programs such as **ExPASY [htp://www.expasy.ch/tools/pi_tool.html](http://www.expasy.ch/tools/pi_tool.html) and [http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim\compo-p.html](http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim\compo-p.html), may be used to predict the isoelectric point of the antibody or fragment.
The antibody molecules of the present invention suitably have a high binding affinity, in particular in the nanomolar range. Affinity may be measured using any suitable method known in the art, including BIAcore, as described in the Examples herein, using isolated natural or recombinant FcRn or a suitable fusion protein/polypeptide. In one example affinity is measured using recombinant human FcRn extracellular domain as described in the Examples herein (SEQ ID NO:94). In one example affinity is measured using the recombinant human FcRn alpha chain extracellular domain (SEQ ID NO:94) in association with β2 microglobulin (β2M) (SEQ ID NO:95). Suitably the antibody molecules of the present invention have a binding affinity for isolated human FcRn of about lnM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 500pM or lower (i.e. higher affinity). In one embodiment the antibody molecule of the present invention has a binding affinity of about 250pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 200pM or lower. In one embodiment the present invention provides an anti-FcRn antibody with a binding affinity of about 100pM or lower. In one embodiment the present invention provides a humanised anti-FcRn antibody with a binding affinity of about 100pM or lower. In one embodiment the present invention provides an anti-FcRn antibody with a binding affinity of 50pM or lower.

Importantly the antibodies of the present invention are able to bind human FcRn at both pH6 and pH7.4 with comparable binding affinity. Advantageously therefore the antibodies are able to continue to bind FcRn even within the endosome, thereby maximising the blocking of FcRn binding to IgG, see Figure 10 for an illustration of the mechanism.

In one embodiment the present invention provides an anti-FcRn antibody with a binding affinity of 100pM or lower when measured at pH6 and pH7.4.

The affinity of an antibody or binding fragment of the present invention, as well as the extent to which a binding agent (such as an antibody) inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard et al. (Ann. KY. Acad. Sci. 51:660-672 (1949)) or by surface plasmon resonance (SPR) using systems such as BIAcore. For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al, Cancer Res. 53:2560-65 (1993)).

In the present invention affinity of the test antibody molecule is typically determined using SPR as follows. The test antibody molecule is captured on the solid phase and human FcRn alpha chain extracellular domain in non-covalent complex with β2M is run over the captured antibody in the mobile phase and affinity of the test antibody molecule for human FcRn determined. The test antibody molecule may be captured on the solid phase chip surface using any appropriate
method, for example using an anti-Fc or anti Fab' specific capture agent. In one example the affinity is determined at pH6. In one example the affinity is determined at pH7.4.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for FcRn. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang et al., J. Mol. Biol, 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutator strains of E. coli (Low et al., J. Mol. Biol, 250, 359-368, 1996), DNA shuffling (Patten et al, Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al, J. Mol. Biol, 256, 77-88, 1996) and sexual PCR (Crameri et al, Nature, 391, 288-291, 1998). Vaughan et al. (supra) discusses these methods of affinity maturation.

In one embodiment the antibody molecules of the present invention block human FcRn activity. Assays suitable for determining the ability of an antibody to block FcRn are described in the Examples herein. Suitable assays for determining whether antibodies block FcRn interaction with circulating IgG molecules as described in the Examples herein. A suitable assay for determining the ability of an antibody molecule to block IgG recycling in vitro is described herein below.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom et al, Controlled Drug Delivery, 2nd Ed., Robinson et al, eds., 1987, pp. 623-53; Thorpe et al, 1982, Immunol. Rev., 62:1 19-58 and Dubowchik et al, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00 195, WO 89/01476 and WO 03/03 1581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.
Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to *(e.g. kills)* cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracion dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimitabolites *(e.g. methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents *(e.g. mechlorethamine, thioea chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitoul, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines *(e.g. daunorubicin (formerly daunomycin) and doxorubicin), antibiotics *(e.g. dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-

mitotic agents *(e.g. vincristine and vinblastine)*.

Other effector molecules may include chelated radionuclides such as $^{111}$In and $^{90}$Y, Lu$^{177}$, Bismuth$^{213}$, Californium$^{252}$, Iridium$^{192}$ and Tungsten$^{188}$/Rhenium$^{188}$, or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumour necrosis factor, a-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e. g*. angiotatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include $^{125}$I, $^{131}$I, $^{111}$In $^{99}$Tc.
In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

In one embodiment a half-life provided by an effector molecule which is independent of FcRn is advantageous.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethylene glycol), poly(propylene glycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethylene glycol) such as methoxypoly(ethylene glycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylase, dextran, glycogen or derivatives thereof.

In one embodiment the polymer is albumin or a fragment thereof, such as human serum albumin or a fragment thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.
Suitable polymers include a polyalkylene polymer, such as a poly(ethylene glycol) or, especially, a methoxypoly(ethylene glycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

In one example antibodies for use in the present invention are attached to poly(ethylene glycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971, WO2008/038024). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached.

Multiple sites can be used to attach two or more PEG molecules.

Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an a-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment, Fab' fragment or diFab which is PEGylated, i.e. has PEG (poly(ethylene glycol)) covalently attached thereto, e.g. according to the method disclosed in EP 0948544 or EP 1090037 [see also "Poly(ethylene glycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethylene glycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to
each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

Particular PEG molecules include 2-\([3-(N\text{-maleimido})propionamido]ethyl\) amide of N,N'-bis(methoxypoly(ethylene glycol) MW 20,000) modified lysine, also known as PEG2MAL40K (obtainable from Nektar, formerly Shearwater).

Alternative sources of PEG linkers include NOF who supply GL2-400MA3 (wherein \(m\) in the structure below is 5) and GL2-400MA (where \(m\) is 2) and \(n\) is approximately 450:

That is to say each PEG is about 20,000Da.

Thus in one embodiment the PEG is 2,3-Bis(methylpolyoxyethylene-oxy)-l-\([3-(6\text{-maleimido}-1\text{-oxohexyl})amino]propyloxy\) hexane (the 2 arm branched PEG, \(-\text{CH}_2\) \(\_\text{NHCO(\text{CH}_2)_3\text{s-MAL}}\), Mw 40,000 known as SUNBRIGHT GL2-400MA3.

Further alternative PEG effector molecules of the following type:

are available from Dr Reddy, NOF and Jenkem.

In one embodiment there is provided an antibody which is PEGylated (for example with a PEG described herein), attached through a cysteine amino acid residue at or about amino acid 226 in the chain, for example amino acid 226 of the heavy chain (by sequential numbering), for example amino acid 226 of SEQ ID NO:36.
In one embodiment the present disclosure provides a Fab'PEG molecule comprising one or more PEG polymers, for example 1 or 2 polymers such as a 40kDa polymer or polymers.

Fab'-PEG molecules according to the present disclosure may be particularly advantageous in that they have a half-life independent of the Fc fragment. In one example the present invention provides a method treating a disease ameliorated by blocking human FcRn comprising administering a therapeutically effective amount of an anti-FcRn antibody or binding fragment thereof wherein the antibody or binding fragment thereof has a half life that is independent of Fc binding to FcRn.

In one embodiment there is provided a Fab' conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment there is provided a scFv conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment the antibody or fragment is conjugated to a starch molecule, for example to increase the half life. Methods of conjugating starch to a protein as described in US 8,017,739 incorporated herein by reference.

In one embodiment there is provided an anti-FcRn binding molecule which:

- Causes 70% reduction of plasma IgG concentration,
- With not more than 20% reduction of plasma albumin concentration, and/or
- With the possibility of repeat dosing to achieve long-term maintenance of low plasma IgG concentration.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule of the present invention. Suitably, the DNA sequence encodes the heavy or the light chain of an antibody molecule of the present invention. The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.
Examples of suitable DNA sequences are provided in herein.

Examples of suitable DNA sequences encoding the 1519 light chain variable region are provided in SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:90. Examples of suitable DNA sequences encoding the 1519 heavy chain variable region are provided in SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:92.

Examples of suitable DNA sequences encoding the 1519 light chain (variable and constant) are provided in SEQ ID NO:23, SEQ ID NO:75 and SEQ ID NO:91.

Examples of suitable DNA sequences encoding the 1519 heavy chain (variable and constant, depending on format) are provided in SEQ ID NOs:37, 38 and 76 (Fab'), SEQ ID NO:72 or 85 (IgG1), SEQ ID NO: 44 or 93 (IgG4P) and SEQ ID:88 (IgG4).

Accordingly in one example the present invention provides an isolated DNA sequence encoding the heavy chain of an antibody Fab' fragment of the present invention which comprises the sequence given in SEQ ID NO:37. Also provided is an isolated DNA sequence encoding the light chain of an antibody Fab' fragment of the present invention which comprises the sequence given in SEQ ID NO:23.

In one example the present invention provides an isolated DNA sequence encoding the heavy chain and the light chain of an IgG4(P) antibody of the present invention in which the DNA encoding the heavy chain comprises the sequence given in SEQ ID NO:44 or SEQ ID NO:93 and the DNA encoding the light chain comprises the sequence given in SEQ ID NO:75 or SEQ ID NO:91.

In one example the present invention provides an isolated DNA sequence encoding the heavy chain and the light chain of a Fab-dsFv antibody of the present invention in which the DNA encoding the heavy chain comprises the sequence given in SEQ ID NO:51 or SEQ ID NO:80 and the DNA encoding the light chain comprises the sequence given in SEQ ID NO:47 or SEQ ID NO:79.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody of the present invention. Suitably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively and suitable signal sequences. In one example the vector comprises an intergenic sequence between the heavy and the light chains (see WO03/048208).

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current

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

Suitable types of Chinese Hamster Ovary (CHO cells) for use in the present invention may include CHO and CHO-Ki cells including dhfr- CHO cells, such as CHO-DG44 cells and CHO-DXB11 cells and which may be used with a DHFR selectable marker or CHO-Ki-SV cells which may be used with a glutamine synthetase selectable marker. Other cell types of use in expressing antibodies include lymphocytic cell lines, e.g., NSO myeloma cells and SP2 cells, COS cells.

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

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The antibodies and fragments according to the present disclosure are expressed at good levels from host cells. Thus the properties of the antibodies and/or fragments are conducive to commercial processing.

Thus there is provided a process for culturing a host cell and expressing an antibody or fragment thereof, isolating the latter and optionally purifying the same to provide an isolated antibody or fragment. In one embodiment the process further comprises the step of conjugating an effector molecule to the isolated antibody or fragment, for example conjugating to a PEG polymer in particular as described herein.

In one embodiment there is provided a process for purifying an antibody (in particular an antibody or fragment according to the invention) comprising the steps: performing anion exchange chromatography in non-binding mode such that the impurities are retained on the column and the antibody is eluted.

In one embodiment the purification employs affinity capture on an FcRn column.
In one embodiment the purification employs cibacron blue or similar for purification of albumin fusion or conjugate molecules.

Suitable ion exchange resins for use in the process include Q.FF resin (supplied by GE-Healthcare). The step may, for example be performed at a pH about 8.

The process may further comprise an intial capture step employing cation exchange chromatography, performed for example at a pH of about 4 to 5, such as 4.5. The cation exchange chromatography may, for example employ a resin such as CaptoS resin or SP sepharose FF (supplied by GE-Healthcare). The antibody or fragment can then be eluted from the resin employing an ionic salt solution such as sodium chloride, for example at a concentration of 200mM.

Thus the chromatograph step or steps may include one or more washing steps, as appropriate.

The purification process may also comprise one or more filtration steps, such as a diafiltration step.

Thus in one embodiment there is provided a purified anti-FcRn antibody or fragment, for example a humanised antibody or fragment, in particular an antibody or fragment according to the invention, in substantially purified from, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

Purified form as used supra is intended to refer to at least 90% purity, such as 91, 92, 93, 94, 95, 96, 97, 98, 99%, w/w or more pure.

Substantially free of endotoxin is generally intended to refer to an endotoxin content of 1 EU per mg antibody product or less such as 0.5 or 0.1 EU per mg product.

Substantially free of host cell protein or DNA is generally intended to refer to host cell protein and/or DNA content 400µg per mg of antibody product or less such as 100µg per mg or less, in particular 20µg per mg, as appropriate.

The antibody molecule of the present invention may also be used in diagnosis, for example in the in vivo diagnosis and imaging of disease states involving FcRn.

As the antibodies of the present invention are useful in the treatment and/or prophylaxis of a pathological condition, the present invention also provides a pharmaceutical or diagnostic composition comprising an antibody molecule of the present invention in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody molecule of the invention for the manufacture of a medicament. The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable excipient.
The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody molecule of the present invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients or non-antibody ingredients such as steroids or other drug molecules, in particular drug molecules whose half-life is independent of FcRn binding.

The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100mg/Kg.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Therapeutic doses of the antibodies according to the present disclosure show no apparent toxicology effects in vivo.

In one embodiment of an antibody or fragment according to the invention a single dose may provide up to a 70% reduction in circulating IgG levels.

The maximal therapeutic reduction in circulating IgG may be observed about 1 week after administration of the relevant therapeutic dose. The levels of IgG may recover over about a six week period if further therapeutic doses are not delivered.

Advantageously, the levels of IgG in vivo may be maintained at an appropriately low level by administration of sequential doses of the antibody or fragments according to the disclosure.

Compositions may be administered individually to a patient or may be administered in combination (e.g. simultaneously, sequentially or separately) with other agents, drugs or hormones.

In one embodiment the antibodies or fragments according to the present disclosure are employed with an immunosuppressant therapy, such as a steroid, in particular prednisone.
In one embodiment the antibodies or fragments according to the present disclosure are employed with Rituximab or other B cell therapies.

In one embodiment the antibodies or fragments according to the present disclosure are employed with any B cell or T cell modulating agent or immunomodulator. Examples include methotrexate, microphenylolate and azathioprine.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the extent of the inflammation present and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) and/or long lasting pharmacodynamics (PD) profile it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

In one embodiment the dose is delivered bi-weekly, i.e. twice a month.

Half life as employed herein is intended to refer to the duration of the molecule in circulation, for example in serum/plasma.

Pharmacodynamics as employed herein refers to the profile and in particular duration of the biological action of the molecule according the present disclosure.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polyactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutically compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative,
stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pi of the protein is in the range 8-9 or above then a formulation pH of 7 may be appropriate. Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one example the pharmaceutical formulation at a pH in the range of 4.0 to 7.0 comprises: 1 to 200mg/mL of an antibody molecule according to the present disclosure, 1 to 100mM of a buffer, 0.001 to 1% of a surfactant, a) 10 to 500mM of a stabiliser, b) 10 to 500mM of a stabiliser and 5 to 500 mM of a tonicity agent, or c) 5 to 500 mM of a tonicity agent.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.


In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according
to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 1 to 5 μη. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellant gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellant gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellant gases may be used on their own or in mixtures thereof.

Particularly suitable propellant gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellant-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The antibody of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium
citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulizable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solution buffer.

The antibodies disclosed herein may be suitable for delivery via nebulisation.

It is also envisaged that the antibody of the present invention may be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled in situ.

The present invention also provides an antibody molecule (or compositions comprising same) for use in the control of autoimmune diseases, for example Acute Disseminated Encephalomyelitis (ADEM), Acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, ANCA-associated vasculitis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome (APS), Autoimmune angioedema, Autoimmune aplastic anemia, Autoimmune dysautonomia, Autoimmune hepatitis, Autoimmune hyperlipidemia, Autoimmune immunodeficiency, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune thrombocytopenic purpura (ATP), Autoimmune thyroid disease, Autoimmune urticarial, Axonal & nal neuropathies, Balo disease, Behcet's disease, Bulloss pemphigoid, Cardiomyopathy, Castleman disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogans syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Dilated cardiomyopathy, Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic angiocentric fibrosis,
Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evans' syndrome, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis (GPA) see Wegener's, Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia, Idiopathic hypocomplementemic tubulointestinal nephritis, Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related disease, IgG4-related sclerosing disease, Immunoregulatory lipoproteins, Inflammatory aortic aneurysm, Inflammatory pseudotumour, Inclusion body myositis, Insulin-dependent diabetes (type 1), Interstitial cystitis, Juvenile arthritis, Juvenile diabetes, Kawasaki syndrome, Kuttner's tumour, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus (SLE), Lyme disease, chronic, Mediastinal fibrosis, Meniere's disease, Microscopic polyangiitis, Mikulicz's syndrome, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal fibrosclerosis, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's), Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Ormond's disease (retroperitoneal fibrosis), Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paraproteinemic polyneuropathies, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus vulgaris, Periaortitis, Periarteritis, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia, POEMS syndrome, Polycystic nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatic, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Psoriasis, Psoriatic arthritis, Idiopathic pulmonary fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynauds phenomenon, Reflex sympathetic dystrophy, Reiter's syndrome, Relapsing polychondritis, Restless legs syndrome, Retroperitoneal fibrosis (Ormond's disease), Rheumatic fever, Rheumatoid arthritis, Riedel's thyroiditis, Sarcoidosis, Schmidt syndrome, Scleritis, Sclerodema, Sjogren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombotic, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, Transverse myelitis, Ulcerative colitis, Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vesiculobullous dermatosis, Vitiligo, Waldenstrom Macroglobulinaemia, Warm idiopathic haemolytic anaemia and Wegener's granulomatosis (now termed Granulomatosis with Polyangiitis (GPA)).

In one embodiment the antibodies or fragments according to the disclosure are employed in the treatment or prophylaxis of epilepsy or seizures.

In one embodiment the antibodies or fragments according to the disclosure are employed in the treatment or prophylaxis of multiple sclerosis.

In embodiment the antibodies and fragments of the disclosure are employed in alloimmune disease/indications which includes:
• Transplantation donor mismatch due to anti-HLA antibodies
• Foetal and neonatal alloimmune thrombocytopenia, FNAIT (or neonatal alloimmune thrombocytopenia, NAITP or NAIT or NAT, or foeto-maternal alloimmune thrombocytopenia, FMAITP or FMAIT).

Additional indications include: rapid clearance of Fc-containing biopharmaceutical drugs from human patients and combination of anti-FcRn therapy with other therapies - IVIg, Rituxan, plasmapheresis. For example anti-FcRn therapy may be employed following Rituxan therapy.

In embodiment the antibodies and fragments of the disclosure are employed in a neurology disorder such as:

• Chronic inflammatory demyelinating polineuropathy (CIDP)
• Guillain-Barre syndrome
• Paraproteinemnic polineuropathies
• Neuromyelitis optica (NMO, NMO spectrum disorders or NMO spectrum diseases), and
• Myasthenia gravis.

In embodiment the antibodies and fragments of the disclosure are employed in a dermatology disorder such as:

• Bullous pemphigoid
• Pemphigus vulgaris
• ANCA-associated vasculitis
• Dilated cardiomypathy

In embodiment the antibodies and fragments of the disclosure are employed in an Immunology, haematology disorder such as:

• Idiopathic thrombocytopenic purpura (ITP)
• Thrombotic thrombocytopenic purpura (TTP)
• Warm idiopathic haemolytic anaemia
• Goodpasture's syndrome
• Transplantation donor mismatch due to anti-HLA antibodies

In one embodiment the disorder is selected from Myasthenia Gravis, Neuro-myelitis Optica, CIDP, Guillame-Barre Syndrome, Para-proteinemic Poly neuropathy, Refractory Epilepsy, ITP/TTP, Hemolytic Anemia, Goodpasture's Syndrome, ABO mismatch, Lupus nephritis, Renal Vasculitis, Sclero-derma, Fibrosing alveolitis, Dilated cardio-myopathy, Grave's Disease, Type 1 diabetes, Auto-immune diabetes, Pemphigus, Sclero-derma, Lupus, ANCA vasculitis, Dermato-myositis, Sjogren's Disease and Rheumatoid Arthritis.

In one embodiment the disorder is selected from autoimmune polyendocrine syndrome types 1 (APECED or Whitaker's Syndrome) and 2 (Schmidt's Syndrome); alopecia universalis; myasthenic crisis; thyroid crisis; thyroid associated eye disease; thyroid ophthalmopathy; autoimmune diabetes; autoantibody associated encephalitis and/or encephalopathy; pemphigus
The antibodies and fragments according to the present disclosure may be employed in treatment or prophylaxis.

The present invention also provides a method of reducing the concentration of undesired antibodies in an individual comprising the steps of administering to an individual a therapeutically effective dose of an anti-FcRn antibody or binding fragment thereof described herein.

In one embodiment the present disclosure comprises use of antibodies or fragments thereof as a reagent for diagnosis, for example conjugated to a reporter molecule. Thus there is provided antibody or fragment according to the disclosure which is labelled. In one aspect there is provided a column comprising an antibody or fragment according to the disclosure.

Thus there is provided an anti-FcRn antibody or binding fragment for use as a reagent for such uses as:

1) purification of FcRn protein (or fragments thereof) - being conjugated to a matrix and used as an affinity column, or (as a modified form of anti-FcRn) as a precipitating agent (e.g. as a form modified with a domain recognised by another molecule, which may be modified by addition of an Fc (or produced as full length IgG), which is optionally precipitated by an anti-Fc reagent)

2) detection and/or quantification of FcRn on cells or in cells, live or fixed (cells in vitro or in tissue or cell sections). Uses for this may include quantification of FcRn as a biomarker, to follow the effect of anti-FcRn treatment. For these purposes, the candidate might be used in a modified form (e.g. by addition of an Fc domain, as in full length IgG, or some other moiety, as a genetic fusion protein or chemical conjugate, such as addition of a fluorescent tag used for the purposes of detection).

3) purification or sorting of FcRn-bearing cells labeled by binding to candidate modified by ways exemplified in (1) and (2).

Also provided by the present invention is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block FcRn activity and in particular the ability of the cells to recycle IgG. Such an assay may be useful for identifying inhibitors of FcRn activity, such as antibody molecules or small molecules and as such may also be useful as a batch release assay in the production of such an inhibitor.

In one aspect there is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:
a) coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),

b) contacting the cells under mildly acidic conditions such as about pH5.9 with a test molecule and an IgG to be recycled by the cell for a period of time sufficient to allow binding of both the test molecule and the IgG to FcRn, optionally adding the test molecule before the IgG to be recycled and incubating for a period of time sufficient to allow binding of the test molecule to FcRn.

c) washing with a slightly acidic buffer, and

d) detecting the amount of IgG internalised and/or recycled by the cells.

In one aspect there is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:

a) coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),

b) contacting the cells under mildly acidic conditions such as about pH5.9 with a test antibody molecule and an IgG to be recycled by the cell for a period of time sufficient to allow binding of both the test antibody molecule and the IgG to FcRn, optionally adding the test antibody molecule before the IgG to be recycled and incubating for a period of time sufficient to allow binding of the test antibody molecule to FcRn.

c) washing with a slightly acidic buffer to remove unbound IgG and test antibody molecule,

and

d) detecting the amount of IgG recycled by the cells.

Suitable cells include Madin-Darby Canine Kidney (MDCK) II cells. Transfection of MDCKII cells with human FcRn alpha chain and human β2 microglobulin (β2M) has previously been
described by Claypool et al., 2002, Journal of Biological Chemistry, 277, 31, 28038-28050. This paper also describes recycling of IgG by these transfected cells.

Media for supporting the cells during testing includes complete media comprising MEM (Gibco #21090-022), 1 x non-essential amino acids (Gibco 11140-035), 1 x sodium pyruvate (Gibco #11360-039), and L-glutamine (Gibco # 25030-024).

Acidic wash can be prepared by taking HBSS+ (PAA #H15-008) and adding 1M MES until a pH 5.9 +/- 0.5 is reached. BSA about 1% may also be added (Sigma # A9647).

A neutral wash can be prepared by taking HBSS+ (PAA #H15-008) and adding 10M Hepes pH 7.2 +/- 0.5 is reached. BSA about 1% may also be added (Sigma # A9647).

Washing the cells with acidic buffer removes the unbound test antibody and unbound IgG and allows further analysis to be performed. Acidic conditions used in step (b) encourage the binding of the IgG to FcRn and internalisation and recycling of the same.

The amount of test antibody or fragment and IgG on only the surface of the cells may be determined by washing the cells with neutral wash and analysing the supernatant/washings to detect the quantity of test antibody or IgG. Importantly a lysis buffer is not employed. To determine the amount of IgG internalised by the cells the antibody may first be removed from the surface of the cell with a neutral wash and the cells lysed by a lysis buffer and then the internal contents analysed. To determine the amount of IgG recycled by the cells the cells are incubated under neutral conditions for a suitable period of time and the surrounding buffer analysed for IgG content. If the surface and internal antibody content of the cell is required then the cell can be washed with acid wash to maintain the antibody presence on the cell surface, followed by cell lysis and analysis of the combined material.

Where it is desired to measure both internalisation and recycling of the IgG samples are run in duplicate and testing for internalisation and recycling conducted separately.

A suitable lysis buffer includes 150mM NaCl, 20mM Tris, pH 7.5, 1mM EDTA, 1mM EGTA, 1% Triton-X 100, for each 10ml add protease inhibitors/phosphate inhibitors as described in manufacturer's guidelines.

Typically the IgG to be recycled is labelled, in one example a biotinylated human IgG may be used. The IgG can then be detected employing, for example a streptavidin sulfo-tag detection antibody (such as MSD #r32ad-5) 25mL at 0.2ug/mL of MSD blocking buffer. Blocking buffer may comprise 500mM Tris, pH7.5. 1.5M NaCl and 0.2% Tween-20 and 1.5% BSA.

Alternatively the IgG may be pre-labelled with a fluorophore or similar label.

In one embodiment a suitable surface is a plastic plate or well such as a 96 well plate or similar, a glass slide or a membrane. In one example cells are coated onto the surface at a density that results in the formation of a monolayer.
In one embodiment the assay described herein is not a measurement of transcytosis of an antibody top to bottom across a membrane with a pH gradient there-across, for example acid conditions one side of the membrane and neutral conditions on the underside of the membrane.

In one example the test antibody or fragment and IgG may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding.

In one example the test antibody or fragment may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding before addition of the IgG to be recycled. Subsequently the IgG to be recycled by the cell may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding.

Neutral conditions facilitate release of the IgG into the supernatant.

Comprising in the context of the present specification is intended to meaning including.

Where technically appropriate embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Technical references such as patents and applications are incorporated herein by reference.

The present invention is further described by way of illustration only in the following examples, which refer to the accompanying Figures, in which:

**Figure 1** shows certain amino acid and polynucleotide sequences.

**Figure 2** shows alignments of certain sequences.

**Figure 3** shows a comparison of binding on human MDCK II for a Fab’ fragment according to the present disclosure and a PEGylated version thereof.

**Figure 4** shows a Fab’ fragment according to the present disclosure and a PEGylated version thereof inhibiting IgG recycling on MDCK II cells.

**Figure 5** shows a PEGylated Fab’ fragment according to the present disclosure inhibits apical to basolateral IgG trancytosis in MDCK II cells.

**Figure 6** shows a comparison of binding of cyno monkey MDCK II for a Fab’ fragment according to the present disclosure and a PEGylated version thereof.

**Figure 7** shows a PEGylated Fab’ fragment according to the present inhibiting IgG recycling on MDCK II cells for human and cyno monkey versions thereof.

**Figure 8** shows the effect of a single dose of a PEGylated Fab’ molecule according to the disclosure on plasma IgG levels in cynomolgus monkeys.

**Figure 9** shows the effect of four weekly doses of a PEGylated Fab’ molecule according to the disclosure on plasma IgG levels.

**Figure 10** shows a diagrammatic representation of antibody recycling function of FcRn inhibited by a blocking protein.

**Figure 11** shows flow cytometry based human IgG blocking assay using purified gamma 1 IgG antibodies.
Figure 12 shows Fab'PEG single/intermittent IV doses in normal cynomolgus monkeys 20mg/Kg days 1 and 67 IgG pharmacodynamics.

Figure 13 shows Fab'PEG: repeat IV doses in normal cynomolgus monkeys 4x 20 or 100 mg/Kg per week IgG pharmacodynamics.

Figure 14 shows Fab'PEG single/intermittent IV doses in normal cynomolgus monkeys -20 mg/Kg and 100 mg/Kg days 1 and 67 IgG Pharmacodynamics.

Figure 15 shows plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 20mg/Kg 15 19.g57 Fab'PEG.

Figure 16 shows plasma IgG levels in 4 cynomolgus monkeys receiving 10 IV doses of 20mg/Kg 15 19.g57 Fab'PEG, one every 3 days.

Figure 17 shows the effect of two 30mg/Kg IV doses of 15 19.g57 IgG4P on the endogenous plasma IgG in cynomolgus monkeys.

Figure 18 shows the effect of 30 mg/Kg if followed by 41 daily doses of 5mg/Kg 15 19.g57 IgG4P on plasma IgG in cynomolgus monkeys.

Figure 19 shows the result of daily dosing with vehicle on the plasma IgG in cynomolgus monkeys.

Figure 20 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'PEG or PBS IV.

Figure 21 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 IgGl or IgG4 or PBS IV.

Figure 22 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'-human serum albumin or PBS IV.

Figure 23 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 FabFv or PBS IV.

Figure 24 shows the increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab or Fab'PEG or PBS IV.

Figure 25 shows a bispecific antibody fusion protein of the present invention, referred to as a Fab-dsFv.

30 EXAMPLES

The following immunizations were performed in order to generate material for B cell culture and antibody screening:

Sprague Dawley rats were immunized with three shots of NIH3T3 mouse fibroblasts co-expressing mutant human FcRn (L320A; L321A) (Ober et al., 2001 Int. Immunol. 13, 1551-1559) and mouse β2M with a fourth final boost of human FcRn extracellular domain.

Sera were monitored for both binding to mutant FcRn on HEK-293 cells and for its ability to prevent binding of Alexafluor 488-labelled human IgG. Both methods were performed by flow cytometry. For binding, phycoerythrin (PE)-labelled anti mouse or rat Fc specific secondary reagents were used to reveal binding of IgG in sera.

B cell cultures were prepared using a method similar to that described by Zubler et al. (1985). Briefly, B cells at a density of approximately 5000 cells per well were cultured in bar-coded 96-well tissue culture plates with 200 μl/well RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS (PAA laboratories ltd), 2% HEPES (Sigma Aldrich), 1% L-Glutamine (Gibco BRL), 1%, penicillin/streptomycin solution (Gibco BRL), 0.1% β-mercaptoethanol (Gibco BRL), 2-5%
activated rabbit splenocyte culture supernatant and gamma-irradiated EL-4-B5 murine thymoma cells (5x10^4/well) for seven days at 37°C in an atmosphere of 5% CO_2.

The presence of FcRn-specific antibodies in B cell culture supernatants was determined using a homogeneous fluorescence-based binding assay using HEK-293 cells transiently transfected with mutant FcRn (surface-stabilised) as a source of target antigen. 10 ul of supernatant was transferred from barcoded 96-well tissue culture plates into barcoded 384-well black-walled assay plates containing 5000 transfected HEK-293 cells per well using a Matrix Platemate liquid handler. Binding was revealed with a goat anti-rat or mouse IgG Fey-specific Cy-5 conjugate (Jackson). Plates were read on an Applied Biosystems 8200 cellular detection system. From 3800 x 96-well culture plates, representing 38 different immunized animals, 9800 anti-human FcRn binders were identified. It was estimated that this represented the screening of approximately 2.5 billion B cells.

Following primary screening, positive supernatants were consolidated on 96-well bar-coded master plates using an Aviso Onyx hit-picking robot and B cells in cell culture plates frozen at -80°C. Master plates were then screened in a Biacore assay in order to identify wells containing antibodies of high affinity and those which inhibited the binding of human IgG to FcRn (see below).

Biomolecular interaction analysis using surface plasmon resonance technology (SPR) was performed on a BIAcore T200 system (GE Healthcare). Goat anti-rat IgG, Fc gamma (Chemicon International Inc.) in 10mM NaAc, pH 5 buffer was immobilized on a CM5 Sensor Chip via amine coupling chemistry to a capture level of approx. 19500 response units (RU) using HBS-EP* as the running buffer. 50mM Phosphate, pH6 + 150mM NaCl was used as the running buffer for the affinity and blocking assay. B cell culture supernatants were diluted 1 in 5 in 200mM Phosphate, pH6 +150mM NaCl. A 600s injection of diluted B cell supernatant at 5μl/min was used for capture by the immobilized anti-rat IgG,Fc. Human FcRn at 100μg/mL was injected over the captured B cell culture supernatant for 180s at 30μl/min followed by 360s dissociation. Human IgG (Jackson ImmunoResearch) was injected over for 60s with 180s dissociation at 30μl/min.

The data was analysed using T200 evaluation software (version 1.0) to determine affinity constants (KD) of antibodies and determine those which blocked IgG binding. As an alternative assay, master plate supernatants were also screened in a cell-based human IgG blocking assay. 25 ul of B cell culture supernatant from master plates were added to 96 well U-bottomed polypropylene plate. Mutant hFcRn-transfected HEK-293 cells (50,000 cells per well in 25 ul PBS pH6/1% FCS) were then added to each well and incubated for 1 hour at 4°C. Cells were washed twice with 150 ul of PBS media. Cells were then resuspended in 50 ul/well PBS/FCS media containing human IgG labelled with Alexafluor 488 or 649 at 7.5ug/ml and incubated 1 hour at 4°C. Cells were then washed twice with 150 ul of media and then resuspended in 35 ul/well of PBS/FCS media containing 1% formaldehyde as fixative. Plates were then read on a FACS Canto 2 flow cytometer. Example data is given in Figure 11.

To allow recovery of antibody variable region genes from a selection of wells of interest, a deconvolution step had to be performed to enable identification of the antigen-specific B cells in a given well that contained a heterogeneous population of B cells. This was achieved using the
Fluorescent foci method. Briefly, Immunoglobulin-secreting B cells from a positive well were mixed with streptavidin beads (New England Biolabs) coated with biotinylated human FcRn and a 1:1200 final dilution of a goat anti-rat or mouse Fey fragment-specific FITC conjugate (Jackson). After static incubation at 37°C for 1 hour, antigen-specific B cells could be identified due to the presence of a fluorescent halo surrounding that B cell. These individual B cells, identified using an Olympus microscope, were then picked with an Eppendorf micromanipulator and deposited into a PCR tube. Fluorescent foci were generated from 268 selected wells.

Antibody variable region genes were recovered from single cells by reverse transcription polymerase chain reaction (RT)-PCR using heavy and light chain variable region-specific primers. Two rounds of PCR were performed on an Aviso Onyx liquid handling robot, with the nested 2° PCR incorporating restriction sites at the 3′ and 5′ ends allowing cloning of the variable regions into a mouse γ1 IgG (VH) or mouse kappa (VL) mammalian expression vector. Paired heavy and light chain constructs were co-transfected into HEK-293 cells using Fectin 293 (Invitrogen) and cultured in 48-well plates in a volume of 1 ml. After 5-7 days expression, supernatants were harvested and antibody subjected to further screening.

PCR successfully recovered heavy and light chain cognate pairs from single B cells from 156 of the selected wells. DNA sequence analysis of the cloned variable region genes identified a number of unique families of recombinant antibody. Following expression, transient supernatants were interrogated in both human IgG FACS blocking (described above) and IgG recycling assays. In some cases, purified mouse γ1 IgG was produced and tested (data labeled accordingly).

The recycling assay used MDCK II cells (clone 34 as described in Examples 4 and 5 below) over-expressing human FcRn and beta 2 microglobulin plated out at 25,000 cells per well of a 96 well plate. These were incubated overnight at 37°C, 5% CO₂. The cells were washed with HBSS+ Ca/Mg pH 7.2+1% BSA and then incubated with 50μl of varying concentrations of HEK-293 transient supernatant or purified antibody for 1 hour at 37°C, 5% CO₂. The supernatant was removed and 500ng/ml of biotinylated human IgG (Jackson) in 50μl of HBSS+ Ca/Mg pH 5.9 +1%BSA was added to the cells and incubated for 1 hour at 37°C, 5% CO₂. The cells were then washed three times in HBSS+ Ca/Mg pH 5.9 and 100μl of HBSS+ Ca/Mg pH 7.2 added to the cells and incubated at 37°C, 5% CO₂ for 2 hours. The supernatant was removed from the cells and analysed for total IgG using an MSD assay with an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD). The inhibition curve was analysed by non-linear regression to determine IC50 values.

Based on performance in these assays a family of antibodies was selected comprising the six CDRs given in SEQ ID NOs 1 to 6. Antibody CA170_015 19 had the best activity and was selected for humanisation.

Example 1 Humanisation Method

Antibody CA170_015 19 was humanised by grafting the CDRs from the rat antibody V-regions onto human germline antibody V-region frameworks. In order to recover the activity of the antibody, a number of framework residues from the rat V-regions were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair et al.
Alignments of the rat antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figures 2A and 2B, together with the designed humanised sequences. The CDRs grafted from the donor to the acceptor sequence are as defined by Kabat (Kabat et al., 1987), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair et al., 1991 Humanised antibodies. WO91/09967). Human V-region VK1 2-l-(l) A30 plus JK2 J-region (V BASE, http://vbase.mrc-cpe.cam.ac.uk) was chosen as the acceptor for the light chain CDRs. Human V-region VH3 1-3 3-07 plus JH4 J-region (V BASE, http://vbase.mrc-cpe.cam.ac.uk) was chosen as the acceptor for the heavy chain CDRs.

Genes encoding a number of variant heavy and light chain V-region sequences were designed and these were constructed by an automated synthesis approach by Entelechon GmbH. Further variants of both heavy and light chain V-regions were created by modifying the VH and VK genes by oligonucleotide-directed mutagenesis. These genes were cloned into a number of vectors to enable expression of humanised 1519 Fab' in mammalian and E. coli cells. The variant chains, and combinations thereof, were assessed for their expression in E. coli, their potency relative to the parent antibody, their biophysical properties and suitability for downstream processing, leading to the selection of the gL20 light chain graft and gH20 heavy chain graft. The final selected gL20 and gH20 graft sequences are shown in Figures 2A and 2B, respectively. This V-region pairing was named 1519.g57.

The light chain framework residues in graft gL20 are all from the human germline gene, with the exception of residues 36, 37 and 58 (Kabat numbering), where the donor residues Leucine (L36), Phenylalanine (F37) and Isoleucine (158) were retained, respectively. Retention of these three residues was essential for full potency of the humanised Fab'. The heavy chain framework residues in graft gH20 are all from the human germline gene, with the exception of residues 3, 24, 76, 93 and 94 (Kabat numbering), where the donor residues Proline (P3), Valine (V24), Serine (S76), Threonine (T93) and Threonine (T94) were retained, respectively. Retention of these five residues was important for full potency of the humanised Fab'.

For expression in E. coli, the humanised heavy and light chain V-region genes were cloned into the UCB expression vector pTTOD, which contains DNA encoding the human C-kappa constant region (Klm3 allotype) and the human gamma-1 CHI-hinge region (Glml7 allotype). The E.coli FkpA gene was also introduced into the expression plasmid, as co-expression of this chaperone protein was found to improve the yield of the humanised Fab' in E. coli strain MXE016 (disclosed in WO201/086136) during batch-fed fermentation, using IPTG to induce Fab' expression. The 1519 Fab' light and heavy chains and FkpA polypeptide were all expressed from a single multi-cistron under the control of the IPTG-inducible tac promoter.

For expression in mammalian cells, the humanised light chain V-region genes were cloned into the UCB-Celltech human light chain expression vector pMhCK, which contains DNA encoding the human Kappa chain constant region (Rm3 allotype). The humanised heavy chain V-region genes were cloned into the UCB-Celltech human gamma-4 heavy chain expression vector pMhg4P FL, which contains DNA encoding the human gamma-4 heavy chain constant region with the hinge stabilising mutation S241P (Angal et al, Mol Immunol. 1993, 30(1): 105-8). Co-transfection of light and heavy chain vectors into HEK293 suspension cells was achieved using
293 Fectin (12347-019 Invitrogen), and gave expression of the humanised, recombinant 1519 antibodies.
Example 1A Preparation of 1519.g57 Fab'-PEG conjugate

Fab' expressed in the periplasm of E.coli was extracted from cells by heat extraction. Fab' purified by Protein G affinity purification with an acid elution. Fab' reduced and PEGylated with 40kDa PEG (SUNBRIGHT GL2-400MA3). PEG is covalently linked via a maleimide group to one or more thiol groups in the antibody fragment. PEGylation efficiency was confirmed by SE-HPLC. Fab'PEG was separated from un-PEGylated Fab' and diFab' by cation exchange chromatography. Fractions analyzed by SE-HPLC and SDS-PAGE. Pooling carried out to minimize levels of impurities. Final sample concentrated and diafiltered into desired buffer.

Example IB Preparation of 1519.g57 Fab' (Anti human FcRn) conjugated with human serum albumin

Anti human FcRn Fab' 1519.g57 was chemically conjugated with human serum albumin (recombinant derived) which was then used for animal studies.

- Human serum albumin: Recombumin from Novozyme (Cat No: 200-010) presented as 20%w/v solution produced recombinantly in Saccharomyces cerevisiae.
- 1519.g57Fab': 3Omg/ml presented in 0.1M Sodium Phosphate, 2mM EDTA, pH6.0 (reduction buffer)
- 1,6-Bismaleimidohexane (BMH) from Thermo Fisher (Cat No: 22330)

Reduction of Albumin:

Albumin was reduced using freshly prepared cysteamine hydrochloride (Sigma cat no: 30078) which was prepared in reduction buffer. To the albumin solution cysteamine hydrochloride was added at 10 fold molar excess and then incubated at 37°C water bath for 30 minutes. Following reduction the solution was desalted using PD10 columns (GE Healthcare Cat. No: 17-0851-01) to remove any excess reducing agent.

Addition of BMH linker:

A stock solution of 1,6-bismaleimidohexane was prepared in glass vial using dimethylformamide. The solution was vortexed to ensure complete dissolution of BMH.

BMH solution was added to the desalted reduced albumin solution at 10 fold molar excess with respect to albumin concentration. The solution was then incubated at 37°C for 30 minutes followed by overnight incubation at room temperature on a roller to ensure proper mixing. A white precipitate was seen which was spun down using bench top centrifuge.

After the completion of the reaction the solution was desalted using PD10 columns.

Reduction of 1519.g57 Fab'

1519.g57 Fab' was reduced using freshly prepared cysteamine hydrochloride (Sigma cat no: 30078) which was prepared in reduction buffer. To the 1519.g57 Fab' solution cysteamine hydrochloride was added at 10 fold molar excess and then incubated at 37°C water bath for 30 minutes. Following reduction the solution was desalted using PD10 columns (from GE Healthcare Cat. No: 17-0851-01) to remove any excess reducing agent.
Mixing of reduced Fab and albumin-BMH
Equal amounts (in molar terms) of the reduced Fab' and albumin- linker was added and incubated at room temperature overnight on a roller mixer.

Affinity purification:
The above mix was then affinity purified using Blue Sepharose which bound to albumin-Fab conjugate and free albumin. Purification was carried out according to manufacturer's instruction which is briefly described here:
Blue sepharose was reconstituted in DPBS pH7.4 and washed thrice with PBS. Following washing the mixture of Fab and linker linked albumin was added and incubated at room temperature for 1 hour on a roller mixer. After incubation the matrix was washed again with PBS to remove any unbound materials and then eluted with PBS7.4 containing 2M KCl.

Size exclusion purification:
The affinity purified material contained albumin conjugated to Fab along with some unreacted HSA. This required further clean-up and this was achieved using size exclusion chromatography (S200 16X60 from GE Healthcare). The final pooled fractions were presented in DPBS pH7.4. The final 1519,g57Fab-HSA conjugate was concentrated up to 20mg/ml in DPBS pH7.4 and analyzed on analytical size exclusion chromatography (Agilent Zorbax GF250 and GF450 in tandem) and was found to be predominantly monomeric conjugate. Endotoxin assay was also carried out and the sample was found to be below the specified lower limit of endotoxin content.

Example 2 Screening of Fab' & Fab'PEG candidate molecules in the IgG recycling assay
To determine the ability of the candidate Fab'PEG molecules to block FcRn activity in a functional cell assay, the molecules were screened in the IgG recycling assay (described in more detail in Example 5). Briefly, MDCK II clone 34 cells were pre-incubated with candidate Fab' or Fab'PEG before addition of biotinylated human IgG in an acidic buffer. The cells were washed to remove all excess IgG and then incubated in a neutral pH buffer to facilitate release of IgG into the supernatant. The amount of IgG released into the supernatant was measured by MSD assay and EC50 values calculated. The EC50 values of humanised Fab' and Fab'PEG candidate molecules that inhibit IgG recycling are shown in the table below. Upon PEGylation there is a loss of potency for all candidate antibodies, however the extent of this varies depending on candidate.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fab' EC50 (nM)</th>
<th>(n)</th>
<th>Fab'PEG EC50 (nM)</th>
<th>(n)</th>
<th>Fold Change in EC50 after pegylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA170_0519,g63</td>
<td>1.91</td>
<td>3</td>
<td>5.25</td>
<td>3</td>
<td>2.7</td>
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<tr>
<td>CA170_0519,g57</td>
<td>2.06</td>
<td>7</td>
<td>6.64</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>CA170_0519,g2</td>
<td>4.22</td>
<td>2</td>
<td>11.01</td>
<td>4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Mean EC50 values for Fab' and Fab'PEG molecules in the IgG Recycling assay.
MDCK II clone 34 cells stably transfected with human FcRn and beta 2 microglobulin were at 25,000 cells per well in a 96 well plate and incubated overnight at 37°C, 5% C0₂. The cells were
incubated with candidate Fab' or Fab'-PEG in HBSS + (Ca/Mg) pH 5.9 + 1% BSA for 1 hour at 37°C, 5% CO₂ before addition of 500 ng/ml of biotinylated human IgG (Jackson) and incubation for a further 1 hour. The cells were washed with HBSS + pH 5.9 and then incubated at 37°C, 5% CO₂ for 2 hours in HBSS + pH 7.2. The supernatant was removed from the cells and analysed for total IgG using an MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC50. Table 1 represents combined data from 2 to 7 experiments.

**Example 3  Affinity for hFcRn binding**

Biomolecular interaction analysis using surface plasmon resonance technology (SPR) was performed on a Biacore T200 system (GE Healthcare) and binding to human FcRn extracellular domain determined. Human FcRn extracellular domain was provided as a non-covalent complex between the human FcRn alpha chain extracellular domain (SEQ ID NO:94) and β2 microglobulin (β2M) (SEQ ID NO:95). Affinosure F(ab')₂ fragment goat anti-human IgG, F(ab')₂ fragment specific (for Fab'-PEG capture) or Fc fragment specific (for IgGl or IgG4 capture) (Jackson ImmunoResearch Lab, Inc.) in 10mM NaAc, pH 5 buffer was immobilized on a CM5 Sensor Chip via amine coupling chemistry to a capture level between 4000 - 5000 response units (RU) using HBS-EP + (GE Healthcare) as the running buffer. 50mM Phosphate, pH6 + 150mM NaCl + 0.05%P20 or HBS-P, pH7.4 (GE Healthcare) was used as the running buffer for the affinity assay. The relevant antibody, either anti-hFcRn Fab'-PEG, IgGl or IgG4P was diluted to 5µg/ml (Fab'-PEG), 0.5µg/ml (IgGl) or 4µg/ml (IgG4) in running buffer. A 60s injection of Fab'-PEG or IgGl or IgG4 at 10µl/min was used for capture by the immobilized anti-human IgG, F(ab')₂. Human FcRn extracellular domain was titrated from 20nM to 1.25nM over the captured anti-FcRn antibody (Fab'-PEG, IgGl or IgG4) for 300s at 30 µl/min followed by 1200s dissociation. The surface was regenerated by 2 x 60s 50mM HC1 at 10µl/min.

The data was analysed using T200 evaluation software (version 1.0).

**Table 2 Affinity data for anti-hFcRn 1519.g57 Fab'-PEG at pH6**

<table>
<thead>
<tr>
<th>1519.g57Fab'-PEG</th>
<th>ka (MV)</th>
<th>kd (s⁻¹)</th>
<th>KD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.37E+05</td>
<td>1.59E-05</td>
<td>3.63E-11</td>
</tr>
<tr>
<td>2</td>
<td>4.20E+05</td>
<td>2.01E-05</td>
<td>4.78E-11</td>
</tr>
<tr>
<td>3</td>
<td>4.35E+05</td>
<td>1.43E-05</td>
<td>3.29E-11</td>
</tr>
<tr>
<td>4</td>
<td>4.37E+05</td>
<td>2.75E-05</td>
<td>6.30E-11</td>
</tr>
<tr>
<td>5</td>
<td>4.33E+05</td>
<td>1.28E-05</td>
<td>2.97E-11</td>
</tr>
</tbody>
</table>

| 4.32E+05 | 1.81E-05 | 4.19E-11 |
In these experiments the Fab'PEG had an average affinity of around 42pM at pH6 and around 56pM at pH7.4.

### pH7.4

<table>
<thead>
<tr>
<th>1519.g57</th>
<th>ka (MV)</th>
<th>kd (s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (M)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgGl</td>
<td>3.80E+05</td>
<td>1.25E-05</td>
<td>3.29E-11</td>
<td>33</td>
</tr>
<tr>
<td>IgG4P</td>
<td>3.68E+05</td>
<td>1.26E-05</td>
<td>3.43E-11</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 3A Affinity data for anti-hFcRn 1519.g57 as IgGl and IgG4P at pH7.4 (average of three experiments)

### pH6

<table>
<thead>
<tr>
<th>1519.g57</th>
<th>ka (MV)</th>
<th>kd (s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (M)</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgGl</td>
<td>4.56E+05</td>
<td>1.01E-05</td>
<td>2.21E-11</td>
<td>22</td>
</tr>
<tr>
<td>IgG4P</td>
<td>4.43E+05</td>
<td>1.00E-05</td>
<td>2.26E-11</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 3B Affinity data for anti-hFcRn 1519.g57 as IgGl and IgG4P at pH6 (average of three experiments)

Tables 3A and 3B show the affinity of the full length antibodies is consistent with that observed for the Fab'PEG at both pH6 and pH7.4.

### Example 4  Cell-based potency

Cell-based assays were performed using Madin-Darby Canine Kidney (MDCK) II cells which had been stably transfected with a human FcRn and human B2M double gene vector with a Geneticin selection marker. A stable cell clone was selected that was able to recycle and
transcytose human IgG and this was used for all subsequent studies. It will be referred to as MDCK II clone 34.

**Cell based Affinity of CA170_01519.g57 Fab'PEG for human FcRn**

Quantitative flow cytometry experiments were performed using MDCK II clone 34 cells and AlexaFluor 488-labelled CA170_01519.g57 Fab' or CA170_01519.g57 Fab'PEG. Specific binding of antibody to FcRn across a range of antibody concentrations was used to determine $K_D$. The analyses were performed in both neutral and acidic buffers to determine whether environmental pH comparable to that found in blood plasma (pH7.4) or endosomes (pH6) had any effect on the antibody binding.

Figure 3 shows representative binding curves for CA170_01519.g57 Fab'(Figure 3A) and Fab'PEG (Figure 3B). The mean $K_D$ values (n = 2 or 3) were 1.66nM and 6.5nM in neutral buffer, and 1.59nM and 5.42nM in acidic buffer, respectively (see Table 4).

**Table 4 - Mean $K_D$ values (nM) for CA170_01519.g57 Fab' and Fab'PEG on MDCK II clone 34 cells.**

<table>
<thead>
<tr>
<th>Antibody format</th>
<th>Human FcRn pH 7.4</th>
<th>Human FcRn pH 6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1519.g57 Fab'</td>
<td>1.66</td>
<td>1.59</td>
</tr>
<tr>
<td>1519.g57 Fab'PEG</td>
<td>6.5</td>
<td>5.42</td>
</tr>
</tbody>
</table>

Figure 3 shows CA170_01519.g57 Fab' (A) and CA170_01519.g57 Fab'PEG (B) binding on MDCK II clone 34 cells in acidic and neutral pH.

MDCK II clone 34 cells were incubated in Facs buffer (PBS with 0.2% w/v BSA, 0.09% w/v NaN3) for 30 mins prior to the addition of Alexa-fluor 488-labelled CA170_01519.g57 Fab' or Fab'PEG for 1 hour in Facs buffer at either pH 7.4 or pH 6. The final antibody concentrations ranged from 93nM to 0.002nM. The cells were washed in ice cold Facs buffer then analysed by flow cytometry using a Guava flow cytometer (Millipore, UK). Titration data sets were also produced for isotype control antibodies for each antibody format to determine non-specific binding. The number of moles of bound antibody was calculated using interpolated values from a standard curve generated from beads comprised of differing amounts of fluorescent dye.

Geometric mean fluorescence values were determined in the flow cytometric analyses of cells and beads. Non-specific binding was subtracted from the anti-FcRn antibody values and the specific binding curve generated was analysed by non-linear regression using a one-site binding equation (Graphpad Prism®) to determine the $K_D$. Data is representative of 2 or 3 experiments. CA170_01519.g57 Fab'PEG can bind human FcRn expressed on cells at both acidic and neutral pH and the determined $K_D$ values are approximately 3.5 to 4 fold below the equivalent Fab' molecule.

**Example 5 Functional cell based assays**

CA170_01519.g57 Fab'PEG inhibits the recycling of human IgG
FcRn expression is primarily intracellular (Borvak J et al. 1998, Int. Immunol, 10 (9) 1289-98 and Cauza K et al. 2005, J. Invest. Dermatol, 124 (1), 132-139), and associated with endosomal and lysosomal membranes. The Fc portion of IgG binds to FcRn at acidic pH (<6.5), but not at a neutral physiological pH (7.4) (Rhagavan M et al. 1995) and this pH-dependency facilitates the recycling of IgG.

Once it is taken up by pinocytosis and enters the acidic endosome, IgG bound to FcRn will be recycled along with the FcRn to the cell surface, whereas at the physiologically neutral pH the IgG will be released. (Ober RJ et al. 2004, The Journal of Immunology, 172, 2021-2029). Any IgG not bound to FcRn will enter the lysosomal degradative pathway.

An in vitro assay was established to examine the ability of CA170_01519.g57 Fab'PEG or Fab' to inhibit the IgG recycling capabilities of FcRn. Briefly, MDCK II clone 34 cells were incubated in the presence or absence of CA170_01519.g57 Fab' or CA170_01519.g57 Fab'PEG before addition of biotinylated human IgG in an acidic buffer (pH 5.9) to allow binding to FcRn. All excess antibody was removed and the cells incubated in a neutral pH buffer (pH 7.2) which allows release of surface-exposed, bound IgG into the supernatant. The inhibition of FcRn was followed using an MSD assay to detect the amount of IgG recycled and thus released into the supernatant.

Figure 4 shows CA170_01519.g57 inhibits IgG recycling in MDCK II clone 34 cells.

MDCK II clone 34 cells were plated at 25,000 cells per well in a 96 well plate and incubated overnight at 37°C, 5% CO₂. The cells were incubated with CA170_01519.g57 Fab' or Fab'PEG in HBSS+ (Ca/Mg) pH 5.9 + 1% BSA for 1 hour at 37°C, 5% CO₂ before addition of 500 ng/ml of biotinylated human IgG (Jackson) and incubation for a further 1 hour. The cells were washed with HBSS+ pH 5.9 then incubated at 37°C, 5% CO₂ for 2 hours in HBSS+ pH 7.2. The supernatant was removed from the cells and analysed for total IgG using an MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC50. The graph represents combined data from 6 or 7 experiments.

As shown in Figure 4 CA170_01519.g57 Fab’ and CA170_01519.g57 Fab’PEG inhibit IgG recycling in a concentration dependent manner with mean EC50 values (n= 6 or 7) of 1.937nM and 6.034nM respectively. Hence the CA170_01519.g57 Fab'PEG is approximately 3 fold less potent than CA170_01519.g57 Fab' in inhibiting IgG recycling.

CA170_01519.g57 Fab’PEG inhibits the transcytosis of human IgG

FcRn can traffic IgG across polarised epithelial cell layers in both the apical to basolateral and basolateral to apical directions and thus plays an important role in permitting IgG to move between the circulation and lumen at mucosal barriers (Claypool et al. 2004 Mol Biol Cell 15(4): 1746-59).

An in vitro assay was established to examine the ability of CA170_01519.g57 Fab’PEG to inhibit FcRn dependent IgG transcytosis. Briefly, MDCK II clone 34 cells were plated in a 24 well transwell plate and allowed to form monolayers over 3 days. The cells were then pre-incubated with CA170_01519.g57 Fab’PEG on the apical surface before the addition of
biotinylated human IgG in an acidic buffer which facilitates binding to FcRn. The human IgG is transcytosed through the cells from the apical to basolateral side and released into a neutral buffer in the lower chamber. Levels of IgG on the basolateral side were then measured using an MSD assay.

Figure 5 shows CA170_01519.g57 Fab'PEG inhibits apical to basolateral IgG transcytosis in MDCK II clone 34 cells. MDCK II clone 34 cells were plated at 500,000 cells per well of a 24 well transwell plate and incubated for 3 days at 37°C, 5% CO₂ until monolayers were formed. The pH of the apical compartment was adjusted to 5.9 and the basolateral side to 7.2 in an HBSS⁺(Ca/Mg) buffer + 1% BSA. Cells on the apical compartment were pre-incubated with CA170_01519.g57 Fab'PEG for 1 hour before addition of 2μg/ml biotinylated human IgG (Jackson) at the indicated concentrations for 4 hours at 37°C, 5% CO₂. The basolateral medium was then collected and total IgG measured by MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC₅₀. The graph represents combined data from 3 experiments.

In summary Figure 5 shows that CA170_01519.g57 Fab'PEG can inhibit the apical to basolateral transcytosis of human IgG in a concentration dependent manner with an EC₅₀ value of 25.5nM (n=3).

Summary of in vitro effects of CA170_01519.g57 Fab'PEG
CA170_01519.g57 Fab'PEG inhibits both IgG recycling and transcytosis. The EC₅₀ of 6nM achieved in the IgG recycling assay is comparable to the cell affinity binding data in which Kᵦ values of 6.5nM in neutral buffer and 5.42nM in acidic buffer were obtained. CA170_01519.g57 Fab'PEG does show a slight reduction in potency compared to the Fab' alone, but compared to many of the other candidate molecules assessed showed showed the lowest drop in potency between the two formats (see supra). In the IgG transcytosis assay an EC₅₀ of 25.5nM was obtained. The data in this section have clearly shown that CA170_01519.g57 Fab'PEG can inhibit human FcRn function.

Example 6 Cross reactivity of CA170_01519.g57 Fab'PEG with non-human primate FcRn.
To validate the use of CA170_01519.g57 Fab'PEG in a non-human primate PK/PD study and pre-clinical toxicology, its relative affinity and functional potency with cynomolgus macaque FcRn was examined. MDCK II cells stably transfected with cynomolgus macaque FcRn and B2M (MDCK II (cm)) were used for the following studies alongside the previously described MDCK II cells stably transfected with human FcRn and B2M (MDCK II clone 34).

Cell based affinity of CA170_01519.g57 Fab'PEG for cynomolgus monkey FcRn
To determine the cell based binding affinity of CA170_01519.g57 Fab'PEG for cynomolgus monkey FcRn, quantitative flow cytometry experiments were performed using MDCK II (cm) cells and AlexaFluor 488-labelled CA170_01519.g57 Fab’ or Fab’PEG. Specific binding of antibody to cynomolgus macaque FcRn across a range of antibody concentrations was used to
determine $K_D$. Antibody binding was performed in both neutral and acidic pH to determine the effect of binding FcRn in neutral blood plasma or acidic endosomes and to therefore determine any effect pH may have on CA170_01519.g57 binding to cynomolgus macaque FcRn. **Figure 6** shows CA170_01519.g57 Fab' (A) and CA170_01519.g57 Fab'PEG (B) binding on MDCK II (cm) cells in acidic and neutral pH.

MDCK II (cm) cells were incubated in Facs buffer (PBS with 0.2% w/v BSA, 0.09% w/v NaN3) for 30 mins prior to the addition of Alexa-fluor 488 labelled CA170_01519.g57 Fab' or Fab'PEG for 1 hour in Facs buffer at either pH 7.4 or pH 6. The final antibody concentrations ranged from 93 nM to 0.002 nM. The cells were washed in ice cold Facs buffer then analysed by flow cytometry using a Guava flow cytometer (Millipore, UK). Titration data sets were also produced for isotype control antibodies for each antibody format to determine non specific binding. The number of moles of bound antibody was calculated by using interpolated values from a standard curve generated from beads carrying varying amounts of fluorescent dye. Geometric mean fluorescence values were determined in the flow cytometric analyses of cells and beads. Non-specific binding was subtracted from the anti-FcRn antibody values and the specific binding curve generated was analysed by non-linear regression using a one-site binding equation (Graphpad Prism®) to determine the $K_D$. Data is representative of between 2 and 3 experiments.

**Table 5 Mean $K_D$ values (nM) for CA170_01519.g57 Fab' & Fab'PEG on MDCK II (cm) cells.**

<table>
<thead>
<tr>
<th>Antibody format</th>
<th>Cyto FcRn pH 7.4</th>
<th>Cyto FcRn pH 6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1519.g57 Fab'</td>
<td>1.16</td>
<td>1.09</td>
</tr>
<tr>
<td>1519.g57 Fab'PEG</td>
<td>8.15</td>
<td>5.01</td>
</tr>
</tbody>
</table>

Figure 6 shows representative binding curves for CA17001519.g57 Fab' (Figure 6A) and Fab'PEG (Figure 6B) binding to cynomolgus macaque FcRn. The mean $K_D$ values obtained for CA17001519.g57 Fab' and Fab'PEG are shown in Table 5. These values are comparable to the $K_D$ values obtained for CA170_01519.g57 Fab' and Fab'PEG binding to human FcRn (see table 4).

**CA170_01519.g57 Fab'PEG inhibits the recycling of cynomolgus monkey IgG**

To determine if CA170_01519.g57 Fab'PEG is functionally active in blocking cynomolgus monkey FcRn, MDCK II (cm) cells were used to examine the ability of CA170_01519.g57 Fab'PEG to inhibit the recycling of cynomolgus macaque IgG as described previously for the human FcRn assay. The assay was run alongside representative human assays to allow for a comparison between the two.

Briefly, MDCK II cells (clone 34 or cm) were pre-incubated with CA170_01519.g57 Fab'PEG before addition of biotinylated human (h) or cynomolgus macaque (c) IgG in an acidic buffer to allow binding to FcRn. All excess CA170_01519.g57 Fab'PEG and biotinylated IgG were removed and the cells incubated in a neutral pH buffer to allow release of IgG into the...
supernatant. The inhibition of FcRn was assessed by detecting the amount of IgG present in the supernatant by MSD assay and percent inhibition calculated. As shown in Figure 7, CA170_01519.g57 Fab/PEG can inhibit both human and cynomolgus macaque IgG recycling in a concentration dependent manner, with EC50 values of 8.448nM and 5.988nM respectively. Inhibition of FcRn by CA170_01519.g57 Fab/PEG in the human and cynomolgus macaque assays are comparable, although it appears slightly more potent against the cynomolgus FcRn.

Table 6

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>EC50 (nM)</th>
<th>95% CI (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1519.g57 Fab'PEG</td>
<td>8.448</td>
<td>5.988</td>
</tr>
<tr>
<td>519.g57 Fab'PEG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7 shows CA170_01519.g57 inhibits IgG recycling in MDCK II clone 34 cells & MDCK II (cm) cells. MDCK II clone 34 and MDCK II (cm) cells were plated at 25,000 cells per well in a 96 well plate and incubated overnight at 37°C, 5% CO2. The cells were pre- incubated with CA170_01519.g57 Fab' or Fab/PEG in HBSS+ (Ca/Mg) pH 5.9 + 1% BSA for 1 hour at 37°C, 5% CO2 before addition of 500 ng/ml of biotinylated human or cyno IgG and incubated for a further 1 hour. The cells were then washed with HBSS + pH 5.9 and incubated at 37°C, 5% CO2 for 2 hours in HBSS + pH 7.2. The supernatant was removed from the cells and analysed for total IgG using an MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC50. The graph represents combined data from 2 experiments.

Example 7 Effect of 01519g Fab PEG in cynomolgus monkey

This was a study of the effect of the administration of 015 19g Fab PEG in cynomolgus monkeys, in single, intermittent or repeated dosing regimens. 015 19g Fab PEG was administered by intravenous infusion, as a single dose or in repeat doses to groups of four cynomolgus monkeys as indicated in Table 7. Plasma IgG and the pharmacokinetics of the 015 19g Fab PEG were monitored by immunoassay (see Table 7A for immunoassay methods) and LC-MS/MS. Assay of plasma albumin was conducted at Covance.

Table 7

<table>
<thead>
<tr>
<th>Phase</th>
<th>Group</th>
<th>Antibody</th>
<th>Dose (mg/kg)</th>
<th>Dosing Regimen</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>Single Dose</td>
<td>Redose at 67 days</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fab PEG</td>
<td>20</td>
<td>Single Dose</td>
<td>Redose at 67 days</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fab PEG</td>
<td>100</td>
<td>Single Dose</td>
<td>Redose at 67 days</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Control</td>
<td>0</td>
<td>Repeat Dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Fab PEG</td>
<td>20</td>
<td>Repeat Dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Fab PEG</td>
<td>100</td>
<td>Repeat Dose</td>
<td></td>
</tr>
</tbody>
</table>
### Table 7A Plasma IgG, PK and ADA immunoassay methods

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Immunooassay</th>
<th>Method</th>
</tr>
</thead>
</table>
| PD         | Total plasma IgG | 1) Coat immunoassay plate with F(ab')2 goat anti-human Fcγ  
2) Incubate with sample.  
3) Reveal with horseradish peroxidase conjugated F(ab')2, goat anti-human IgG F(ab')2 & the addition of TMB substrate. |
| PK         | Fab PEG PK    | 1) Coat immunoassay plate with FcRn.  
2) Incubate with sample.  
3) Reveal with biotin conjugated murine IgG1 anti-PEG  
/Streptavidin-horseradish peroxidase conjugate & the addition of TMB substrate alternatively reveal with MSD sulfotagged goat anti-human kappa & the addition of MSD read buffer |

### Effect on plasma IgG concentration

Immunooassay and LC-MS/MS plasma IgG data were in good agreement. Plasma IgG was reduced by the administration of Fab PEG (see Fig 12 and Figure 14). For both Phase I dose groups, a single dose of Fab PEG reduced plasma IgG by approximately 70-80%, reaching a nadir at approximately 7 days and returning to pre-dosing levels by day 63. Redosing at day 67 achieved similar results.

For both Phase II dose groups, 4 weekly doses of the Fab PEG reduced plasma IgG by approximately 70-80%, again reaching a nadir at about 7 days after the first dose. The results are shown in Figure 13.

### Example 8 Effect of CA170_01519.g57 Fab'PEG and CA170_01519.g57 IgG4P in cynomolgus monkeys

The effects of CA170_01519.g57 Fab'PEG and CA170_01519.g57 IgG4P on endogenous plasma IgG were determined in cynomolgus monkeys. Animals were dosed as indicated in Table 8, with 4 animals per treatment group. Plasma IgG and the pharmacokinetics of the anti-FcRn entities were monitored by immunoassay (see Table 8A for immunoassay methods) and LC-MS/MS.

### Table 8 Treatment regimens in cynomolgus monkeys

<table>
<thead>
<tr>
<th>Anti-FcRn</th>
<th>Dose (mg/kg)</th>
<th>Dosing Regimen</th>
<th>Route</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fab'PEG</td>
<td>20</td>
<td>Day 0 &amp; 65</td>
<td>i.v.</td>
<td>15</td>
</tr>
<tr>
<td>Fab'PEG</td>
<td>20</td>
<td>Every 3 days, day 0-27</td>
<td>i.v.</td>
<td>16</td>
</tr>
<tr>
<td>IgG4P</td>
<td>30</td>
<td>Day 0 &amp; 63</td>
<td>i.v.</td>
<td>17</td>
</tr>
<tr>
<td>IgG4P</td>
<td>30 &amp; 5</td>
<td>30mg/kg on day 0, 5mg/kg daily day 1-41</td>
<td>i.v.</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>Daily day 0-41</td>
<td>i.v.</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 8A  Plasma IgG and PK immunoassay methods

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Immunoassay</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>Total plasma IgG</td>
<td>1) Coat immunoassay plate with F(ab')2 Goat anti-human Fcγ.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) Incubate with sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) Reveal with horseradish peroxidase conjugated F(ab')2, goat anti-human IgG F(ab')2 and the addition of TMB substrate.</td>
</tr>
<tr>
<td>PK</td>
<td>Fab’PEG PK</td>
<td>1) Coat MSD streptavidin plate with biotinylated FcRn.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) Incubate with sample.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) Reveal with MSD sulfo-tagged goat anti-human kappa and the addition of MSD read buffer.</td>
</tr>
</tbody>
</table>

Effect on plasma IgG concentration.

Imunoassay and LC-MS/MS plasma IgG data were in good agreement. Plasma IgG was reduced by the administration of anti-FcRn Fab’PEG or anti-FcRn IgG4P (see Figures 15 and 16 and Figures 17 and 18 respectively; see Figure 19 for control). For both anti-FcRn entities, a single dose reduced plasma IgG by approximately 70-80%, reaching a nadir at approximately 7 days and returning to pre-dosing levels by day 62. Redosing at day 63 or day 65, as described achieved similar results.

Repeated dosing of anti-FcRn Fab’PEG or IgG4P reduced plasma IgG by approximately 60-80% and maintained the level of IgG for the duration of the dose period. Again, the nadir was reached at about 7 days after the first dose. The results are shown in Figure 16 and 18.

Example 9 Effect of CA170_01519.g57 Fab’PEG, CA170_01519.g57 IgGl, CA170_01519.g57 IgG4P, CA170_01519.g57 Fab’HSA, CA170_01519.g57 FabFv and CA170_01519.g57 Fab in hFcRn transgenic mice

The effect of various different formats of antibody CA170_01519.g57 on the clearance of human IVIG was determined in human FcRn transgenic mice. The formats tested were CA170_01519.g57 Fab’PEG, CA170_01519.g57 IgGl, CA170_01519.g57 IgG4P, CA170_01519.g57 Fab’HSA, CA170_01519.g57 FabFv and CA170_01519.g57 Fab and the results and are shown in Figures 20, 21, 22, 23 and 24 respectively. The single doses of active compound were as shown in the Figures. In order to detect their effects on the clearance of human IgG (IVIG), the mice were injected with 500mg/kg human IVIG which was quantified by LCMSMS in serial plasma samples withdrawn from the tails of the mice at intervals. Blocking of hFcRn by each of the different antibody formats tested resulted in accelerated clearance of hIVIG and lower concentrations of total IgG were observed compared to control mice.

Anti-FcRn treatment enhances the clearance of hlgG in hFcRn transgenic mice

Humanised FcRn transgenic mice (B6.Cg-FcgrttmlDcr Tg(FCGRT)32Dcr/DcrJ, JAX Mice) were infused intravenously with 500mg/kg human IgG (Human IgGl 10% Gamunex-c, Talecris Biotherapeutics). 24 hours later animals were dosed with vehicle control (PBS) or anti-FcRn intravenously as a single dose. Tail tip blood samples were taken at -24, 8, 24, 48, 72, 144 and 192 hours relative to anti-FcRn treatment. Serum levels of human IgG in the hFcRn mouse and
the pharmacokinetics of FcRn inhibitors were determined by LC-MS/MS. Data presented in figures 20 to 24 are mean ± SEM with 3-6 mice per treatment group.

**Quantification of human IgG, endogenous cynomolgus IgG and FcRn inhibitors by LC-MS/MS**

Human IgG, cynomolgus IgG and FcRn inhibitors (1519.g57 Fab'PEG, 1519.g57 IgG4P, 1519.g57 IgGl, 1519.g57 FabFv, 1519.g57 Fab and 1519.g57 Fab'HAS) were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis following tryptic digestion.

Quantitation was achieved by comparison to authentic standard material spiked at known concentrations into blank matrix, with spiked horse myoglobin used as the internal standard.

Unique ("proteotypic") peptides for all analytes of interest investigated were selected and both samples and calibration samples were tryptically digested as outlined below.

In brief, tryptic digest of 5 μl serum samples was performed overnight using Sequencing Grade Modified Trypsin (Promega, Southampton, UK) following denaturation with acetonitrile / tris (2-carboxyethyl) phosphine and carbamido-methylation with iodoacetamide (all from Sigma-Aldrich, Poole, UK).

Analytes were separated using an Onyx Monolithic C18 column (100x4.6 mm, Phenomenex, Macclesfield, UK) with a gradient of 2 to 95 % (v/v) water/acetonitrile (0.1 % formic acid) delivered at 1.5 mL/min over 6 minutes.

The injection volume was 10 μL; all of the eluent was introduced into the mass spectrometer source.

The source temperature of the mass spectrometer was maintained at 600 °C and other source parameters (e.g. collision energy, declustering potential, curtain gas pressure etc.) were optimized to achieve maximum sensitivity for each peptides of interest. Selective transitions for each proteotypic peptide of interest were monitored.

**Example 10: Crystallography and binding epitope.**

The crystal structure of 1519g57 Fab' and deglycosylated human FcRn extracellular domain (alpha chain extracellular domain (SEQ ID NO:94) in association with beta2 microglobulin SEQ ID NO:95) was determined, with the FcRn oligosaccharide excluded in order to facilitate crystallization. 1519.g57 Fab' was reacted with 10-fold molar excess of N-ethyl maleimide to prevent formation of diFab' and any existing diFab' removed by SEC (S200 on Akta FPLC).

Human FcRn extracellular domain was treated by PNGaseF to remove N-linked sugars. For this, the FcRn sample concentration was adjusted using PBS (pH7.4) to 5mg/ml and a total volume of 1ml. 200 units of PNGaseF (Roche) was added to this solution of human FcRn. This was incubated at 37°C for -18 hours, following which the extent of deglycosylation was checked using SDS PAGE. Upon completion of the reaction the deglycosylated FcRn was buffer exchanged into 50mM Sodium Acetate, 125mM NaCl, pH6.0.

The complex was formed by incubation of a mixture of reagents (Fab':FcRn:: 1.2:l, w/w) at room temperature for 60minutes, and then purified using SEC (S200 using Akta FPLC). Screening was performed using the various conditions that were available from Qiagen (approximately 2000 conditions). The incubation and imaging was performed by Formulatrix
Rock Imager 1000 (for a total incubation period of 21 days). The result of screening is shown in Tables 9, 10 and 11.

Table 9: The result of crystallisation screening, showing the crystal used for X-ray analysis.

<table>
<thead>
<tr>
<th>Crystallization experiment type</th>
<th>Sitting drop, vapour diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallization condition</td>
<td>0.1M Sodium citrate pH 5.5, 11%PEG6000</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>10mg/ml</td>
</tr>
<tr>
<td>Drop volume/ratio</td>
<td>0.4μl Protein + 0.4μl Reservoir</td>
</tr>
<tr>
<td>Crystal growth time</td>
<td>8-21 days</td>
</tr>
<tr>
<td>Cryoprotection</td>
<td>Crystals were harvested from the drop, transferred to cryoprotection buffer (70% reservoir + 30% ethylene glycol) and flash-frozen in liquid nitrogen (-180°C) within 10 seconds.</td>
</tr>
</tbody>
</table>

Comments

![Picture of crystal in drop](image)

![Pictures of crystal frozen in the loop (red square is X-ray beam)](image)

Table 10: Conditions for collection and processing of X-ray analysis data.

<table>
<thead>
<tr>
<th>X-ray source</th>
<th>Diamond Light Source, Beamline I04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment Type</td>
<td>Single-wavelength</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.9795Å</td>
</tr>
<tr>
<td>Processing Software</td>
<td>Mosflm/Scala</td>
</tr>
<tr>
<td>Resolution Limits</td>
<td>35.00 – 2.90 Å</td>
</tr>
<tr>
<td>Space group</td>
<td>P3(_2) 2 1</td>
</tr>
<tr>
<td>Unit Cell parameters</td>
<td>a = 150.10 Å, b = 150.10 Å, c = 89.15 Å</td>
</tr>
<tr>
<td></td>
<td>α = 90.00 °, β = 90.00 °, γ = 120.00 °</td>
</tr>
<tr>
<td>Completeness</td>
<td>99.9% (100.0%)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>6.7 (6.8)</td>
</tr>
<tr>
<td>l/a(l)</td>
<td>13.4 (4.8)</td>
</tr>
<tr>
<td>R(_{merge})</td>
<td>9.2% (36.3%)</td>
</tr>
</tbody>
</table>
Table 11 Structure determination and refinement.

<table>
<thead>
<tr>
<th>Structure determination method</th>
<th>Molecular Replacement</th>
<th>Program(s) used</th>
<th>Phaser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure template</td>
<td>Structure FcRn receptor from PDB 3M17 and previously solved Fab-3DVN</td>
<td>Refmac5</td>
<td>Phaser</td>
</tr>
<tr>
<td>Refinement program</td>
<td>Refmac5</td>
<td>Resolution limits</td>
<td>30.00-2.9</td>
</tr>
<tr>
<td>$R$ factor</td>
<td>23.2%</td>
<td>Free $R$ factor</td>
<td>28.4%</td>
</tr>
<tr>
<td>Number of non-hydrogen atoms</td>
<td>- 6125 protein atoms</td>
<td>- 6125 protein atoms</td>
<td>- 2 Acetate ions (4 atoms each)</td>
</tr>
<tr>
<td></td>
<td>- 27 waters in AU</td>
<td>- 2 Cl ions</td>
<td>- 2 Na$^+$ ions</td>
</tr>
<tr>
<td>RMSD bond length</td>
<td>0.009Å</td>
<td>RMSD bond angle</td>
<td>1.338°</td>
</tr>
<tr>
<td>Ramachandran allowed</td>
<td>98.6%</td>
<td>Ramachandran outliers</td>
<td>1.4%</td>
</tr>
<tr>
<td>Comments</td>
<td>Rebuilt using CCP4/Coot.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no obvious change in FcRn structure upon binding of 1519g57 Fab' (comparing this complex with published structures of FcRn). From the crystal structure it the secondary structure content was calculated to be: a-helix 9.4%; β-sheet 45.2%; 3-10 turn 2.5%.

The residues interacting with 1519g57 Fab' were all in the FcRn a chain (not β2M) and are indicated below in bold. The residues concerned encompass all but 1 of the residues critical for binding Fc. 1519g57 binds in a region that overlays the Fc-binding region, suggesting that blockade of FcRn by 1519g57 Fab' is by simple competition, the anti-FcRn being effective by virtue of its superior affinity.

AESHLSLLY HTAVSSAPG TPAFWVSGWL GPOQQYLSYN LRGHAEPCGA WVKNQVWSW Y WKEKTDDLRI KEKLFLEAFK ALGGKGPYTL QGLLGCHELQ GNTSVPTAKF ALNGEEE M NF DLKQGTWGGD WPEA LAISQR WQQQDKAANK ELTFLFFSCP HRRH ELEGR RG NLEWKEPP SM RLKARPSS PGFSVLTCSA FSFYPPEQL RFLRGLAAAG TGGDFGPNS DGSFHAASSSL TVKSGDEH HY CCIVQHAGLA QPLRVELESPAKSS

The FcRn a chain sequence, showing residues involved in interaction with 1519g57 Fab' (bold) and residues critical for interaction with Fc of IgG (underlined). All but 1 of the latter are included in the former.
Claims:

1. An anti-FcRn antibody or binding fragment thereof comprising a heavy chain or heavy chain fragment having a variable region, wherein said variable region comprises one, two or three CDRs independently selected from SEQ ID NO: 1. SEQ ID NO: 2 and SEQ ID NO: 3.
2. An anti-FcRn antibody or binding fragment thereof according to claim 1, wherein CDR H1 has the sequence given in SEQ ID NO: 1.
3. An anti-FcRn antibody or binding fragment thereof according to claim 1 or 2, wherein CDR H2 has the sequence given in SEQ ID NO: 2.
4. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 3, wherein CDR H3 has the sequence given in SEQ ID NO: 3.
5. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 4, wherein the antibody or binding fragment further comprises a light chain or fragment thereof having a variable region comprising one, two or three CDRs independently selected from SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.
6. An anti-FcRn antibody or binding fragment thereof according to claim 5, wherein CDR L1 has the sequence given in SEQ ID NO: 4.
7. An anti-FcRn antibody or binding fragment thereof according to claim 5 or 6, wherein CDR L2 has the sequence given in SEQ ID NO: 5.
8. An anti-FcRn antibody or binding fragment thereof according to any one of claims 5 to 7, wherein CDR L3 has the sequence given in SEQ ID NO: 6.
9. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 8, wherein the antibody is humanized.
10. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 9 having a heavy chain comprising the sequence given in SEQ ID NO: 29 and a light chain comprising the sequence given in SEQ ID NO: 15.
11. An anti-FcRn antibody or binding fragment thereof which binds human FcRn comprising a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 80% identity or similarity to the sequence given in SEQ ID NO: 29 and wherein the variable domain of the light chain comprises a sequence having at least 80% identity or similarity to the sequence given in SEQ ID NO: 15.
12. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 11, wherein the antibody is a scFv, Fv, Fab or Fab' fragment.
13. An anti-FcRn antibody Fab' fragment according to claim 12 having a heavy chain comprising the sequence given in SEQ ID NO: 36 and a light chain comprising the sequence given in SEQ ID NO: 22.
14. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 13, wherein the antibody or binding fragment is conjugated to a polymer for example selected from starch, albumin and polyethylene glycol.
15. An anti-FcRn antibody or binding fragment thereof according to claim 14, wherein the polymer is PEG, for example with a molecular weight in the range 5 to 50kDa.
16. An anti-FcRn antibody according to any one of claims 1 to 11, wherein the antibody is a full length antibody.
17. An anti-FcRn antibody according to claim 16 wherein the full length antibody is selected from the group consisting of an IgG1, IgG4 and IgG4P.
18. An anti-FcRn antibody according to claim 16 or claim 17 having a heavy chain comprising the sequence given in SEQ ID NO:72 or SEQ ID NO:87 or SEQ ID NO:43 and a light chain comprising the sequence given in SEQ ID NO:22.
19. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 11 wherein the antibody or binding fragment thereof is a Fab-dsFv having a heavy chain comprising the sequence given in SEQ ID NO:50 and a light chain comprising the sequence given in SEQ ID NO:46 or SEQ ID NO:78.
20. An anti-FcRn antibody or binding fragment thereof having a binding affinity for human FcRn of 100pM or less.
21. An anti-FcRn antibody or binding fragment thereof according to claim 20 wherein the binding affinity for human FcRn is 100pM or less when measured at pH6 and at pH7.4.
22. An anti-FcRn antibody or binding fragment thereof which binds to the same epitope of human FcRn as the antibody of claim 10.
23. An anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises at least one amino acid selected from the group consisting of residues V105, P106, T107, A108 and K109 of SEQ ID NO:94 and at least one residue selected from the group consisting of P100, E115, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, G129, D130, W131, P132 and E133 of SEQ ID NO:94.
24. An anti-FcRn antibody or binding fragment thereof which cross-blocks the binding of the antibody of claim 10 to human FcRn or is cross-blocked from binding human FcRn by the antibody of claim 10.
25. An anti-FcRn antibody or binding fragment thereof according to any one of claims 20 to 24 which is humanized or fully human.
26. An anti-FcRn antibody or binding fragment thereof according to any one of claims 22 to 25 which has a binding affinity for human FcRn of 100pM or less.
27. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 26 which binds human FcRn.
28. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 27 which blocks binding of human IgG to human FcRn.
29. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 28 which does not bind β2 microglobulin.
30. An assay for testing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:
   a. coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),
b. contacting the cells under mildly acidic conditions such as about pH 5.9 with a
test antibody molecule and an IgG to be recycled by the cell for a period of
time sufficient to allow binding of both the test antibody molecule and IgG to
FcRn,
c. washing with a slightly acidic buffer, and
d. detecting the amount of IgG internalised and/or recycled by the cells.
31. The assay according to claim 30 wherein the test antibody molecule is added
before the IgG to be recycled and incubated for a period of time sufficient to allow
binding of the test antibody molecule to FcRn before addition of the IgG to be
recycled.
32. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody
according to any one of claims 1 to 29.
33. A cloning or expression vector comprising one or more DNA sequences according to
claim 32.
34. A vector according to claim 33, wherein the vector comprises (i) the sequence given
in SEQ ID NO:37 and the sequence given in SEQ ID NO:23 or (ii) the sequence given
in SEQ ID NO:80 and the sequence given in SEQ ID NO:79 or (iii) the sequence
given in SEQ ID NO:93 and the sequence given in SEQ ID NO:91.
35. A host cell comprising one or more cloning or expression vectors according to claim
33 or claim 34.
36. A process for the production of an antibody having binding specificity for human
FcRn, comprising culturing the host cell of claim 35 and isolating the antibody.
37. A pharmaceutical composition comprising an anti-FcRn antibody or binding fragment
thereof as defined in any one of claims 1 to 29 in combination with one or more of a
pharmacologically acceptable excipient, diluent or carrier.
38. A pharmaceutical composition according to claim 37, additionally comprising other
active ingredients.
39. An antibody or binding fragment thereof as defined one in any one of claims 1 to 29
or a composition as defined in claim 37 or 38 for use in therapy.
40. An antibody or binding fragment thereof as defined in any one of claims 1 to 29 or a
composition as defined in claim 37 or 38, for use in the treatment of an autoimmune
disease, such as myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica,
Guillain-Barre syndrome, lupus, and thrombotic thrombocytopenic purpura.
41. A method of treating a patient comprising administering a therapeutically effective
amount of an antibody or binding fragment thereof as defined in any one of claims 1
to 29 or a composition as defined in claim 37 or claim 38.
42. A method according to claim 41, wherein the treatment is for an autoimmune disease
such as myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barre
syndrome, lupus, and thrombotic thrombocytopenic purpura.
FIGURE 1

CA170_1519 Ab sequences

CDRH1
GFTFSNYGMOV  SEQ ID NO: 1

CDRH2
YIDSDGDNTYRRDSVKG  SEQ ID NO: 2

CDRH3
GIVRPFPLY  SEQ ID NO: 3

CDRL1
KSSQSLVGASGKTYLY  SEQ ID NO: 4

CDRL2
LVSTLDS  SEQ ID NO: 5

CDRL3
LQGTHFPHT  SEQ ID NO: 6

Rat Ab 1519 VL region  SEQ ID NO: 7
DVVMQTPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1519 VL region  SEQ ID NO: 8

gatgttgtga tgacccagac tccactgtct ttgctgggtg ccccttgaca
accagcctcc atctcttgca agtcaagtca gagcctcgta ggtgctaagtg
gaaagacata tttgtatgg ttatcaca gaagccggcgc gttctcaaga
cgactaatct atctgtgtgc cacactgggcac tctggaatcc ctgataggtt
cagtgccagt ggacgcagaga cagattttac tcttaaaatc ggcagagttg
aagccgatga tttggaggtt tattactgct tgcgaagtac acatrtttct
ccacagtttg gaggctggac caagctggga attgaa

Rat Ab 1519 VL region with signal sequence underlined and italicised  SEQ ID NO: 9

MMSPAQFLFL LMLWIQGTSG DVVMQTPLS LSVALGQPAS ISCKSSQSLV
GASGKTYLYW LFQRSGQSPK RLIYLVSTLD SGIPDRFSGS GAETDFTLKI
RRVEADDLGV YYCLQGTHFP HTFGAGTKLE LK
FIGURE 1A

Rat Ab 1519 VL region with signal sequence underlined and italicised

atgatgagtc ctgcccagtt cctgttttctg ctgatgctct ggattcaggg
aaccagtgtg gatgctgttga tgacccagac tccacgtgtct ttgtcggggtt
cctctggaca acacgcctcc atcctcttga agtcaagtc gaacctcgtga
gtgctagtgg gaaagacaata ttgttatgg ttatttcaaga ggtccggcca
gtctccaaga cgactaatct atctggtgtgc cacactggac ctctggaattc
cgtatgagtt cagttgagctg ggagcagaga cagatlttatc tctttaaatc
cgccagagtgg aacccgagtaa tttgaggttt tattactgtc tgcaagttac
cacttttct cacacggtttg gagctgggac caagctggaa ttgaaa

Rat Ab 1519 VH region

EVPLVESGGG SVOPGSMKL SCVSVGFTFS NYGMVWVQPA PKKLGFEVAY
IDSDGDNTRY SDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGGQ TTVTVS

Rat Ab 1519 VH region with signal sequence underlined and italicised

atggtgcggc tcgggtgagtc tggggcgcgcc tcagtcgcagc ctgggaggtgc
catgaacatc tccctgtctag ttcagacgat cacttctcgg aattaatgccg
tggctcggtc cggccaggtct ccaagaaggg ttcttctggt ggctgaatctat
attgtctctg atgtgttacaa tcttacctgc cggagctgcca tgaaggccggc
attcactact ccgacaaaat atgcaaaaaag cccctattat tttcgaatttg
acagtctcag tgtcttaggg acggccacat ttactctgtag aacagggagatt
gtccggcctct ttctctattg ggcccaaggg accacgggta ccgtctcctg

Rat Ab 1519 VH region with signal sequence underlined and italicised

MDISLSLAF LVLFKIGVCR E VPLVESGGGS VOPGSMKLCS CVSVGFTFSN
YGMVWVQRQAP PKKLGFEVAYY DSVDGNTRYR DSVKGRFTIS RNNAKSTLYL
QMDLSLRESYT ATYVCTTGGT RPFLYWGGGT TTVTVS

Rat Ab 1519 VH region with signal sequence underlined and italicised

atggtgcggtc gttgtaagagc tggggccgctg ccgctgccagc cggagcgcagc
gtgggtctgg gtcggctgtgc gcgcggtctca gtcgcggccttg
ccaggtctac caaactccac ttgtgtgctct cggatttctat cctatttcgtt
tatgggcatgg tcggggctcg cccagctcga aagaagggtc ttggagtctgt
cgcataatc atctgtcatttg gttcatcagt gccagctgca gatccgctgta
agcgggcgatt cacactctcc acagaaataat caaaaaagcag cccattttgtc
caaatggaca gttcgttgcag gtaaggacag ggcacattttg accttcaaac
aggattgtgc gggcgcctcctc tgtatggggg ccagagaaacc cgggtcaggc
ttctcg
FIGURE 1B

1519 gL20 V-region SEQ ID NO: 15
DIOQTQSPPS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKFGKAPK
RLYLVLSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLOQHTFP
HTFGQQGTKLE IK

1519 gL20 V-region (E. coli expression) SEQ ID NO: 16
gatatccaga tgacccagag tcaaqcagct tcctcccaca gcgtagggca
tcgtgtgact attacctgta aagctccca gtccctgttg ggtgcaagcg
gcacaaccct cctgtcacttg ctcttcctcag aaccggcgaag gcctcggaa
acgcctgatct atctggtgttc taccctggtat agcggatttc cggctcgttt
tccgggtgca cggagcggga ccgaaatctg gtcgaccatt agctcctcct
acgcccggagga ctttgcttacc tattactgcc tccagggccac tcattttcgc
cacacttttcg gccaggggtac caaactggaa atcaca

1519 gL20 V-region (mammalian expression) SEQ ID NO: 17
gatatccaga tgacccagag ccacatctgc tctatccgctt ccgttggtga
tcgcctgacaa atacgtgta aqagctccca atctctctgtg ggtgcaagct
gcacaaccct cctgtcacttg ctcttcctcag aaccggcgaag gcctcggaa
acgcctgatct atctggtgttc taccctggtat agcggatttc cggctcgttt
tccgggtgca cggagcggga ccgaaatctg gtcgaccatt agctcctcct
acgcccggagga ctttgcttacc tattactgcc tccagggccac tcattttcgc
cacacttttcg gccaggggtac caaactggaa atcaca

1519 gL20 V-region with signal sequence underlined and italicized (E. coli expression) SEQ ID NO: 18
MKKTAIAIAV ALAGFATVAQ ADIOQTQSPPS LSASVGDRVT ITCKSSQSL
VGASGKTYLY WLFQKPGKAP KRLYLVSTLD SGIPSRFSGS GSGTEFTLTI
ISSLQPEDFA TYYCLOQHTFP PHTFGQQGTKLE EIK

1519 gL20 V-region with signal sequence underlined and italicized (E. coli expression) SEQ ID NO: 19
atgaaaaagaa cagctatcgc aattgcagtg gctttggcttg gttttcgcttac
cgtagcggcag gtctgatatcc agatgacccca gatgcaaacgc agttctctccg
cacgctaggg cgatcgtgtg actattacct gtaaaagctcc ccaagccccg
gtgggtgcaag cgggcaaaaa ctacctcttac gggtctctctc agaaaccggg
cacaagctcgc aaacgtcctga ccctattcgtg gctctaccttg gataagcggta
tgctctctcg tttctcgggt gacggtactgg gttcggatatt cagcgtaccc
attagcctccc tccagcggcga ggacatttgt actattacct gctctcaggg
cacttatatt cccgacacttt tcggccaggg taccacactg aaaaactcaa

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FIGURE 1C

1519 gL20 V-region with signal sequence underlined and italicized (mammalian expression)
SEQ ID NO: 20
MSVPPTQVLGL LLLLWLDARC DIQMTQSPSS LSASVGDRVT ITCKSSQSLV
GASGKTLYW LFQKPGKAPK RLIIYLVSTLD SGIPRSFGS GSGTEFTLTI
SSLQPEDFAT YYCLQGTHFP HTFQGQTKLE IK

1519 gL20 V-region with signal sequence underlined and italicized (mammalian expression)
SEQ ID NO: 21
atgtctgtcc ccacccaagt cctcggactc ctgctactct gccttacaga
tgccagatgc gataatccaga tcgccccagag cccatctgcttt ttatccgcttt
cgcgggtcga tcgcttgccaca atacctgtga agagctccca atctctcggtg
ggtggcgagtg gcaagacctta ctctgtcactgg ctctttcagaa agctgagcga
ggcaccacaa cgggtgtactt atctgggtgc ttaccctggcact ctctgggtacg
cgctcaagatt ttcccgattc gggagcggaa ctgagttcacc actcaagatt
tcatcgtgc cacccgggagga ctttgcacactctgcctttcagaatcagctgaa

1519 gL20 light chain (V + constant) SEQ ID NO: 22
DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTLYW LFQKPGKAPK
RLIYLVSTLD SGIPRSFGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP
HTFQGQGTKLE IKRTVAAVSF FIFPSPSEQL KSGTASVVL LNNFYPREAK
VQWKVDNALQ SGNSEQSVTE QDSKDSYTSL SSSLTLKAD YEJKHVKYACE
VTHQGLSSPV TKSFNRCGEC

1519 gL20 light chain (V + constant, E. coli expression) SEQ ID NO: 23
gataatccaga tcgacccagag ttccagacgatg ctgcttgccca gcgttggcga
tcgggttgact tatacttgta aagctcttga gttctcgggttt ggtcagccag
cacaaacctta ccttgatcctg ctctttcagaa aaccggcggaa agctccgaaa
ccctcttgatct atctttgtgct tacccttgatc aagctttttcgc gcgttggcatt
tccctgatttc ggtccgtggtgt cagagttcctt cgtctcgcttc gcgttggcgtg
cacatttgcac cgggttgtcgc tgtatctgta atcccaatcag ggtagccata
cccctctgtgc ttcgaatctcc cccctctgta gagaatcggaa aaatctggaa
tctgctcgttc tgataatcttctgtctcctgc agagatcgttca
gtacagttga aggtggataa gcgcccccaaa tcggtgtactt ccacagagacag
tgctcagacg cagacagacg cagacagacg cagacagacg cagacagacg
tgcagctgag caaacgacag ctagagaaac acacaagttca cggctggcga
gtcaccatcc gaggccctag ctcaggacta aaccaaggtg ttataagagg ggaagtt
FIGURE 1D

1519 gL20 light chain (V + constant, mammalian expression)  SEQ ID NO: 24

gatatccaga tgacccagag tccagagagt ctctccgcca gcgtagccga
tcgtgtgact attactctgt aagctctcga gttcctggtg ggtgcaagcg
gaaaaacctta cctgtacttg ctctccagaa aacgaggcaaa agctcccaaa
cgcctctgtct attcggtgtgc taccctgggt tagcgttatcc ctgtctcttg
tctcgggtacg ggtagcggtta cccgattcact gctgaccatt gcctccctcc
tagccggagga cttgctacc tattactgcc tccagggcgc tcattttcgc

cacactttccg gcacaggtac ccaagctggaa atccaaacgtg cggtagcgcg
cccacattgtc ttcattcctc cgcacatcga taagcaggtt caaattcggaa
ctgctctctgt tgctgctgctg ctaataact tctatcccaag aagggccaaa
gtacagtggaa aggtggataa cgcctcccaag tccggttaact cccagggagag
tgctcactagag caggacagca cggacagcac ctacagcctc gcagcagcacc
tgacgctgag caaacagcac taccgaaacc acaaaagtota cgcctgcgaa
gtccaccatc aggcccctgag ctcgccgctg tcaaacagggagagttg

1519 gL20 light chain with signal sequence underlined and italicized (E. coli expression)  SEQ ID NO: 25

MKKTAIAIAAV ALAGFATVAQ ADIOMTQPSL SLSASVGDRV TITCKSSQSL
VGASGKTYLY WLFQKPGKAP KRLIYLVSTL DSGPSSRFSG SGSGETFLLT
ISSLQPEDFA TYYCLRQTHF PHTFGQGKTL EIKRTVAAPS VFIFFPSDEQ
LKSGTASVVC LLNNFYPREA KVQWKVDNAL QSGNSQESVT EQDSKDSTYS
LSSTLTLRSA DYEHHKVYAC EVTHQGLSSP VTKSFNRGEC

1519 gL20 light chain with signal sequence underlined and italicized (E. coli expression)  SEQ ID NO: 26

atgaaaaagga cagctatcgc aattgcagttg gctctgggctg gtttgcgtac
cgtaggccaa ggtgatatacc agatgaccca gagtcacagc agtctctccg
cacagctagg cgatcgtggtg actattacct gttaaaagctc ccaagctctg
tgcgggtgcaa gcggcaaaac ctacgctgtac tggctctcc tgaaccccgag
ccaaagctccg aaacgcctga tctatctgtg gtctaccctgt gatagcggta
ctgggttcctg ttcacctctg agcgttacgc gccaggaatt caggcttgacc
tattgccccc tccagccgca ggcattttgt actattact gctctccgagg
cactcatattt cccgacacatt tcggcagagg taccaaaaagt gaaatcaaac
gtacggtagt gcgcccctat gcattctatct cccgcccatac tggatgacag
ttgaatcttg gaacctgcctc ttggttctgtgct ctcgtcataa accttctatcc
cagagtagc ccagttacag ggaaagggga taacgcccctc caatccggcta
acctccagga cagttctcaaa gacgaggac acaaggacag caccttacagc
cctcagcagc cccctgagct gaccaagagc gactacgaga aacacaagt
ttcagccctgc gaagtcaccacc atccagggctgt cgtctccac gtaaacaaga

gttttatag aggaggagtgt
**FIGURE 1E**

1519 gL20 light chain with signal sequence underlined and italicized (mammalian expression) SEQ ID NO: 27

```plaintext
MSVPTQVGL LLLLWTDARC DIQMTQSPSS LSASVGDRTV ITCKSSQSLV
GASGKTYLYW LFQKFPGKAPK RLIYLVSTLD SGIPSRSFGS GSGTEFTLTI
SSLQPEDFAT YYCLOGTHFP HTFGQGKLE IKRTVAAPSV FIFPPSDEQL
KSGTASVVCL LNNFYPREAK VQWKVDNALO SGNSQESVTE QDSKDSTYSL
SSTLTLSKAD YEHHKVYACE VTHQGLSSPV TKSFNRGEC
```

1519 gL20 light chain with signal sequence underlined and italicized (mammalian expression) SEQ ID NO: 28

```plaintext
atgtctgtcc ccacccaaagt cctcgagactc ctgctactct ggcttacaga
tgccccagatgc qatactccaga tgtccacagq cccatctgac tttacctggctt
cctgttggtga tcgctgtgaca attacggtga agagctccca atctctcggtg
ggtcgaagtg gcacagacta tctgctactg cttcttcaga agcccttgcaaa
gccacccaaa cgctgtgctct atctggtgtc tacccttgac tctggtgatac
tctcagcgatt tccctctgtc gcagctcggaa ctgaggtcac acgtcagatt
tctagctgtgc aacccgcagga cttgtctacc tactctctgc ggacagccac
tcattccccct caccctttcg gcacggggac aaaaactcga aatcaacgct
cgcagcgggc ccacattgtc tctcctttcc cgccctctcga tgcagctgttg
aaatctggaa ctgctccttgt tggctgtgctg ctgaataact ctttatcccaag
gcgccaaa gtacaggtga aggctggataa cgccctccaa tgcggtaact
cccagggag gtctcagagag cagacagaca aggacagcac ctacagccctc
tgacagctgag caaagcgac acagagccac acagagctct cgcctgcgaa
gtcaccccact aggctggcaag ctgcgccccagc acacacaggg agaggt
g
```

1519 gH20 V-region SEQ ID NO: 29

```plaintext
EVPLVESGGG LVQPQGSSLRL SCAVSGGTFS NYGMPVVRQGA PKGKLEWVAY
IDSDGDNXYY RDSVKGRPTI SRDNALKSSLY LQMNSLRAED TAVYYCTTG1
VRFPFLYWGQQ TLTVS
```

1519 gH20 V-region (E. coli expression) SEQ ID NO: 30

```plaintext
ccgtgcgtcgc tgggtgcagtc cggaggcggg cttctgccagc tggaggaggag
ccccctctcg ctctgcgtcgc acacgccttc aactacgta
ccccctgtgtggt gcctgtcagctt cagagtaaag ggtgataagtg aggctggctagta
atgtcgtcgc acggcgacaa ccactctctg cgcctgcag tggaaaggtcgc
tccctgtccgt ccgccgata cgcctctaca cgcctgcgac ggtgataagtg acggcagtgc
tgctggagatc actgtcgcagct cactggtcgc tgcctgcggt tcgggatttg cggctggattg acacgccttc cttctgcg
g```
1519 gH20 V-region (mammalian expression) SEQ ID NO: 31
gaggtaccac tttggtgaaag cggagggagt gttggtgcagc ctggaggaag
tttacgttcc tctttggtgtg tgtctggcact caccttttccc aattacggaa
tgggtcttggt caqacaagca cctggaaagag gtcttgatagc ggtggccctat
attgacctctg cgccgggacac cagctactat ccgagttcagc tgtgaagagcg
ttcacacatt tcccgaggata acgcacaaagag ctctactgac gcgcacagatga
atagctcgag agcgcagagat actgcggctgtg actatttgcac aacggaagtac
gttaggcctt ttctgtactgc ggacagggcc accttgggta ctgtctcg

1519 gH20 V-region (E. coli expression) SEQ ID NO: 32
MKKTAIAIAV ALAGPATVAQ AEVPLVESGG GLVQPGSLR LSCAVSGFTF
SNYGMWVRQAP AGKGLFWEVA YIDSGDNTY YRDALKGFTG ISRDNKSSL
YLQMNLSLAE DTAVYYCTTTG IVRFPLYWGQ GTLVTS

1519 gH20 V-region (E. coli expression) SEQ ID NO: 33
atggaagaagag ctgcctatagc aattgcaagtg ggcgtcagctg gtttgcacac
cgtgggcgaa gctgtggcttc gcgtggtcga gtctggagggc gggctggtc
aacctggagg gacggctgctg cttctcttttg cagttatcgtt gtcacggttc
tcccaactac gcgtctgtcg gctccgatt aaggtctggag atgggtggcg
atatattgtcc ccgcacggtcga cacacacact tatactcgact
tctgtgaaagg tctgctcacc atttcccgcag ataaacgccaa atccagcctcg
tactctgcaaga tgaacacgctt ggcgtgtgcaa gatactgcgg tgtactatttg
caccacgtcg atggtgctgc cgtttctgta ttggggtoag ggtacccctcg
ttactgtcttc g

1519 gH20 V-region (mammalian expression) SEQ ID NO: 34
MEWSWVFLEP LSVTGTGHEE VPILVESGGL VQPGSRLGS CAVSGFETFSN
YGMVVWQRAP GKGLEWVAYI DSDGDNTYR DSVKGRFTIS RDNKSSLYL
QMNSLRAEDT AVYYCTTGIV RFPFLYWQGQT LTVTS

1519 gH20 V-region with signal sequence underlined and italicized (mammalian expression) SEQ ID NO: 35
atggaatgga gctggtgtttc tcttctcttcct ggtcgaattca attcaggagt
ccatcctcgag gtacccacttg tggaaagcgag aggggtcttc gtcagccccg
gaggedttt acgtctctct tgtgtctgtgt cttcgcttccac cttcttcaat
tacggatgct gctgggtgtc acaagacactt ggaagaggtc tctgatgggtt
ggcctatattg gactctgcag gggcaacacac ccctactcgg gattcggctg
aaggccgctt ccaacactcc cagagataacg ccacaagctc acctgctctc
cagagttaga gctggagagc caggagatctg cccctgctctc attgcacaacag
gggatcgttt aggccttttc tgtactgagcc acaagggacc ttgggtcttg tctcg
FIGURE 1G

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge) SEQ ID NO: 36
EVPLVESGGG LVQPGGSRL SCAVSGFTFS NYGMWVVRQA PGKGLEVWAY
IDSGDNTYY RDSVKGRFTI SRDNKSSLY LQMNSLRAED TAVYYCTTGI
VRRFVLYWGQG TLTVVTSSAST KGPSVFPLAP SSKSTSGGTA ALGCLV KDYF
PEPVTVSWNS GALTSGVHTF PAUVSSGLY SSLSVTVPS SSSLGTQTYIC
NVNHKPSNTK VDKKVEPKSC DKTHTCAA

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge, E.coli expression) SEQ ID NO: 37
 gaggttccgc tgtgctgagtc tgtggagcgggg ctttgtccagc cttggaggag
 cctgagtctc ttcttgctgag tatctgctgct caactgtccct aactacggta
 tgggttggtg tgtctggagct ccaaggtcag gttcaggtat gttgggcgtat
 attgacctcg acggcgccact cacactactg cgccgactttg tggaaaggctg
 cttcaccatt tccggcgaga acgcaggata caagcgcttac cttgcaatga
 acacgcttcct gcgtggtgat gctgcggttg tgtgcggtgc gctgcggtgc
 gtcgctccct tgttttgtttg ggtctgcgag acctctgcgta cttgtctcag
 cgcctctaca aagggcccat cgctcttccc cctggcaccac tccctccaga
 gcacctctgg gggcagccagg gccctttggct gcctttgccaa ggactacttc
 cccgaaccgg tgtgctgcgtg tgggactactc ggcgcctcag cccgagcgtg
 gcaccccttc cccggccagc cagctgtcctc aggactctac cccctccagc
 gcggctggcag cgctgcccttc aaccagctgc gacccaggag ccccgcttcac
 cacgtgaaatc acaagccccag caacaccaag gtggacaaga aagttgaggct
 caaatcttgg gacaaacaatc acacatgctgc cgcg

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge, mammalian expression) SEQ ID NO: 38
 gaggttacac ccctgtggaaag cggagggagt ctttgtgcagc cttggaggaag
 ctttacgttcc ttcttggtctg tgtctggctt cactctttcc aattacggaa
 tgggtctgggt cagacagact cttggaaaag gttctgatag gttggcctat
 attgactctcg acggggccact cacactactg cgggattccag tggaaaggacg
 cttcacaatc tccggcqaga caagccggag acgtctgtac cttgcaatga
 atagcttgag agcggagagc actggcggtgt acttagcgac aacggaatc
 gtctgccttg tttgtgtactg ggcagcaggc acctctgtta cttgtctcag
 cgcctctaca aagggcccat cgctcttccc cctggcaccac tccctccaga
 gcacctctgg gggcagcagcg gccctttggct gcctttgccaa ggactacttc
 cccgaaccgg tgtgctgcgtg tgggactactc ggcgcctcag cccgagcgtg
 gcaccccttc cccggccagc cagctgtcctc aggactctac cccctccagc
 gcggctggcag cgctgcccttc aaccagctgc gacccaggag ccccgcttcac
 cacgtgaaatc acaagccccag caacaccaag gtggacaaga aagttgaggct
 caaatcttgg gacaaacaatc acacatgctgc cgcg
FIGURE 1H

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (E. coli expression) SEQ ID NO: 39

MKKTAIAIAV ALAGFATVAQ AEVPLVESGG GLVQPSSGLR LSCAVSGFTF
SNYGMVWRQ APGKGLEWVA YIDSDGNTY YRDSVKGRFT ISRDNAXSSL
YLMQNSLRRAE DTAVYCTTG IVRPFYWGQ GDLTVSSAS TKGPSVPLA
PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPALQSSGL
YSLSVVTVDP SSSLGTQTYI CNVNHKSNTKV KDVKKPEKSC CDKHTC

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (E. coli expression) SEQ ID NO: 40

atgaagaaga ctgctatagc aatgcagcgt ggcgctagctg tttcgccac
cgctggcgca aatgcggttc cgcgtgtcga gtgcggaggg gggctgttcc
agccctggag ggacacctgcct ctctcttttgat cagatcttg cttcgacggt
ctcgaactcgt gtatgtgtgtg ggctttcgtgct caatccaggt aagctgtgga
atggggtgcc tgtatttgact ccagccggcga caacatctac tctcgagact
ctgtgaaagg tgccttcacc aatcctccgct ataagctcgatactctggtgtaa
tgctgtgggtctaatg gccctgtgcct gattctgtgcttg ctctctcttca
ctcctgtgca aacggtggtac ggtccagtctt gttcggcgca cccctgggct
cccttgtgct ccagcctggtc tggsgggtggct gctgtgcttgc gcagcttgggt
caagactacct ttcgcggtgt gcggccccag atgtgacggt gcctgtgcggctc
tgagggcgcc ggctgcagcgt ttcgcgctgtg gcgctggtgggcc tctctctacta
tctctcttct ccagcgtgtgct gcgctgttgc ctctctctgtc gaagtctggtgca
agagattgca ggcctaatct tgcagcaaaaa ctacagcttg cgccctggc

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (mammalian expression) SEQ ID NO: 41

MEWSWVFLFF LSVTTGVHSE VPLVESGGGL VQPGLGLRLS CAVSGFTFSN
YGMVWRQQAP GKGLEWVAYI DSDGNTYIYR DSVKGRFTIS RDNAXSSLYL
QMSLRAEDT AVYYCTTGIV RFLYWGQGT LVTVDSSASTK GPSVPLAPS
SKSTSGGTAA LGLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSSVTVSPS SLGTQTYICN VNHKSNTKV DKKVEPKSCD KHTC

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FIGURE 11

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (mammalian
equivalent) SEQ ID NO: 42

```
atggaatgga gctgggtcatt tctcctcttc ctgcacgtaa ctacaggagt
ccatcctcag gtacactctg tggaaagccg aggaggtcct gttgacgctg
gaggaagtct acgtctctct tgggctgtgt ctggtcttcac cttctccaat
tacggaatgg tctggtgcag acaagcaccct ggaaggggtc ttkaagtggtt
ggccttatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aagggacgctt caccaacctcc ccagataacg ccaagacgct acgttagctg
cagatgaata gctgtaggcc cagagatact gccgtgtact attgacacac
gggaaatgctt aggcccttttc tgtactgggg acagggcacg ttgggtactg
ttcqacgccg tttctacaaag ggcctcatcgc ttctccccct ggcacccctcc
tcgaagagca cctctgggag cacagcggcc ctgggctgcc tgtgaagagga
tactccccca gaccccggtga cgttgctgctg gaactcaagg gcctgaccca
gccgcgtgca caccctcccg gctgtctctac agtctctcagg acctactccc
tccacagcagc tgtgaccgct gccctccacgc agcttgggca ccagaccta
catctgcacac gtaatcacca agcccaagca caccaaggtc gacaagagaa
ttgagcccaaa atcccttgcg ccatgccccacctaaactcaca catgcgccgcg
g```

1519gH20 IgG4 heavy chain (V + human gamma-4P constant) SEQ ID NO: 43

```
EVPLVESGGG LVQPQGQLRL SCAVSGFTFS NYGMVWVRQA PGKQLEWVAY
IDSGDNTYY YSVDVGRFTI SRDNAKSSLY LQMNLSRAED TAVYYCTTGI
VRFPFLYWGQG TLTVVASSAT KGPSVFPLAP CSRSTSESTA ALGCLVKDYF
PDPVTWSNS GALTSGVHTF PAVLQSSGLY SLSSVVTPVS SSLGKKTYTC
NVDHKSNTK VDKRVESKY PCCPCPAPE FLGGPSVFLF PPKPKDTLMI
SRTPETCVV VDFSQSEDPEV QFNWYVDGVE VHHNATKPRE EQFNSTYRRV
SRTLTLHQD WLNKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP
SQEEMTKNQV STLCLVKGFY PSDLAVEWES NQPOYNXYKT TPFLVLDSDGS
FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NYTQLSLSL SLGK
```
FIGURE 1J

1519gH20 IgG4 heavy chain (V + human gamma-4P constant, exons underlined)  SEQ ID NO: 44

qaggtaaccac ttgtggaaga cggaggaggt ctgtgacagc ctggaggaag
tttactttcct ttctgtgtcct gtcttggcct caccttcctc aattcggaa
tggtctgggtc caagaccaagca ccctgaaaggg ctctttgaagtg gttggcctat
attgactctgg acggggagca caacactaat gcggatatgg cgaaagggagc
ttcacatctt cccgccgaata aagcccaaqag ctcactgttac ctcgcaagtga
atagcgctgac agccgaggat actcgctgtct actattgcac aacgggaatcc
gtttagcccttt ttctgtacttg ggacagagggc acctttggtta ctgtctcgag
ccgttctacaa aagggcccat cctgttcccc cctggtgccc cgccctcagga
gcaccctcga qagacacagcc ggccttgggtc gctgtgaatca ggaattatcc
cccgaacccgc tggagtgtgtc ttggaacactca gcgctgctcag ccagcggggtc
gcacaccttc gagcgttgtgc tttacccttc agaagctacttc gctctcacgca
ccgttggtcgc cgtgcctctcc aagcagcttg ccagcaagagc ctacacctgct
aacagtagac caacagccag ccacaccaag tgtgacacaag gaagttgtctga
gagcgcaagca caggagggga gggtgttgctgc tggagaagggc cttcagccct
ccctgctgga cgcaccctccgt cttgctgaccc ccaagccagg gcaagaaagc
atggcccatc tgtcttcctca cccggagggc tctgacaccct ccacatcagtc
cagggagag ggtctctcttg attttttcaca caggctcggg gcagcgcaagc
ccctggtgac cctaccccaac ggccttgccgt caaacgagctt gttgctgctc
tcagacccgt cagagccat atccggggag accctgcccc gcagctaatgc
ccaccccagaa gcggacactc tcctcctcct cagctcagac ccctttctcc
tcccccagatct gtagaattct ccaatcttctt tttgaggttcag gccaaatagc
gttcccctag cccacacatgc ccaggttaagc caaccccaagc ctcgccctcc
agctcaaggc gggaaggttt gcctagagta gctgtcaactc agggcaacgc
ccaggggggc tggctgaogca tccacctctca ttctttctctag ccacagcttg
ccctgtgggg cccattacgt tttccatgtcc ccccccaccc caaaggacac
ttctatgatc tcccggcaccgc tttgaggttac gttgctggtgc gttgacagcta
gccaggaaga ccccgaggttc cagttcaactc gttacgtgga tggctgtcag
ctgtcactag ccaagacaaaa gcggcggggag gacggatcag caacagctata
cgggtgtggag agcgtctcga ccggctgtagg ccagactcgg tcagaacggca
agaggactaa ctgcaaggttc tccacaagag ctcctccgtct ctctcatcgag
aaacacatcat ccaagccagc gggagaggtc caggctggttc cagggcaacoa
tggacagagg tcaagctggc ccacctctctg cctcggagag gacagctgtg
ccacaccttg tccctcaagc gcagcccccgac gacggcagag ggtacacccct
gcccccctaccc caggaggaga tggacaaaaa ccagctcagcg tcagccctggc
ctgtcaaggg cttctcaacc agcgcactcg cctgtgaaagtg ggagaagcaat
gggcgccgg aggacaacact aacagcaccag cccctccgtgc tggacccaga
cgggtgcttc tttcctctacag cagagctaac gcggacagaag agcagtgtgggc
agaggaggaga tgtctttctca tgcctcgttg agcagctggag tgtggtatta
FIGURE 1K
1519gH20 IgG4 heavy chain (V + human gamma-4P constant) with signal sequence underlined and italicised

SEQ ID NO: 45

atggaatgga gctgggtctt tactttcttc ctgtcagtaa ctacagggagt ccattctqag gtaccacttg tggaaagcgq aggaggtctt gtgcagcctg gaggagaatt aqgtctcctct tgtgtcgtgtt ctggcttcac ctcttccaaat tacggaattt tctggtctag agcaagcactt ggaaggggctt ttagaatggtt gcctctatatt gactctgacg gggacaacac ccatactatgg gattccgtgq aagggagcctt cacaatctcc cggagataac ccgaaagcct acgtacactct cagaggtgagc cgaggtatact gccggtgact attgacacac gggaaatcgtt agggctttttc tgtactggggc aacgacagctt ttgggtacttg ctccaggggc gcctccttc ggctccgqagc cacagcggcc ctcgggtcggc tgtgcaagga ctctccccc gacccggtgg cgggtgcggt cgagtccagc cactcccccgc cgtgctttac ccgctccagg acctcactcc ctcgacaggcg tgtgcggtcgg aagccgatcg tgaactccca atctttcttc tgtcagagtt ccataatggtc cccctcagcc accatgcttc ggtacacgaa ccaggcctgc gccctccagc tcgaaggggg acagtgcggtt tgaagggc tgcgtgactc ccgggtgtgc ccctccagc tgcctctacc ccgtcttcgg tacagcaggc ccgcttcctc tgtacagtcc gcagagcctt ctggggtcctc tgtcaggtgt tgcgccgggtc gacaggtccgc gccgggtcgtt aagcctcttt gcagccttcg ctggggtcctc cggagtgcgtt acgttggttt gcaggtgcgtt acgttggttt gcaggtgcgtt acgttggttt
FIGURE 1L

1519gL20 FabFv light chain  
SEQ ID NO: 46
DIQMTQSPSS LSASVGDRTV ITCKSSQSLV GASGKTYLYW LFQKPGBKAPK
RLYLVSTLGD GIPSRSFGS GSGTETFLTI SSLQPEDFAT YYCLQGTHFP
HTFGQQTKLE IKRTVAAASPV FIFPPSDEQL KGTSASVCL LNNFYPREAK
VQWKVNDALQ SGNSQESVTE QDSKSTYSL SSTTTLSDK AD YEKHVYACE
VTHQGLSSPV TKSFRNGECGS GGGGSGGGGS GGGGSQMT QSPSSVSASV
GDRVTITCQS SPSVWSNFLS WYQKPKGPAP KLLIYEASKL TSGVPSRFSG
SGSGTDFTLT ISSSLQPEDFA TYYCGGGYSS ISDTTFGCCT KVEIKRT

1519gL20 FabFv light chain  
SEQ ID NO: 47
GATACTTCCAG TAGCCACAGAC CCAATTCGCCT CATCCATCGCA GAGCAGCA
AGTCAGCAGAC TACAGGATGCG TACACACGGG TGGTGGAGTC AGGTGGTGC
TGGGATCTCTTT TACAGGTCAC ACCAGGATA ACGTGTCGAC GGGGTAGGTT
GGGATGGGTTG GGGGATGGG TTGGGATGGG TGGGATGGG TGGGATGGG
GGGATGGGTTG GGGGATGGG TTGGGATGGG TGGGATGGG TGGGATGGG
GGGATGGGTTG GGGGATGGG TTGGGATGGG TGGGATGGG TGGGATGGG

1519gL20 FabFv light chain with signal sequence underlined & italicised  
SEQ ID NO: 48
MSVPTQVLGL LLLWLDARC DIQMTQSPSS LSASVGDRTV ITCKSSQSLV
GASGKTYLYW LFQKPGBKAPK RLYLVSTLGD GIPSRSFGS GSGTETFLTI
SSLQPEDFAT YYCLQGTHFP HTFGQQTKLE IKRTVAAASPV FIFPPSDEQL
KGTSASVCL LNNFYPREAK VQWKVNDALQ SGNSQESVTE QDSKSTYSL
SSTTTLSDK AD YEKHVYACE VTHQGLSSPV TKSFRNGECGS GGGGSGGGGS
GGGGSQMT QSPSSVSASV GDRVTITCQS SPSVWSNFLS WYQKPKGPAP
KLLIYEASKL TSGVPSRFSG SSGGTDFTLT ISSSLQPEDFA TYYCGGGYSS
ISDTTFGCCT KVEIKRT
FIGURE 1M

1519gL20 FabFv light chain with signal sequence underlined and italicised  

SEQ ID NO: 49

atgtctgtcc ccacccaagt cctcgagactc ctgctactct ggcttacaga
tgccacagatgc gatatccaga tgacccagag cccatctagc ttataccgctt
ccttctgctga tcggctggaca attacgtgta agagctccca atctctctgtg
ggtgcaacatgt gcaagacacta tctgtactgg ctttctcaga agccctggaac
gcaccacaaa ccgctgtatct atctgtggtct taccttggac tgttggtgata
cgctcagatt ttcgccagatct gggagccggaa cttgagctcact actcagcatt
tctacgcgtgc aacccgaggag ctctctgtacc tctacgtgcg ctcagagccac
tcatttccct ctcacactttcg ggcaggggac aaaaactcgaat atcaaccgta
cgtctagggc cccatctgtct ttcatcttcct gcggcatctga tgtgcagttg
aaatcgcgaa cctgtcctgtc tggctgctgtc tgaataact tctatcccgag
agaggcctaa gtacagttgaa agttggataaa cgggctcctaa tccggtaacct
cccagaggag tgtccagacag caggacagca aggcagacac ctcagccttgg
tagagccaccc tgcaggtgtgc ttcagtcagac tctcagcagac ctcagtcagc
tgctggtgga ggcagttgcct ggtggtggtgc gctagggggtc cggaggggtc
cggaggtggcg gttcagacat acaaatgacc cagagcctctg ctcggttatc
cgctgctcgtt gcggcataggg ttacatttaa atgtccttaag cttctctagcg
tctgggagaaaa cggagcctgag gaaacgctgag caggtcgtcagc ttcgctgtga
attacgtggc tgtcgtgatag gacagactttc accttggtatc ctcggtgagg tgtatagtac
attacgtgact ctcaggtgatc agcagactttc accttggtatc ctcggtgagg tgtatagtac
ataaggtgta gcacattttcg gttcagttgc ttcagtcagc ttcggtgagg tgtatagtac

1519gH20 FabFv heavy chain  

SEQ ID NO: 50

EVPLVESGGG LVQFGGSSRL SCAVSGFTFS NYGMVWVRQA PGKGELOWAY
IDSDGNTYY RDSVKGRFTI SRDANKSLY LQMNSLRAED TAVYCYTTGI
VRPFLYWGQG TLVTSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVYDFY
PEPVTWVSNS GALTSGVHTF PAVLQSSLGLY SLSSVVTVPVS SSLGTQTYIC
NVNHHKPSNTK VDKKEVPKSC SGGGSGGGGG TGGGGEVQL LESSGGGLVQP
GSGSLRLSCAV SGIDLSNYAI NWVRQAPGKC LEWIGITIWAS GTTFYATWAK
GRFTISRDNS KNTVYLQMS SRAEDTAVYY CARTVPGYST APYFDLWGGQ TLVTSS
FIGURE 1N

1519gH20 FabFv heavy chain  
SEQ ID NO: 51

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag  
tttacgcttc tcttctgtcg tgtctttgctt cacctttccc aattacggaa  
ttgtctgggt cagacaagca cctggaaagg gtcttgaatg gtgggcctat  
attgacctcg aacggtgacca cacactactt caagggagcc tgaagccgag  
cctcaacaatc tccgagagata aacgcaagag ctcactgtac ctgcatgatga  
atacgctgag agcccgaggt actgctcttgct actattgcac aacggaatgc  
gtaagccccct tttctgtactg cggacagggc accttggtttc ctgttctgag  
cagctcccaac aagggccccat cgggtctctcc cctgcacccct tctccaaaga  
gacaccttgtg gggcagccagc gccctgtgct gccctgtggat ggcgactacctc  
ccccgacagc tggaggtgct tcggagactca ggtcgccccgt ccagcgccgt  
tccagcccttc cgggtctctct ttaagctttc aaggtactac gccctccagac ctatgctctgc  
anagctgtac acaagccccag caacacacctg atgcagatatc aagttggagcc  
caatattggt aacagtcgag tgtgctcagag tgggagccag acccgttggag  
gttgacagcag ggttcactgtg cttggtcttg gaggaggcct agtccagcct  
gagggagccc tgcgtctctct tgtctgaagta aacggcatcg accttgagca  
nttacgacatc aacggtgcttg cgaagaccct gggaaagtgt ttgaagttgga  
tcggtatatg atgagccagc cggagcagcct ttgtatctac atgggctgagag  
gggagggtta cattaggccag gcacagaatgc aaaaaacccag tgtatctcctc  
aatgacacttc ttgcagacag aggacagcagc ggtgtactat tcgtgctgca  
cgtcctccagg ttatagcact gcacccctact tcgatctgtg gggacaaagg  
accccttggtg acctttcag t

1519gH20 FabFv heavy chain with signal sequence underlined and italicised  
SEQ ID NO: 52

**MEWSWVFFPE LSVTTGVHSE** VPLVESGGCL VQPGGL SLRLS CAVSGFTPSN
YGMMWVRQAP GKGLEWYAVI DSDGDNTYJR DSVKGRFTIS RDNAKSSLYL
QMSLRAEDT AVYYCTTGIV RPFLYWGGQT LVTSSASTK GPSVPPLAPS
SKSTSGGTAAG LGCLV KDYFP EPVTWSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVWTVPSN SLHTQTYICN VNHKPSNTKV DKKVEPKSCS GGGSGGGGGT
GGGSEVQLLL ESSGLLVQPG GLSRACAVS GIDLSNYAIN WVRQAPKCL
EWIGIIWASG TTFYATWAKG RFTISRDNK TNYLQMNSL RAEDTAVYYC
ARTPGYSTA PYFDLWGQGT LVTSS

15/59
FIGURE 1P

1519gH20 FabFv heavy chain with signal sequence underlined & italicised  SEQ ID NO: 53

atggaatgga gctgggtcttt tctctctctc ctgtcagtaa ctacaggagt
ccattcttag gttaccacttg tggaaagcgg aggaggtcctg gtgcagcctg
gaggaagttt acgtccctct tgtcgctgtgt ctggtctcctc cttctctcat
tacggaatgg tcggggctcag aacaagcactt ggaagggtcctt ttgaatgggt
ggcctatatatt gactctgcag gggacaacac ctactatccg gtatccgcgta
aagggcctctt ccaatactcc cagagataac ccaagagcctc acgtgacttg
cagatgaata gcctgagacgc cgaggatact gccgtgtcact attgcaacaac
gggatcgctt aggccttttt tcgtactctgg acagggccac ttggttaactg
tctcgacgct gcgtcacaagag ggcctcatcg gtctcctcctt ggcaccctcc
tccaaagagca cctctggggtg cacaagcggcc ctgggctgccc tgtcacaagga
catacttcccc gaaacaggtga ggggtgtccctg gaaactcaggt gcctctggca
gcggcgttca cacctttccg gctgctctcat acgtctctagc acctctactcc
cgagcagcgc tgggtgaccggt gcctctctccg agctcgggca ccaagaccta
catctgcaaca gtagatccaca agcccaagcag cacaagcgaat gataagaaag
ttgagcccaa atctttgagttt ggagggttgg gctcaagttgg aggcgggacc
ggtgaggagt gcagcagctg tcaacttcctt ggtcttgctgc gaggccgtgt
ccagctctgga ggggagccctgc gtctctcttg tcagtaaagc gcatacggcc
tgaccaattt gcggctatcacc tgggtgagact gctgcggcgg aaggtgttta
gaatggatcg gtataaatatg gcgcagcttgga acgcacctttt atgcctacatg
ggcgaagag agtttcaacg ttagccccac caatagccaa aacaccctgt
atctcaacaat gaaactccttg ccagcagggg acagggcgcgt gcctactttgt
gtcgcaactg tcccgaggtta tagcactgca cccacttctcg atctgtgggg
acaagggcgg cctgggtcagct tgtcaagt

Human VK1 2-1(1) A30 JK2 acceptor framework  SEQ ID NO: 54
DIQMTQPSSS LSASVGDRTT ITCRASQGIR NDLGWYQQKP GKAQKRLLYA
ASSLQSGVPS RFSGSGSGTE FTLTISLSQP EDFATYYCLQ HNSYFYGTFQ GTKLEIK

Human VK1 2-1(1) A30 JK2 acceptor framework  SEQ ID NO: 55
gacattccaga tgacccagcgtc tccatcctccc ctgtctgcatcttgaggaga
cagagtcacc atcaacttgccc gggcagatcga gggcattagca aatgatttag
gtcggtatcag gcaagaaaccg gggaaagcccctaagcgccttgctcgatcgtc
gtcacaggttg cggcctatcgc agtctccagcg gcgtgctgggac
tgggacagaa ttcactctcca caatcagcag cctgcaaccttg gagaattttg
cacactatgta ctgcatacg catatctagtctt ttccttacac tttggcccag
gggaccaagcttgagatcaca a
**FIGURE 1Q**

**Human VH3 1-3 3-07 JH4 acceptor framework**  
SEQ ID NO: 56  
EVQLVESGGG LVQPGGSRL SCAASGFTFS SYWMSWVRQA PGKGLEWVAN  
IKQDGEKYY VDSVKGRFTI SRDNKNSLH LQMNSLRAED TAVYVCARYF  
DYWGQGTLVLS VS

**Human VH3 1-3 3-07 JH4 acceptor framework**  
SEQ ID NO: 57  
gaggtgacagc tggtggagtc tgaggagggc ttgtccagcc ctgggggtgc  
cctgagactc tctctggcag ctctctgatt cacctattgt agctattgga  
tgagctgggt cccaggcagct cccagggaggg ggctggaggtg gtgggccacc  
ataaagcaag atgggattga gaataactat qtggtactcttg tgaagggcccq  
atccacagtcc aggagagac caagccaaaga ctctctgtat ctggcaaatga  
acaagccttag attcagagac acggcggtgtg attactgtgc gagatacttt  
gactacttgg gccagggacac cctggtcacc gtctcc

**Rat Ab 1548 VL region**  
SEQ ID NO: 58  
DVVMQTQPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK  
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLOGTDFP  
HTFGAGTKLE IK

**Rat Ab 1548 VL region**  
SEQ ID NO: 59  
gatgttggtga tgacccagac tccactgtct ttggtcgggtt cccttggacaa  
accaggccccc atctctttgca agtcaagtca gacgtctgtggtc gtgtctagtg  
gaagacatca ctttcattgg ttttttcaga ggtccggtca gttctccaaag  
cgactaatct atctctgtgtc cacactggac tctggaatcc ctgataggttt  
cagtggcaegt gcagcagaga cagatcttac tctttaatac cgcagagttg  
aagccagatga tttgggagtctt tattactgtct tgcaaggtac acatattttct  
cacacgctttg gaggctggagc caagctggaa ataaaa

**Rat Ab 154 VH region**  
SEQ ID NO: 60  
EVPLVESGGG SVQPGRSMLK SCSVSGFTFS NYGMVWVRQA PKKGGLEWVAY  
IDSDGNDNTYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCCTTG  
VRPFLYWGQG VMVTVS
FIGURE 1R

Rat Ab 1548 VH region  SEQ ID NO: 61

gaggtggcgc ctttgttgagtc tgggggccggc tcagtgcaagc ctgggaggtc
catgaacatc tctctgttag tctccagatt cactttcagt aattatgca
tggtctggtt cccgccaagct ccaagaaggg gttcggagtg ggtcgcataat
attgattcttg atgggtgaat tatcttactac cgagatccgg tgaagggccg
attcaactac tccaagaata atgcacaagag cacccctatat ttgcaaatggg
acagttctgg gttcgagggac acgggccactt attactgtact aacagggatt
gtccgggccct ttctctattg gggccaaagga gtcattggtca cagttctcg

Rat Ab 1644 VL region  SEQ ID NO: 62

DVVMQTTPCLS LSVAIGQPAS ISCKSSQSLV GASGKYLYW LFQRSGQSPK
RLYLVSTLDS GIPDPFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1644 VL region  SEQ ID NO: 63

gatgttttgtga tgaccgcagac tccactgtct tttgctgggtg ccaattttgaca
accagcctcc atctctttgca agtcaagtca gacgctgcta ggtgctagttgg
gaagaagcata ttgtatattg tattttcaga ggttccggccaa gtttccaagag
cgacttaatct atcctgggttc cacatctggac tcctggatctt cttgattttctt
caagctgcagtg gagaactagga cagatatttcac ttttaaaatc gcagaagtgg
aagccgatga tttgggagtt tattactgtct tgcaaggtac acattttttcct
ccacagttgtg ggtctgggac caagctggaa actgaa

Rat Ab 1644 VH region  SEQ ID NO: 64

EVPLVESGGG SVQPGRSTKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IGSDGDNIVYY RDSVKGRTFI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWQGG TTFTVS

Rat Ab 1644 VH region  SEQ ID NO: 65

gaggtggcgc cttgttgagtc tgggggccggc tcagtgcaagc ctgggaggtc
cacgaaaactc tctctgttag tctccagatt cactttcagt aactatgca
tggtctggtt cccgccaaggct ccaagaaggg gttcggagtg ggtcgcataat
attgattcttg atgggtgaat tatcttactac cgagatccgg tgaagggccg
attcaactac tccgaaaaata atgcacaagag cacccctatat ttgcaaatggg
acagttctgg gttcgagggac acgggccactt attactgtac aacagggatt
gtccgggccct ttctctattg gggccaaagga accacggtca cctgcttcg
Figure 1S

Rat Ab 1496 VK region  SEQ ID NO: 66
DVVMQTQPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1496 VK region  SEQ ID NO: 67
gatgtgtgctg tgcagctcttgct tttgctggttg ccccttgagca
accaqctcct atctcttggca aqtcagctcga gacqctctgta ggtqctaggtg
gaaagcata tttgtatttg tttatctcaga gttcggcgcga gttgcctcgaag
cgaactaactc atctctgtgctc caacacttgac tctggaaatc ctgatagttt
cagttcgcaat ggaacgcagaga cagattttactttaaaatc gcagcaggtg
caagccqgatg tttgctggatt tattactgtc tgcagcagat acatatttacct
cacagctttggagctcagctggac ccagctggaa cctgaa

Rat Ab 1496 VH region  SEQ ID NO: 68
EVLLVESGGG SVQPRGRSMK SCVSVSFTFS NYGMVWVRQA PKKGLEWVAY
IDSDGDNYYY RDSVKGRFTI SRNNAKSTLY LQMDLSLRSED TATYYCTTGI
VRPFLYWGGQ TMVTVS

Rat Ab 1496 VH region  SEQ ID NO: 69
gagttgctgc tgtgctgggta tcgaggggcc gcctgctgagtct gttgagggagtc
caagaaactc tctgtgtagtctt cagctggtaatc tattatgacat ctaagctgaa
sgtgcaggtc cccctgcagct ccaagagaggg tctgtgagttgtctcagcatat
attgaatccttatgtgcata taccgactac ccgtagcctt gtaagggcccg
aatcactatc tccagagata atgcaaaaaag caccctatat tggcaaatgg
acagttgtgtgcttgagagagtgcagccacatcttattacgctac cacaggagatt
gtgcgagccccttctttctttggcagcaagagcattgagctgca cctcctcg

1519gH20 IgGl heavy chain (V + human gamma-1 constant)  SEQ ID NO: 72
EVPLLVESGGG LVQPSSGLRL SCAVSGFTFS NYGMVWVRQA PKKGLEWVAY IDSDGDNYYY
RDSVKGRFTI SRDNKASSLY LQMNSLRAED TAVYYCTTGI VRPFLYWGGQ TLTVTVSSAST
KGPSFPLPAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY
SLSSVTVPS SSLGTQTYIC NVWHKPSNTK VDKKVEPKSC DKTHTCPFPC APELLGGPSV
FLFPKPKIDT IMSRTPTEVF CQVVDVSHED PEVKFNWVYD GVEVHNAKTK PREEQNYST
RVSFLTVLH QDVLNGKEYK CKVSNKALPA PIEKTISAKA GQPREPQVYLT LPSSRDELTK
NQVSLTLCVK GFYPSDIAVE WESNGQPHMN YKTPPVLDS DGSSFLYSKL TVDKSRWQQG
NVSFSCSVMHE ALHNHYTQKS LSLSPGK
Figure 1T

1519gH20 IgG1 heavy chain (V + human gamma-1 constant, exons underlined) SEQ ID NO:73
gagttaccac ttgtgaaag cggaggaggt ttctgagcct cggaggagga tttacgttctc
tcttggtctg ttgctggtctt caccttcctcc aattacggga tggctggtgct gacagaacga
cctggaaaggg ctccgaaatgg ttggcccttat atttgctctcg agccctgcttg cacactat
ccggattcgc tgaaagggag ccctacaatc tcccgagaga aagccaaagag ttcacctgtac
tctgagatag atacgctgag agcggcaggt actgctggtgct actatgcaac aacggaaatc
gtttgcccttt ttctgtacttg ggacagaggg acctttggtta ctctctcctag cgctctctaca
aagggcccat cgtgtctctcc cttggcaggcc tctcctcaag aacaatctgg ccgcacacccc
gcctgtggtcg gccttggtcag ggtcatcttc cccggaaccc tggagcttgtgc gtgggaacctca
ggccccctga ccacgcccggt gcacaccttc cccgctctcc tacagctctca agccctcctac
tccctcagca gctttggtgac ctctctctcc agcagcttggc ccaccccaag cccagtctagc
aacgtgatac aacaagccgag caacacacag gcgtgacacaga aagttttgtga gaggccagca
caggagggga ggttgtctgc tggaagccag gctcagcgcct cctgctggtga gcaatcggcc
catgacgcg cagctgccgg gcacagcaagcc agggcggctc tgcttctctca cccgagagcc
cttgccgccc ccactgacgt ccaggggagag ggtctctctgg ctccccccca aggccttggg
caggcagcag ctagttgccc tccaagcagg cccctgcacac gacgggagcg ggtctgtggtct
ccagacgccg aagagccata tccggggaga cctgcccct gcacactacg caaccccaag
ccaaactcct ccctcctcctg agctcggagca cctctcctcc tccccacatc gagaatccccc
caatctctcc tctgcaaggcc ccaatcttg tgcacaacaac caaacatgcc caccctggcc
aggtgacccaa gcggcgccct gcgcctccag cttacagggc gcacgtgctgc cttagagtacgc
tcgactccag gcagccggcc cagcgccggtg ccgacagcgc cactcctcatc ttctctccag
cacccagact cctgggaggg cccgctagctct tctctctcct cccaaacccc aagccagccccc
catgctctct cccgagccct tgggtcagttt ggggctgtggt ggacgtggtgct gcagaggccc
ctgtgatcag gttgaacttt gacgtgagcct gcgagagagt gcgatagcc gcgctgcagc gcgcctggag ccgcaagcacc
tccctggag cctggaggaag cccatctcctc aacacaccccg tggagccagcc tgggttgccag gggccacacttg
gacagcgcgc ggtcgccccc acctctctgc ctgagaagtg ccgagtttc agcatacttc ccacccagcc
cctccgagca accacagcag gaccacagtg tacacctgct ccacccctcag gacgtgagctg
accaagagcc aggtcacgct ccgatctgcc gcgtgagcct gcggcatccct gcgcctgcagc
gttggtcgag ccgcaactgg gcggcgcggcc aacaactaca gcagccacgc ccctctgtgcg
gactcggag gcctctctcc cctctacccg aagggcgagc gcgctcctcc tggagcagag caggggaagc
tctctctcag ctctgtgctag cgtgagatgc ttgagcattc gcgactcagc ctacccgcag
aagacgcttcct cccctgcctcc gggtaaa
1519gH20 IgG1 heavy chain (V + human gamma-1 constant) with signal sequence underlined and italicized

SEQ ID NO: 74

taggaatgga gctgggtcttt tctctctctc cagtcagtaa cacagggagt ccattctgag
gtccacactg tgggaagcccg aggggttctt ggcagcctcg gaggaagttt acgtcctctc
tgtgtgtgt ctggctctc ac tctctctctc tagcagtaa gtcgagctg tctgagctc
ccagaggtgc ttcctatatt gactctgacg gggcaacaac ctaactatcgq
gatcctgtaa aagggcagg t caaacttccc cagagaataac ccaaggacgct actgtgactg
cagatgata gctctgagac gcagagatact gcgcgtactt atgcacacac gggatcgtt
aggctctcttc tctacttgggg acagggcacc ttgggttactg tctcgagcgc ttctcacaag
ggcccatcgg tctctccccc gccaccccct ccacacagca cctctgtgggg cagacgagcc
tctggctgcc tctgctacagc ctacctccccc gccacggtga cgggtctggtg gaacctacgg
gccgctgccgc cccaccccag ctcctccagct aacccctcag ctgctggacac gtcagagcgc
tctctcagc gcctcccaag tggagtgca tataacttac cagacgccccct ccgctctagg
taagccacgc cagacgtcgt ccacctccctg aagccggcag acgtgcccct aatgtcagct
catccaggg cagcgcctcag ccgggtgcttg acagctccac ctctccatct ctctccacac
tctgctctgc cgggggacgcc ctacacgccct gcagcctggctt gctctccccc cccaaaccac gacacccctca
tgatcctcag gcacccctag gtcacatgcg tgggtgtgga cggagacccac gaaacccttg
aggtgacagtta caacctgtagc gttgagctgc taatgcacaa acaagccggc
gggagagcga cagcaccacgc aggtgagctgc tcgaggttcg ggtgagttgc ataaccctcag cggagacccac
tcgtccctgg ccacacgcgg gcagcaggtc gcagcagctg aacccctcag cgacacccctca
tgatccagag cggagagcga cggagagcga cggagagcga cggagagcga acaagccggc
gggagagcga cagcaccacgc aggtgagctgc tcgaggttcg ggtgagttgc ataaccctcag cggagacccac
tcgtccctgg ccacacgcgg gcagcaggtc gcagcagctg aacccctcag cgacacccctca
**Figure 1V**

1519 gL20 light chain (V + constant, mammalian expression alternative) SEQ ID NO: 75

gatctccaga tgacccagag cccatctagc ttatccgctt ccgttgggtga
tgcgctgagca attacgtgta agagctccca atctctctgtg ggtgcaatgtg
gcgaagcttc tctgtacctg aagttctgcga ggcacccaaat
cggctgtatct atccgctgtc tacctctgagc tggagtgcgtt
tttggctgtct tttgatcagc atcaatattc aatctgctg

gccttcaccat cagcagaactg ccctggagaag tggctgtagc cggctgtatt

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge, mammalian expression one base change from SEQ ID NO: 38) SEQ ID NO: 76

gaggtctaccac ttgtggagaag cggaggggtt ccgttgctaca ctgtggaagag
tttacgtcttc ttgagtcgctt tttgctgccc aataagagaa tgcctgagat

ttgctgtggtt cagcagattgg ctctgacaag ggtgtgtagc ggtgctgtatt

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (mammalian expression one base change from SEQ ID NO: 42) SEQ ID NO: 77

atggaatggga gctgggtcttt tctctcttttc tggctgatgaa actaggttggtt

ccttcctcag tcaaccacttg ttggagagcc aggggttcttt tggctgagatg

gaggtcagctt tgtgctgctt tgggctgctt cttctctctc atctgtcttct

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**Figure 1W**

1519gL20 FabFv light chain (alternative sequence to SEQ ID NO: 46)  
SEQ ID NO: 78

```plaintext
DIQMTQSPPSS LSASVDRVT ITCKSSQSLV GASGKTYLYW LFQPKGKAPK
RLYLVSTLD SGIPSFSGS GSGTFTLTI SSLQPEDFAT YYCLQGTHFP
HTFGQGTKLE IKRTVAAPSV FIPPSSEQL KSGTASVCL LNNFYPREAL
VQWKVDNALQ SGNESVSVE QSDKSTYSL SSLTLLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGECG GGGSGGGGSG GGGSDIMQTQ SPSSVSASVG
DRVITICTQSS PSWNSNLWY YQQKPKGAPK LLIYEEKLT SGVPSRFSGS
GSGTDFTLTI SSLQPDFTAT YYCCGGYSSI SDTTFGCIGK TEIKRT
```

1519gL20 FabFv light chain (alternative sequence to SEQ ID NO: 47)  
SEQ ID NO: 79

```plaintext
gacattccaga tgacccagtc cccctccagc ctgtccgcct ccgtggggca
cagagtggacc atccacatcg atgccttcaaa gtagcctgct cccctggtgt
gcagcatgc gaagggaaac gacagcagct cgtgctgaga cggagggcgc
cttctggat gtagcctggt ccagccctgct gatggcctgt caaggggcgg
```

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FIGURE 1X

1519gH20 FabFv heavy chain (alternative sequence to SEQ ID NO: 51) SEQ ID NO: 80
gaggtgcctcc tggtgggaatac tggcgccgga ctgtgctacg cttggcctac
cctgagacttg tctcgccgcgt gtcgcccgtt cactttctcc aactacgcca
tgtgtctggtg ccgacaggtc cctggcaagg gactggaatg gttgcctac
atcgatcccg acgggacaa cacactaact cgagctcgcg tgaagggccg
gttcaccatc tccggggacg acggcaagtc ctccccgtcgt ctcgagatga
actccctgctg ggccccggagc acgggctgtat actactgcac caccggcatc
gtgcggtccct ctctggtactg gggcaggggc accctgtgca cctgtgcttc
tgctctctaca aaggccccata cggttctcccc cctgccaccc cctctccaaga
gcacctcttg gggcacaagcg gcctgggtct gcctggtcaaa ggaactacttc
cccgaaaccgg tgacaggtgct gtggaaactca ggccgccccg ccagccgcgt
gcacaccttc cccgtgctctc tacagttctcg tggactccat tctctcagca
gcgctggtgac cgtgcaccctcc acgcagcttg gcacccagac ctacatctgc
aacgtgaatc aacaacccca aacaaccaag gtggacaaaga aagttgaccc
caaatcttttg tcgccaggttg gcggtttccgg aggtggccgt acaggtggcg
gttggtccga agtccgctgt cttgatcccg gagccggact cgtgcaaccc
ggagggagtc ttgctgttgc tgtgctgttca ctggataatcg acctagcga
ctgcggcactc aactgggtga gacaggcacc tgggaatagc ctggaatggga
tcgccattat atggtcattg ggggacagct ttatgctgta atggggcaag
ggtgtggatc ccatctcctc gcgtatagat aagaacacag tgtacctgcga
gatgaactcc ctgctgagcag aggataccgc gttttcattat tgtgtcgcgca
cgtgcccagag ttatagcact gcacccctact ttgatcgttg gggcaggggc
actctggtca cggcggtgctc
Figure 1Y (signal sequences underlined and italicised)

Rat Ab 1548 VL region (alternative sequence to SEQ ID NO: 58)  SEQ ID NO: 81
DVVMTQTPLS LSAVGQPAS I5SSKSQSLV GAGGKTYLYW LLQRSGQSPK
RLIYLVSTLD SGIPDFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTNLIE IK

Rat Ab 1548 VL region (alternative sequence to SEQ ID NO: 59)  SEQ ID NO: 82
gatgttggta tgaaccacagc tccactgtct ttgtcgggta ccattggaca
accaccctcc atctcttctta agtcaagtca gagcctcgta ggtgcttgtg
gaaagacata tttgtatggc ttattacaga ggtccgagcc gtctccaaag
cgactaatct atctgggtgta cacactggac tctggaattc ctgataggtt
cagtggcagt ggacgagaga cagatatttc tctttaaatc cgcagacgtgg
aagccgtaga tttgggggtt tattactgtc tgcaggttac acatatttct
ccacagtgctt gaggcggagac caactgagaa ataaaa

Rat Ab 1548 VH region (alternative sequence to SEQ ID NO: 60)  SEQ ID NO: 83
EVPLVESGGG SVQPGRSMKL SCVVSQFTFS NYMVWWRQA PKKGLEWVAY
IGSDGNTRYY RDSVKGRFTI SRNNAKSTM YQMSLRSER TATYYCTTGI
VRFLYWGFG VMVTVS

Rat Ab 1548 VH region (alternative sequence to SEQ IS NO: 61)  SEQ ID NO: 84
gaggtgcgccg tggtggagtc tgggggcgcc gcctagcgagc ctggaggtgc
catgaaactc tcctgtgtag tcctaggatt caccttacgt aactctagca
tggtcctggt ccggccaggtt ccxaaaagag gtctggaggtg ggtgcacatat
attggttctg atggtgataa tattatctac cgagttcccq tgaaagggccg
attcactatc tcagaaata atgcaaaaaa aaccctatat ttgcaaatgg
acagttctgag gtctggagac acggccacctt attattcttg aacaggatt
gtccggccct ttctctactg gggccaagga gtcattgtca cagttctcg
Figure 1Z
1519gH20 IgG1 heavy chain (V + human gamma-1 constant, exons underlined one base
change to SEQ ID NO: 71) SEQ ID NO: 85

```
gaggtaccac tttgtggaag cggaggaggt cttgtgacgc ctggaggaag tttaaagtctc
tttggtctct tggctggcttt cactaagaga tattcctgat gcacactatct
ccggattccq tgaagggagct cttcaacacac tcccgagcata accggcagagt ttactgcgtc
ctgcagatctg ataggctctgg aagcgcggag gatcctcgctag cacttcacct

tctgtgagct cttgttctcag gcacactacac cccaacccgggg ctctcggtgtc ggcaggtggggg
tgcgtggcag ttcctacgac ctcctgctgcag ccccatcctcct ccgctggtgctg
tggaggagct gattcagcag ttttcccttcc cggcagctctg tgcctgcgcc
ggctgctgtgc cgcagcagcag ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg ggcaggtgggg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
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tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
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tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
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tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
tggcagagc tgcctgctgtgc ccccaacccgggg ccccaggtgggg gtcgctgtgctg
1519gH20 IgG1 heavy chain (V + human gamma-4 constant with no mutations) [SEQ NO:72]

1519gH20 IgG4 heavy chain (V + human gamma-4 constant with no P mutations) [SEQ NO:87]
1519gH20 IgG4 heavy chain (V + human gamma-4 constant, exons underlined no P mutations) SEQ ID NO:88

gaggtaccac ttgtggaaag cggagggagt ctgtgtgcagc cttggaggaag
	ttacctgctc tgttggttctg tgtctgttctt cacctctcccc aattacgqaa
ttgtctgggt ctacaccaagc cttggaaagtt gtttggctat
atttgacttctg aacgaggtcaca cacactatct cgggattccg tgaagggacg
tctcacaatc ctcgctagata atgccaagac ctctactcgta atgcacctgag acagcagagat actgccccgtgt actatgcaac acagggaaatc
gttaggccttc ttctgtacttg ggacagaggc accttggttta cgctctctgag
cgtctctcaca aagccagacg ccctgtcccc cctggtgcggc ccctgtcgagga
gcacctccgag gagcagcagc ggcctgggct gcctgttcaca ggactacttc
ccggagcgct ccgacgtgcgc gtcgggactca ggccgctcggc ccagcggccgt
gcacacccctc cccgcttgcc acagctcttc ctcctctcggc ctagctctgag
ggtttgggac ccgctccctcc acagcctggg gcacgaggaac ctctcactgc
aacggtgatacg acaagggcagc ccaaccaaggagt ggagggatggag

gagggcaagca cagggagggga gggtgtctgc tgtgaagcagc gctcagcccc
	ctgcgtcgtcca ggacccgagcc cttgacgcggc ccagccccgcc cagcagcagc
attgccccatc tgtctctcaca cccgagggcc tctgacaccc ccagcctgacg
cggagagagac ccggaggttc cagtctcaact gtcagcctgg gggctgggtga
gttcataatgc cctccgaccc cttcgatgta gcttgctaccc caggagcagc
ccacaggcggg tgtgtcagcc caacactacc tctctctctc agcaccttggag
tctctggggg gaccatcagt cttctctgttc ccccaaaacc ccaagggcactc
tctctgatgc cccgctggacc ctgaggtcagc cttgctttgtg tcggagagtgac
ggccaggaaga ccccgaggttc cagtctcaact ggtacgctgg ggggctgggtga
gttcataatgc cctccgaccc cttcgatgta gcttgctaccc caggagcagc
ccagagttggc agcggttcacc gcatcctgtcc cccgctggga ccagcactgtg
gttgaccaggg agagttggcc ctcaggtcttgcc cttgctttgtg gccaaggggca
ccagagttggc tgtgaggtgc cttggagggc cttggagtgtg gaccgtgcttg

gacgctcggc cccaccccagc cttggagggc cggcagggga ccagcagagc
tgggacttcgg ccagacccagc cggccggaca ctcacgagtgc cggcagggga
tgggacttcgg ccagacccagc cggccggaca ctcacgagtgc cggcagggga

caccccttcttctcctcagcaggtgcagtc ccgacggtgattcttggacttgcc
tggctacgggagctgcctgttctg gggcaggtaggatccagggagggcctttggagg
Figure 1CC

1519gH20 IgG4 heavy chain (V + human gamma-4 constant) with signal sequence underlined and italicised—no P mutation  

SEQ ID NO: 89

atggaatggag gctgggtcttt tcttttttctt ctggcagtaa ctacaggagt
catatctggag gttaccacctg tgtgaaagcgg agggagtctt tgtgacgccctg
gaggagtgtt acgtctctcct tgtgtctgtgt ctggcttac ctcttccaat
tacggcattg gctggtgtcag aaacggacct ggaaagggtc ttgaatgggt
ggccttatat ggctctgaggg gagacaaacc actactacgg gattccgtgag
aaggacgttt cacaatctcc cagagataac gcaagagctc acgtgtactctg
cagatgaata gctctgatagt ggctgatact attcgacaca aagggatcgtt
agccctttttc tgtactgggac acaagggacc aaggtgtactgt
tctctgacgcc ttctacaaag ggcccatccg tctctccccc ctggcgcctgc
tccagagcaactcagagac ctacggcccc ctggctgctgc tgagcagga
ctacttccccc gaaacgttga cgtgtgcgtgt gaaactcaggcgccctgacca
gcggcggtgca cacccctcgc ggtgtctctac gttctctcagg ctcctactcc
tctgacagcg gcctcgcagcg acgttgggcc acaagacctat

cacctgcaac gtagatcaca agcggcagcag caccaggttg gacaagagag
	tacgtgtgac ccgcaagcag ggaagggaggt tgtgctgctgc aagcgacggt
cagccccctt gctgtggacgc acggcggtgct cagacccccca gagcgccggca
goagcggagtgg gccattctgtg ctcctcactgc ggacgcttct gccacccagct
ctcatgcccc gtagcggtgttt ttcttcacctgc gtcgccccagc
gcagacggct gtagccccctt acggccagcc ctcgctgacac agggccaggt
gtcgctccta gcacggccag cagcgcatctg ccggagggc ccggtccctgta
cctagccca ccccaagggc cacaacttcc aactcctcag ctcagacacc
ttcctccctc cccagatctga taaaactccca atctctctctc tgcagacgtc
aaataaggctc cccagatccgac aatacgccca ggtagccca ccagggctcc
gccctccagc tcaagggcggg caggtgtcagc tagagtactgc tgcactctccag

gacaggcccc acgggtgtgc tgcagcatct gccctcgcagc acctctcgc ctcttcgcctc
tcgtgggtt gagcagacttgc ctggtgggtggt tagttgggtgcg

gaggtgcagct caggtggtggtc tccaatccag gcagttgtgctg gcgggacgaga
cgtgctcgag cagatgctgct cggcagagc cagggaggc ctcgctttgta
caggtggatgt ccagagggctt cagaaaaacct gctccgggaa
caggtgggtt gataatggtt tgggagcccag caggggacgg cgggagggc tggagaattg
gcagcagctt caggtgggttg gcagttgcct ggagccccct cctctccgtcct
acggacaaag accaatctcctc aggcagacaag ggagaacagt gtagagattg
gaactgtgagc agccagaggg gggagagccc aacgccagcg gacccggcc

gccgcaagtg cgcggagcag ccggtcggc ccccccggtg tggagagtgcgc

gcgtgtgccat ccctctgagc ccagagggcc gggcgagagc cccacaaggg
tacccctggcc cccatccagc gaggagacta ccaaggaac gctcgcggtgtc
dctctgtcctcc tccctcagct ggcctacgc ggctacagggc cttgcgggtg
	acgtggcagc ctggcttgctt ctctcaacg gacatcggcc tggatgggag

gaggtgtgggtt caggggactt gttgtgagctgc tccggtgtgatcattaggctc
tgccacacc tacacacagag acgcctcttc ctggtctgtggtagaa
Figure 1DD

1519 gL20 V-region (mammalian expression alternative to SEQ ID NO: 17)
SEQ ID NO: 90
gacatccaga tgacccagtc cccctccagc ctgtccgcct ccgtaggcga
cagagtcacc atacatgcc aatcttcctca gtcctctggc ggaggtcctcg
gcaagactca cctgtcactgag tgcctccage agccccgcaac ggcccccaag
cggtgcattc aacctggtgtc taacccgttac ccgtcgctcct cctccgggttt
cctcgcgctct ggtcttcgca cgcaggttac cttgaccatc tccagcctgc
gccccgagga cttcgcctacc tactactgct tgcagggcac ccacatccct
cacaccctccg gcccagggcag cacagtggaa atcaagggcg cctggtgccc
tcctccgctg ttccatcttcc caccctctcca cggctctccc aatgtggtac
caggctcttgc cgggtgtctgt gctgagcact ttcaccctcct cgcagccaggt
gagacactcct cctgtggctgt gcagaacctct ctacccctct cgcaggttgc

ten19 gL20 light chain (V + constant, mammalian expression alternative to SEQ ID NO: 24)
SEQ ID NO: 91
gacatccaga tgacccagtc cccctccagc ctgtccgcct ccgtaggcga
cagagtcacc atacatgcc aatcttcctca gtcctctggc ggaggtcctcg
gcaagactca cctgtcactgag tgcctccage agccccgcaac ggcccccaag
cggtgcattc aacctggtgtc taacccgttac ccgtcgctcct cctccgggttt
cctcgcgctct ggtcttcgca cgcaggttac cttgaccatc tccagcctgc
gccccgagga cttcgcctacc tactactgct tgcagggcac ccacatccct
cacaccctccg gcccagggcag cacagtggaa atcaagggcg cctggtgccc
tcctccgctg ttccatcttcc caccctctcca cggctctccc aatgtggtac
caggctcttgc cgggtgtctgt gctgagcact ttcaccctcct cgcagccaggt
gagacactcct cctgtggctgt gcagaacctct ctacccctct cgcaggttgc
1519 gH20 V-region (mammalian expression alternative to SEQ ID NO: 31) SEQ ID NO: 92

gaggtgcgcccttggtggaattctggcgccgacttggtgcagcctggcggtcctc
cctgagactgtgttgccgcttggtcgggttcaccttctcacaactacggca
ttggtctgggcctggacaggctcctggcaagggtactggaatgggtgccctac
atcgactcgcgagggagcacaacccctactacggggactgcttgaggccggttcaccatctcccgccgcaacccgcaagtctccctgtacctgccagatgacacctctgctc
gtgcgcgcctttctgtctgctggccagggacctcctgtcaccgctgcgccatc
gtgcgcgcctttctgtctgctggccagggacctcctgtcaccgctgcgccatc

gaggtgcgcccttggtggaattctggcgccgacttggtgcagcctggcggtcctc
cctgagactgtgttgccgcttggtcgggttcaccttctcacaactacggca
ttggtctgggcctggacaggctcctggcaagggtactggaatgggtgccctac
atcgactcgcgagggagcacaacccctactacggggactgcttgaggccggttcaccatctcccgccgcaacccgcaagtctccctgtacctgccagatgacacctctgctc
gtgcgcgcctttctgtctgctggccagggacctcctgtcaccgctgcgccatc
gtgcgcgcctttctgtctgctggccagggacctcctgtcaccgctgcgccatc

Figure 1FF

1519gH20 IgG4 heavy chain (V + human gamma-4P constant alternative to SEQ ID NO: 44) SEQ ID NO: 93

gaggtgccccc tgttggaatct tggccgaggga cttggcagcc gtggcgggtc
cctcagagctg ctctgggctt caccttcggt caactccgga

tggtcctggt ccgacagggct cctggccagag gagtcggagtg gttggcctac
atcagctccg aaggagacaa cacactactac cggactcccg tgaagggccg
gtgccaccctcc tcccggcagaa aacccaaatttc ctccctgtag ctgaggatga
acctccctgc ggcggagagg acgccggctg tcatccgtgg ccatggccac
gccggcccttc ctggctcttc cccggctgtg gcacccgggc gcacccgggc
ccacccgttg gctctgggtc gcctgggttc gcgtggcttc gcgtggcttc
cctggtggc ccatcggcgcc ccgattcggc ccgcctggct cctgctggct
tccccgggct ccgagcagtc gaggcgtggt cccggcgtgg ccggtgggtgc
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cctggtgctgta ccagcggagt ccgagatggc ctggagccgg ccggtgggtgc

cctggtgctgta ccagcggagt ccgagatggc ctggagccgg ccggtgggtgc

cctggtgctgta ccagcggagt ccgagatggc ctggagccgg ccggtgggtgc

cctggtgctgta ccagcggagt ccgagatggc ctggagccgg ccggtgggtgc

cctggtgctgta ccagcggagt ccgagatggc ctggagccgg ccggtgggtgc

Human β2M (SEQ ID NO:95)

IQKTPQIQVYRSRRHPNPGKPNLNCYVSVQFHPPQIEELKNGKKIPNIEMSDLSDSKDWSFYILAHTEFTPTEDVYA CRVKHVTLEKPTVTWDRDM

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**FIGURE 2A**

LIGHT CHAIN Graft 1519

|   | 1   | 5   | 10  | 15  | 20  | 25  | 30  | abcde | 35  | 40  | 45  | 50  | 55  | 60  | 65  | 70  | 75  | 80  | 85  | 90  | 95  | 100 | 105 |
|---|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Light 1519 | DVMTQTPLSSLVALQGSASICGKSSQLVGSAGKTYLIFNLPFQGSPKRKLILYLVSTLDSGIPDRFSGGAETDFTLKIRRVEADDLGVYYCLQGTHFPHTFGAGTKLEIK |
| VK1 2-1-(1) A30 | DIQNTQSIPSLASASVGVDTITCIPASQGIPN----DLGYIQKPGKAPKRLIYAAASLGPSRSFSGGSGETFLLTISLSQPEDFATYYCLQHNSYPFTFGAGTKLEIK |
| 1519 gL20 | DIQNTQSIPSLASASVGVDTITCIPASQGIPN----LPJKPGKAPKRLIYLVSTLDSGIPDRFSGGAETDFTLKIRRVEADDLGVYYCLQGTHFPHTFGAGTKLEIK |

**Legend**

1519 = Rat variable light chain sequence  
1519 gL20 = Humanized graft of 1519 variable light chain using VK1 2-1-(1) A30 human germline as the acceptor framework.

CDRs are shown in bold/underlined  
Donor residues are shown in bold/italic and are highlighted: L36, F37 and I58
FIGURE 2B

HEAVY CHAIN Graft 1519

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<th>5</th>
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<th>15</th>
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<th>25</th>
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<th>35</th>
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<th>70</th>
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<th>80</th>
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<th>90</th>
<th>95</th>
<th>100</th>
<th>105</th>
<th>110</th>
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Heavy 1519 | EVPLVESGGSVQPGSRMKSCVSGGSFGNYMVQVRQAPKGELEWA VYSDSGDNYRDSVKG | GIVRPFLYWSGQITVT | |
| 3-19 | | | | | | | | | | | | | | | | | | | | | | | | |
| VH3 1-3 3-07 | EVQLVESGGGLVQPSGSRMLSCVSGSGFGNYMVQVRQAPKGELEWA MIPQGSSEDYDSVKG | GIVRPFLYWSGQITVT | |
| 1519gh20 | EVPLVESGGSVQPGSRMKSCVSGGSFGNYMVQVRQAPKGELEWA VYSDSGDNYRDSVKG | GIVRPFLYWSGQITVT | |

Legend

1519 = Rat variable heavy chain sequence
1519gH20 = Humanized graft of 1519 variable heavy chain using VH3 1-3 3-07 human germline as the acceptor framework.

CDRs are shown in bold/underlined
Donor residues are shown in bold/italic and are highlighted: P3, V24, S76, T93 and T94
FIGURE 3A  CA170_01519.g57 Fab’ binding on MDCK II clone 34 cells in acidic and neutral pH.
FIGURE 3B  CA170_01519.g57 Fab’PEG binding on MDCK II clone 34 cells in acidic and neutral pH.

![Graph showing binding of Fab’PEG to MDCK II clone 34 cells at different pH levels.](image)
FIGURE 4 CA170_01519.957 inhibits IgG recycling in MDCK II clone 34 cells

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<th>EC50 (nM)</th>
<th>95% CI (nM)</th>
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<tr>
<td>1519.g57 Fab' PEG (n=6)</td>
<td>6.034</td>
<td>4.614 to 7.891</td>
</tr>
<tr>
<td>1519.g57 Fab' (n=7)</td>
<td>1.937</td>
<td>1.426 to 2.632</td>
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</table>
FIGURE 5 CA170_01519.g57 Fab'PEG inhibits apical to basolateral IgG transcytosis in MDCK II clone 34 cells

% Inhibition

Antibody log nM

CA170_01519.g57 Fab'PEG (n=3)
FIGURE 6A- CA170_01519.g57 Fab’ binding on cynomolgus MDCK II (cm) cells in acidic and neutral pH

A

Specific Bound Antibody (M)

2.0x10^{-9}

1.5x10^{-9}

1.0x10^{-9}

5.0x10^{-10}

0

1.0x10^{-12} 1.0x10^{-11} 1.0x10^{-10} 1.0x10^{-9} 1.0x10^{-8} 1.0x10^{-7} 1.0x10^{-6}

Total (M)

cyno MDCK neutral
cyno MDCK pH6
FIGURE 6B - CA170_01519,g57 Fab’PEG binding on cynomologus MDCK II (cm) cells in acidic and neutral pH

B

Specific Bound Antibody (M)

1.5x10^-0.9

1.0x10^-0.9

5.0x10^-1.0

0

1.0x10^-12 1.0x10^-11 1.0x10^-10 1.0x10^-9 1.0x10^-8 1.0x10^-7 1.0x10^-6

Total (M)

cyno MDCK neutral
cyno MDCK pH6
FIGURE 7 CA170_01519.g57 inhibits IgG recycling in human and cynomolgus MDCK II clone 34 cells and MDCK II (cm) cells.

% Inhibition

Antibody log nM

CA170_01519.g57 Fab'PEG hFcRn:hlgG (n=2)

CA170_01519.g57 Fab'PEG cFcRn:clgG (n=2)
FIGURE 8  Cynomolgus Monkey- single dose of 1519 Fab’PEG on Plasma IgG levels

- Fab’PEG 1x 20 mg/kg
- Fab’PEG 1x100 mg/kg
- 1xControl
Figure 10
Figure 11    Flow Cytometry based human IgG blocking assay using purified gamma 1 IgG Antibodies

Purified HuFcRn Abs for humanisation - Blocking of 488-IgG binding to HuFcRn (mut) on HEK293 cells

<table>
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<tr>
<th>Concentration (ng/ml)</th>
<th>% positive cells</th>
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- [CA170_01519.0]
- [CA170_01522.0]
- [CA170_01521.0]
FIGURE 12  Fab’PEG single/intermittent IV doses in Normal Cyno (4 animals n: 4-7) -1519 Fab’PEG 20mg/Kg days 1 and 67 IgG pharmacodynamics
FIGURE 13  Fab'PEG: repeat IV doses in normal cyno- 4x 20 or 100 mg/Kg (top and bottom respectively) per week IgG pharmacodynamics (individual animals)

Low [IgG] maintained longer with this regimen
FIGURE 14  Fab'PEG single/intermittent IV doses in normal cyno 20 mg/Kg and 100 mg/Kg days 1 and 67 IgG Pharmacodynamics
Figure 15: Change in plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 20mg/Kg 1519-g57 Fab’PEG
Figure 16  Change in plasma IgG levels in 4 cynomolgus monkeys receiving 10 IV doses of 20mg/Kg 1519.g57 Fab’PEG every 3 days.
Figure 17: Change in plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 30 mg/Kg 1519.g57 IgG4 i.v.
Figure 18
Change in plasma IgG levels in cynomolgus monkeys treated with 30 mg/Kg 15,19,57 IgG4P on day 0 followed by
5mg/Kg 15,19,57 IgG4P daily for 41 days
Figure 19  Change in plasma IgG levels in 4 cynomolgus monkeys receiving 42 daily doses of vehicle

- Cyno 5
- Cyno 6
- Cyno 7
- Cyno 8
- Dose

IgG (% baseline) vs. Time (days post dose)
Figure 20: Increased clearance of IV IgG in plasma of hFeRn transgenic mice treated with CA170-1519,57 Fab/PEG or PBS IV.
Figure 21  Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 IgG1 or IgG4 or PBS IV
Figure 22  Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'-human serum albumin or PBS IV

![Graph showing the clearance of IV hIgG in plasma over time with different treatments.](image)

- **Vehicle control**
- **100mg/kg 1519.g57 Fab'HSA**

% initial hIgG vs. Time (hours)
Figure 23  Increased clearance of IV hlgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 FabFv or PBS IV
Figure 24  Increased clearance of IV IgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab or Fab’PEG or PBS IV
First Variable region of light chain VL1
First Variable region of heavy chain VH1 chain
Constant regions Clappa and CH1
Second variable region of light chain VL2
Second variable region of heavy chain VH2
Disulphide bond
A. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or both national classification and IPC

INV. C07K16/28 G01N33/53

ADD.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>Y</td>
<td>Wo 2009/080764 A2 (ABYLNX N V [BE]; H00GENOB00M HENDRICUS RENERUS JACOBUS MATTHEUS [NL]; D) 2 July 2009 (2009-07-02) the whole document page 86 - page 104</td>
<td>1-29, 32-42</td>
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</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:
  *A* document defining the general state of the art which is not considered to be of particular relevance
  *E* earlier application or patent but published on or after the international filing date
  *L* document which may throw doubts on priority claim(s) on which is cited to establish the publication date of another citation or other special reason (as specified)
  *O* document referring to an oral disclosure, use, exhibition or other means
  *P* document published prior to the international filing date but later than the priority date claimed

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

*P* document member of the same patent family

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

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

Date of the actual completion of the international search

17 December 2013

Date of mailing of the international search report

02/01/2014

Authorized officer

Perez-Mato, Isabel
<table>
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<tr>
<td>Y</td>
<td>CHRISTIANSON GREGORY J ET AL: &quot;Monoclonal anti bodyes directed agaist human FcRn and thei r applicati ons&quot;, MABS, vol. 4, no. 2, March 2012 (2012-03), pages 208-216, XP002700027, ISSN: 1942-0862, the whole document</td>
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<td>WARK K L ET AL: &quot;Latest technologi es for the enhancement of anti body affini ty&quot;, ADVANCED DRUG DELIVERY REVIEWS, ELSEVIER BV, AMSTERDAM, NL, vol. 58, no. 5-6, 7 August 2006 (2006-08-07), pages 657-670, XP024892147, ISSN: 0169-409X, DOI: 10.1016/J.ADDR.2006.01.025 [retrieved on 2006-08-07], the whole document</td>
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<td>Y</td>
<td>W02006/106323 Al (UCB SA [BE]; LAWSON ALASTAIR DAVID GRIFFITH [GB]) 12 October 2006 (2006-10-12) the whole document</td>
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<td>Y</td>
<td>E. P. ALTSHULER ET AL: &quot;Generation of recombinant anti bodyes and means for in creasing thei r affini ty&quot;, BIOCHEMISTRY (MOSCOW), vol. 75, no. 13, 1 December 2010 (2010-12-01), pages 1584-1605, XP055069538, ISSN: 0006-2979, DOI: 10.1134/S0006297910130067, the whole document</td>
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Form PCT/ISA/210 (continuation of second sheet) (April 2005)
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<td>Y</td>
<td>CHOWDHURY P S ET AL: &quot;IMPROVING ANTIBODY AFFINITY BY MIMICKING SOMATIC HYPERMUTATION IN VITRO&quot;, NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 17, 1 June 1999 (1999-06-01), pages 568-572, XP000918985, ISSN: 1087-0156, DOI: 10.1038/9872, the whole document</td>
<td>10-29, 32-42</td>
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### INTERNATIONAL SEARCH REPORT

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

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

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

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

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

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

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

| see additional sheet |

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

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

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

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

   1-29, 32-42

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/21 0 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-29, 32-42

directed to anti-FcRn antibodies having certain sequences (or a certain K\text{D}), nucleic acids encoding these, vectors comprising said nucleic acids, host cells comprising said vectors, methods to produce the antibodies using said cells, pharmaceutical compositions comprising the antibodies and medical uses of said antibodies.

2. claims: 30, 31

directed to an assay for testing the ability of a test molecule such as an antibody to block human FcRn activity and in particular to recycle IgG comprising coating non-human mammalian cells expressing human FcRn alpha chain and human beta2 microglobulin onto a surface, contacting the cells with a test antibody and an IgG to be recycled allowing binding of both the test antibody and the IgG to FcRn, washing with an acidic buffer and detecting the amount of IgG internalised and/or recycled by the cells.
<table>
<thead>
<tr>
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<th>Publication date</th>
<th>Patent family member(s)</th>
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<td>US 2007092507 A1</td>
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