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(57) Abrégé/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.

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# 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.

# IgG Recycling Assays

This application is a division of application 2872326 filed May 13, 2013.

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 α 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 IgG1 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-Barré syndrome; Chronic inflammatory demyelinating polyneuropathy; Goodpasture's syndrome; hyperviscosity syndromes; cryoglobulinemia; paraproteinemia; Waldenström 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 alloimune 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 WO2006/118772.

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**

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

**Figure 24** shows the increased clearance of IV hIgG 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):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). 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 WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22583 and WO05/113605). One such example of the latter is a Tri-Fab (or TFM) as described in WO92/22583.

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 Liss, Inc., 1985).

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 methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93(15):7843-78481; WO92/02551; WO2004/051268 and International Patent Application number WO2004/106377.

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; WO91/09967). 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.

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)WGQGTLVTVS (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 2-1-(1) 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 2-1-(1) 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 WO91/09967.

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 aspargine, 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

- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.,* 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.,* 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.,* 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

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 VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g bispecific or may be monospecific (see for example WO 92/22853, WO05/113605, 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 IgG1 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, WO2011/030107, WO2011/061492 and WO2011/086091.

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 IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. 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 CH1 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:

AESHLSLLYH LTAVSSPAPG TPAFWVSGWL GPQQYLSYNS LRGEAEPCGA WVWENQVSWY WEKETTDLRI KEKLFLEAFK ALGGKGPYTL QGLLGCELG**P** DNTS**VPTAK**F ALNG**EEFMNF DLKQ**GTW**GGD WPE**ALAISQR WQQQDKAANK ELTFLLFSCP HRLREHLERG RGNLEWKEPP SMRLKARPSS PGFSVLTCSA FSFYPPELQL RFLRNGLAAG TGQGDFGPNS DGSFHASSSL TVKSGDEHHY CCIVQHAGLA 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, 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 1nM 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., 62:119-58 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 89/00195, WO 89/01476 and WO 03/031581. 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 anthracin 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, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, 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, $\alpha$-interferon, $\beta$-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin 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 and $^{99}$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, polyalkenylene 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(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, 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(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

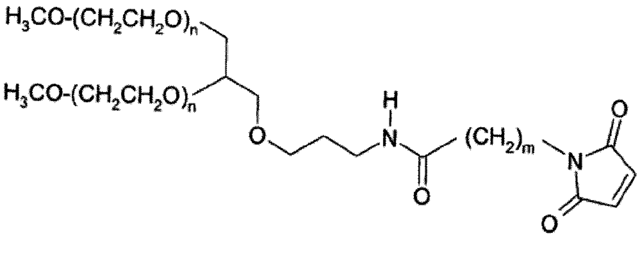
In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located 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 α-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(ethyleneglycol)) covalently attached thereto, *e.g.* according to the method disclosed in EP 0948544 or EP1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) 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-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:

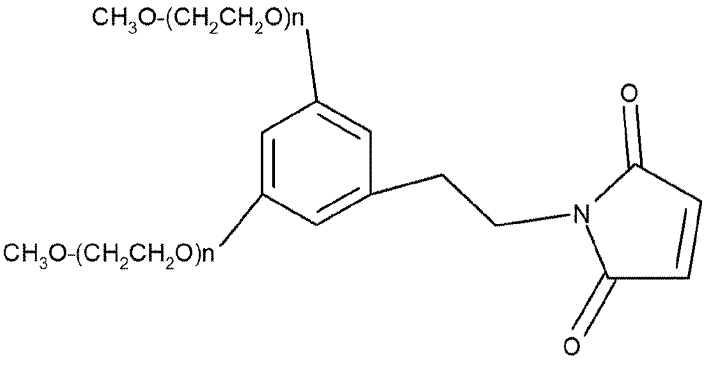


m is 2 or 5

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

Thus in one embodiment the PEG is 2,3-Bis(methylpolyoxyethylene-oxy)-1-{[3-(6-maleimido-1-oxohexyl)amino]propyloxy} hexane (the 2 arm branched PEG, $-CH_2)$ $_3NHCO(CH_2)_5$-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.

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

Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

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

Suitable types of Chinese Hamster Ovary (CHO cells) for use in the present invention may include CHO and CHO-K1 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 CHOK1-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 a 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 purifiying 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-acceptableexcipient.

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, microphenyolate 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, polylactic 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 therapeutic 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.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

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 µm. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellent gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellent 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 propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellent 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 propellent-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/solutionbuffer.

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, Bullous 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 tubulointestitial 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 (type1), 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, Polyarteritis 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, Scleroderma, 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 polyneuropathy (CIDP)
- Guillain-Barre syndrome
- Paraproteinemic polyneuropathies
- 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 cardiomyopathy

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, Guillaume-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 foliaceus; epidermolysis bullosa; dermatitis herpetiformis; Sydenham's chorea; acute motor axonal neuropathy (AMAN); Miller-Fisher syndrome; multifocal motor neuropathy (MMN); opsoclonus; inflammatory myopathy; Isaac's syndrome (autoimmune neuromyotonia), Paraneoplastic syndromes and Limbic encephalitis.

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.

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 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,
d) incubating the cells in a neutral buffer such as about pH 7.2
e) detecting the amount of IgG recycled by the cells by determining the amount of IgG released into the supernatant.

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.

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 cyno 20mg/Kg days 1 and 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 -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 1519.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 1519.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 1519.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 hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'PEG or PBS IV |
| **Figure 21** | shows the increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 IgG1 or IgG4 or PBS IV |
| **Figure 22** | shows the increased clearance of IV hIgG 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 hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 FabFv or PBS IV |
| **Figure 24** | shows the increased clearance of IV hIgG 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. |

## 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 $\beta$2M 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% $\beta$-mercaptoethanol (Gibco BRL), 2-5%

activated rabbit splenocyte culture supernatant and gamma-irradiated EL-4-B5 murine thymoma cells (5x$10^4$/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 Fcγ-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 -80C. 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 100nM 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 ($K_D$) 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 Fcγ 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% $CO_2$. 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% $CO_2$. 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% $CO_2$. 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% $CO_2$ 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_01519 had the best activity and was selected for humanisation.

## Example 1 Humanisation Method

Antibody CA170_01519 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.*

(1991) (Humanised antibodies WO91/09967). 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 2-1-(1) A30 plus JK2 J-region was chosen as the acceptor for the light chain CDRs. Human V-region VH3 1-3 3-07 plus JH4 J-region 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 (I58) 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 (K1m3 allotype) and the human gamma-1 CH1-hinge region (G1m17 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 WO2011/086136) 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 (Km3 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 1B 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': 30mg/ml presented in 0.1M Sodium Phosphate, 2mM EDTA, pH6.0 (reduction buffer)
- 1,6-Bismaleimidohexane (BMH) from Thermofisher (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 $EC_{50}$ values calculated. The $EC_{50}$ 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 1**

| Antibody | Fab' $EC_{50}$ (nM) | (n) | Fab'PEG $EC_{50}$ (nM) | (n) | Fold Change in $EC_{50}$ after pegylation |
|---|---|---|---|---|---|
| CA170_0519.g63 | 1.91 | 3 | 5.25 | 3 | 2.7 |
| CA170_0519.g57 | 2.06 | 7 | 6.64 | 6 | 3.2 |
| CA170_0519.g2 | 4.22 | 2 | 11.01 | 4 | 2.6 |

**Mean $EC_{50}$ 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% $CO_2$. 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% $CO_2$ 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% $CO_2$ 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 $EC_{50}$. 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). Affinipure $F(ab')_2$ fragment goat anti-human IgG, $F(ab')_2$ fragment specific (for Fab'-PEG capture) or Fc fragment specific (for IgG1 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, IgG1 or IgG4P was diluted to 5μg/ml (Fab'-PEG), 0.3μg/ml (IgG1) or 4μg/ml (IgG4) in running buffer. A 60s injection of Fab'-PEG or IgG1 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, IgG1 or IgG4) for 300s at 30μl/min followed by 1200s dissociation. The surface was regenerated by 2 x 60s 50mM HCl 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**

| 1519.g57Fab'-PEG | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) |
|---|---|---|---|
| 1 | 4.37E+05 | 1.59E-05 | 3.63E-11 |
| 2 | 4.20E+05 | 2.01E-05 | 4.78E-11 |
| 3 | 4.35E+05 | 1.43E-05 | 3.29E-11 |
| 4 | 4.37E+05 | 2.75E-05 | 6.30E-11 |
| 5 | 4.33E+05 | 1.28E-05 | 2.97E-11 |
| | **4.32E+05** | **1.81E-05** | **4.19E-11** |

**Table 3 Affinity data for anti-hFcRn 1519.g57 Fab'-PEG at pH7.4**

| 1519.g57Fab'-PEG | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | $K_D$ (M) |
|---|---|---|---|
| 1 | 3.40E+05 | 1.87E-05 | 5.49E-11 |
| 2 | 3.31E+05 | 1.85E-05 | 5.58E-11 |
| 3 | 3.25E+05 | 1.99E-05 | 6.13E-11 |
| 4 | 3.23E+05 | 1.52E-05 | 4.70E-11 |
| 5 | 3.20E+05 | 1.99E-05 | 6.21E-11 |
|  | **3.28E+05** | **1.84E-05** | **5.62E-11** |

In these experiments the Fab'PEG had an average affinity of around 42pM at pH6 and around 56pM at pH7.4.

**pH7.4**

| 1519.g57 | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) | KD (pM) |
|---|---|---|---|---|
| IgG1 | 3.80E+05 | 1.25E-05 | 3.29E-11 | 33 |
| IgG4P | 3.68E+05 | 1.26E-05 | 3.43E-11 | 34 |

**Table 3A Affinity data for anti-hFcRn 1519.g57 as IgG1 and IgG4P at pH7.4 (average of three experiments)**

**pH6**

| 1519.g57 | ka ($M^{-1}s^{-1}$) | kd ($s^{-1}$) | KD (M) | KD (pM) |
|---|---|---|---|---|
| IgG1 | 4.56E+05 | 1.01E-05 | 2.21E-11 | 22 |
| IgG4P | 4.43E+05 | 1.00E-05 | 2.26E-11 | 23 |

**Table 3B Affinity data for anti-hFcRn 1519.g57 as IgG1 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.**

| **Antibody format** | **Human FcRnpH 7.4** | **Human FcRnpH 6.0** |
|---|---|---|
| **1519.g57 Fab'** | 1.66 | 1.59 |
| **1519.g57 Fab'PEG** | 6.5 | 5.42 |

**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 931nM 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% $CO_2$. 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% $CO_2$ 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% $CO_2$ 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 $EC_{50}$. 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 $EC_{50}$ 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% $CO_2$ until monolayers were formed. The pH of the apical compartment was adjusted to 5.9 and the basolateral side to 7.2 in a $HBSS^+$(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 2.5µg/ml biotinylated human IgG (Jackson) at the indicated concentrations for 4 hours at 37°C, 5% $CO_2$. 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 $EC_{50}$. 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 $EC_{50}$ 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 $EC_{50}$ of 6nM achieved in the IgG recycling assay is comparable to the cell affinity binding data in which $K_D$ 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 the lowest drop in potency between the two formats (see *supra*). In the IgG transcytosis assay an $EC_{50}$ 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 (MDCKII (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 931nM 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 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.**

| *Antibody format* | *Cyno FcRnpH 7.4* | *Cyno FcRnpH 6.0* |
|---|---|---|
| *1519.g57 Fab'* | 1.16 | 1.09 |
| *1519.g57 Fab'PEG* | 8.15 | 5.01 |

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)

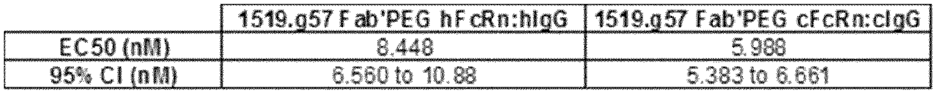
**CA170_01519.g57 Fab'PEG inhibits the recycling of cynomolgus monkey IgG**

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 $EC_{50}$ 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**



| | 1519.g57 Fab'PEG hFcRn:hIgG | 1519.g57 Fab'PEG cFcRn:cIgG |
|---|---|---|
| EC50 (nM) | 8.448 | 5.988 |
| 95% CI (nM) | 6.560 to 10.88 | 5.383 to 6.661 |

**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% $CO_2$. 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% $CO_2$ 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% $CO_2$ 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 $EC_{50}$. 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 01519g Fab PEG in cynomolgus monkeys, in single, intermittent or repeated dosing regimens. 01519g 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 01519g 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** Dose groups in study NCD2241. Dosing was by intravenous infusion. The redose was the same as the first dose in each case. Repeat doses (4 of) were weekly.

| Phase | Group | Antibody | Dose (mg/kg) | Dosing Regimen | Comments |
|---|---|---|---|---|---|
| I | 1 | Control | 0 | Single Dose | Redose at 67 days |
| | 2 | Fab PEG | 20 | Single Dose | Redose at 67 days |
| | 3 | Fab PEG | 100 | Single Dose | Redose at 67 days |
| II | 4 | Control | 0 | Repeat Dose | |
| | 5 | Fab PEG | 20 | Repeat Dose | |
| | 6 | Fab PEG | 100 | Repeat Dose | |

**Table 7A** Plasma IgG, PK and ADA immunoassay methods

| Assay type | Immunoassay | Method |
|---|---|---|
| PD | Total plasma IgG | 1) Coat immunoassay plate with F(ab')$_2$ goat anti-human Fcγ<br>2) Incubate with sample.<br>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.<br>2) Incubate with sample.<br>3) Reveal with biotin conjugated murine IgG1 anti-PEG /.Streptavidin-horseradish peroxidase conjugate & the addition of TMB substrate alternatively reveal with MSD sulfo-tagged goat anti-human kappa & the addition of MSD read buffer |

**Effect on plasma IgG concentration**

Immunoassay 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'PEGand CA170_01519.g57 IgG4P in cynomolgus monkeys

The effects of CA170_01519g.57 Fab'PEG and CA170_01519g.57 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.

| Anti-FcRn | Dose (mg/kg) | Dosing Regimen | Route | Figure |
|---|---|---|---|---|
| Fab'PEG | 20 | Day 0 & 65 | i.v. | 15 |
| Fab'PEG | 20 | Every 3 days, day 0-27 | i.v. | 16 |
| IgG4P | 30 | Day 0 & 63 | i.v. | 17 |
| IgG4P | 30 & 5 | 30mg/kg on day 0, 5mg/kg daily day 1-41 | i.v. | 18 |
| Control | 0 | Daily day 0-41 | i.v. | 19 |

Table 8A Plasma IgG and PK immunoassay methods

| Assay type | Immunoassay | Method |
|---|---|---|
| PD | Total plasma IgG | 1) Coat immunoassay plate with $F(ab')_2$ Goat anti-human Fcγ.<br>2) Incubate with sample.<br>3) Reveal with horseradish peroxidase conjugated $F(ab')_2$, goat anti-human IgG $F(ab')_2$ and the addition of TMB substrate. |
| PK | Fab'PEG PK | 1) Coat MSD streptavidin plate with biotinylated FcRn.<br>2) Incubate with sample.<br>3) Reveal with MSD sulfo-tagged goat anti-human kappa and the addition of MSD read buffer. |

**Effect on plasma IgG concentration.**

Immunoassay 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 IgG1, 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 IgG1, 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 hIgG in hFcRn transgenic mice**

Humanised FcRn transgenic mice (B6.Cg-*Fcgrt*$^{tm1Dcr}$ Tg(FCGRT)32Dcr/DcrJ, JAX Mice) were infused intravenously with 500mg/kg human IgG (Human IgI 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 IgG1, 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 oligsaccharide 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::1.2:1, 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.

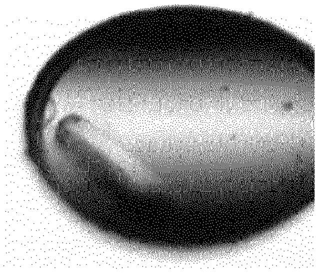
| | | | |
|---|---|---|---|
| Crystallization experiment type | | Sitting drop, vapour diffusion | |
| Crystallization condition | 0.1M Sodium citrate pH 5.5, 11%PEG6000 | | |
| Protein concentration | 10mg/ml | Drop volume/ratio | 0.4ul Protein +<br>0.4ul Reservoir |
| Crystal growth time | 8-21 days | | |
| Cryoprotection | 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. | | |
| Comments | Picture of crystal in drop |  Pictures of crystal frozen in the loop (red square is X-ray beam) | |

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

| | | | |
|---|---|---|---|
| X-ray source | Diamond Light Source, Beamline I04 | | |
| Experiment Type | Single-wavelength | Wavelength | 0.9795Å |
| Processing Software | Mosflm/Scala | | |
| Resolution Limits | 35.00 – 2.90 | Space group | $P3_2\ 2\ 1$ |
| Unit Cell parameters | a = 150.10 Å | b = 150.10 Å | c = 89.15 Å |
| | α = 90.00 ° | β = 90.00 ° | γ = 120.00 ° |
| Completeness | 99.9% (100.0%) | Multiplicity | 6.7 (6.8) |
| $I/\sigma(I)$ | 13.4 (4.8) | $R_{merge}$ | 9.2% (36.3%) |

| | | | |
|---|---|---|---|
| Number of reflections | 172724 (25602) | Number of unique reflections | 25967 (3760) |
| Comments | | | |

*Note: Numbers in parenthesis refer to the outer resolution shell*

Table 11 Structure determination and refinement.

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

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: α-helix 9.4%; β-sheet 45.2%; 3-10 turn 2.5%.

The residues interacting with 1519g57 Fab'were all in the FcRn α 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.

AESHLSLLYH LTAVSSPAPG TPAFWVSGWL GPQQYLSYNS LRGEAEPCGA WVWENQVSWY WEKETTDLRI
KEKLFLEAFK ALGGKGPYTL QGLLGCELG**P** DNTS**VPTAK**F ALNG**EEFMNF DLKQ**GTW**GGD WPE**ALAISQR
WQQQDKAANK ELTFLLFSCP HRLREHLERG RGNLEWKEPP SMRLKARPSS PGFSVLTCSA FSFYPPELQL
RFLRNGLAAG TGQGDFGPNS DGSFHASSSL TVKSGDEHHY CCIVQHAGLA QPLRVELESPAKSS

The FcRn α 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. A method for testing the ability of a test molecule to block 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 with a test molecule and an IgG to be recycled by the cells for a period of time sufficient to allow binding of both the test 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.

2. The method of claim 1, wherein the test molecule is an antibody molecule.

3. The method of claim 1 or 2, wherein the mildly acidic condition of step (b) is about pH5.9.

4. The method of any one of claims 1 to 3, wherein the amount of IgG recycled by the cells is determined by determining the amount of IgG released into the supernatant.

5. The method of claim 4, wherein the cells are incubated in a neutral buffer after step (c) and before step (d).

6. The method of any one of claims 1 to 5 wherein the test molecule is added before the IgG to be recycled and incubated for a period of time sufficient to allow binding of the test molecule to FcRn before addition of the IgG to be recycled.

# FIGURE 1

CA170_1519 Ab sequences

CDRH1
GFTFSNYGMV SEQ ID NO: 1

CDRH2
YIDSDGDNTYYRDSVKG SEQ ID NO: 2

CDRH3
GIVRPFLY 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
DVVMTQTPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1519 VL region SEQ ID NO: 8

gatgttgtga tgacccagac tccactgtct ttgtcggttg cccttggaca
accagcctcc atctcttgca agtcaagtca gagcctcgta ggtgctagtg
gaaagacata tttgtattgg ttatttcaga ggtccggcca gtctccaaag
cgactaatct atctggtgtc cacactggac tctggaattc ctgataggtt
cagtggcagt ggagcagaga cagattttac tcttaaaatc cgcagagtgg
aagccgatga tttgggagtt tattactgct tgcaaggtac acattttcct
cacacgtttg gagctgggac caagctggaa ttgaaa

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

*MMSPAQFLFL LMLWIQGTSG* DVVMTQTPLS LSVALGQPAS ISCKSSQSLV
GASGKTYLYW LFQRSGQSPK RLIYLVSTLD SGIPDRFSGS GAETDFTLKI
RRVEADDLGV YYCLQGTHFP HTFGAGTKLE LK

## FIGURE 1A

Rat Ab 1519 VL region with signal sequence underlined and italicised SEQ ID NO: 10
*atgatgagtc ctgcccagtt cctgtttctg ctgatgctct ggattcaggg*
*aaccagtggt* gatgttgtga tgacccagac tccactgtct ttgtcggttg
cccttggaca accagcctcc atctcttgca agtcaagtca gagcctcgta
ggtgctagtg gaaagacata tttgtattgg ttatttcaga ggtccggcca
gtctccaaag cgactaatct atctggtgtc cacactggac tctggaattc
ctgataggtt cagtggcagt ggagcagaga cagattttac tcttaaaatc
cgcagagtgg aagccgatga tttgggagtt tattactgct tgcaaggtac
acattttcct cacacgtttg gagctgggac caagctggaa ttgaaa

Rat Ab 1519 VH region SEQ ID NO: 11
EVPLVESGGG SVQPGRSMKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGQG TTVTVS

Rat Ab 1519 VH region SEQ ID NO: 12
gaggtgccgc tggtggagtc tgggggcggc tcagtgcagc ctgggaggtc
catgaaactc tcctgtgtag tctcaggatt cactttcagt aattatggca
tggtctgggt ccgccaggct ccaaagaagg gtctggagtg ggtcgcatat
attgattctg atggtgataa tacttactac cgagattccg tgaagggccg
attcactatc tccagaaata atgcaaaaag caccctatat ttgcaaatgg
acagtctgag gtctgaggac acggccactt attactgtac aacagggatt
gtccggccct ttctctattg gggccaagga accacggtca ccgtctcg

Rat Ab 1519 VH region with signal sequence underlined and italicised SEQ ID NO: 13

*MDISLSLAFL VLFIKGVRC*E VPLVESGGGS VQPGRSMKLS CVVSGFTFSN
YGMVWVRQAP KKGLEWVAYI DSDGDNTYYR DSVKGRFTIS RNNAKSTLYL
QMDSLRSEDT ATYYCTTGIV RPFLYWGQGT TVTVS

Rat Ab 1519 VH region with signal sequence underlined and italicised SEQ ID NO: 14

*atggacatca gtctcagctt ggctttcctt gtccttttca taaaaggtgt*
*ccggtgt*gag gtgccgctgg tggagtctgg gggcggctca gtgcagcctg
ggaggtccat gaaactctcc tgtgtagtct caggattcac tttcagtaat
tatggcatgg tctgggtccg ccaggctcca aagaagggtc tggagtgggt
cgcatatatt gattctgatg gtgataatac ttactaccga gattccgtga
agggccgatt cactatctcc agaaataatg caaaaagcac cctatatttg
caaatggaca gtctgaggtc tgaggacacg gccacttatt actgtacaac
agggattgtc cggccctttc tctattgggg ccaaggaacc acggtcaccg
tctcg

## FIGURE 1B

1519 gL20 V-region SEQ ID NO: 15
DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK
RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP
HTFGQGTKLE IK

1519 gL20 V-region (*E. coli* expression) SEQ ID NO: 16
gatatccaga tgacccagag tccaagcagt ctctccgcca gcgtaggcga
tcgtgtgact attacctgta aaagctccca gtccctggtg ggtgcaagcg
gcaaaaccta cctgtactgg ctcttccaga aaccgggcaa agctccgaaa
cgcctgatct atctggtgtc taccctggat agcggtattc cgtctcgttt
ctccggtagc ggtagcggta ccgaattcac gctgaccatt agctccctcc
agccggagga ctttgctacc tattactgcc tccagggcac tcattttccg
cacactttcg gccagggtac caaactggaa atcaaa

1519 gL20 V-region (mammalian expression) SEQ ID NO: 17
gatatccaga tgacccagag cccatctagc ttatccgctt ccgttggtga
tcgcgtgaca attacgtgta agagctccca atctctcgtg ggtgcaagtg
gcaagaccta tctgtactgg ctctttcaga agcctggcaa ggcaccaaaa
cggctgatct atctggtgtc tacccttgac tctgggatac cgtcacgatt
ttccggatct gggagcggaa ctgagttcac actcacgatt tcatcgctgc
aacccgagga ctttgctacc tactactgcc tgcaaggcac tcatttccct
cacactttcg gccaggggac aaaactcgaa atcaaa

1519 gL20 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 18
*MKKTAIAIAV ALAGFATVAQ A*DIQMTQSPS SLSASVGDRV TITCKSSQSL
VGASGKTYLY WLFQKPGKAP KRLIYLVSTL DSGIPSRFSG SGSGTEFTLT
ISSLQPEDFA TYYCLQGTHF PHTFGQGTKL EIK

1519 gL20 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 19
*atgaaaaaga cagctatcgc aattgcagtg gccttggctg gtttcgctac*
*cgtagcgcaa gct*gatatcc agatgaccca gagtccaagc agtctctccg
ccagcgtagg cgatcgtgtg actattacct gtaaaagctc ccagtccctg
gtgggtgcaa gcggcaaaac ctacctgtac tggctcttcc agaaaccggg
caaagctccg aaacgcctga tctatctggt gtctaccctg gatagcggta
ttccgtctcg tttctccggt agcggtagcg gtaccgaatt cacgctgacc
attagctccc tccagccgga ggactttgct acctattact gcctccaggg
cactcatttt ccgcacactt tcggccaggg taccaaactg gaaatcaaa

## FIGURE 1C

1519 gL20 V-region with signal sequence underlined and italicized (mammalian expression)
SEQ ID NO: 20
*MSVPTQVLGL LLLWLTDARC* DIQMTQSPSS LSASVGDRVT ITCKSSQSLV
GASGKTYLYW LFQKPGKAPK RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI
SSLQPEDFAT YYCLQGTHFP HTFGQGTKLE IK

1519 gL20 V-region with signal sequence underlined and italicized (mammalian expression)
SEQ ID NO: 21
*atgtctgtcc ccacccaagt cctcggactc ctgctactct ggcttacaga*
*tgccagatgc* gatatccaga tgacccagag cccatctagc ttatccgctt
ccgttggtga tcgcgtgaca attacgtgta agagctccca atctctcgtg
ggtgcaagtg gcaagaccta tctgtactgg ctctttcaga agcctggcaa
ggcaccaaaa cggctgatct atctggtgtc taccttgac tctgggatac
cgtcacgatt ttccggatct gggagcggaa ctgagttcac actcacgatt
tcatcgctgc aacccgagga ctttgctacc tactactgcc tgcaaggcac
tcatttccct cacactttcg gccaggggac aaaactcgaa atcaaa

1519 gL20 light chain (V + constant) SEQ ID NO: 22
DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK
RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP
HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGEC

1519 gL20 light chain (V + constant, *E. coli* expression) SEQ ID NO: 23
gatatccaga tgacccagag tccaagcagt ctctccgcca gcgtaggcga
tcgtgtgact attacctgta aaagctccca gtccctggtg ggtgcaagcg
gcaaaaccta cctgtactgg ctcttccaga aaccgggcaa agctccgaaa
cgcctgatct atctggtgtc taccctggat agcggtattc cgtctcgttt
ctccggtagc ggtagcggta ccgaattcac gctgaccatt agctccctcc
agccggagga ctttgctacc tattactgcc tccagggcac tcattttccg
cacactttcg gccagggtac caaactggaa atcaaacgta cggtagcggc
cccatctgtc ttcatcttcc cgccatctga tgagcagttg aaatctggaa
ctgcctctgt tgtgtgcctg ctgaataact tctatcccag agaggccaaa
gtacagtgga aggtggataa cgccctccaa tcgggtaact cccaggagag
tgtcacagag caggacagca aggacagcac ctacagcctc agcagcaccc
tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa
gtcacccatc agggcctgag ctcaccagta acaaaaagtt ttaatagagg ggagtgt

## FIGURE 1D

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

gatatccaga tgacccagag tccaagcagt ctctccgcca gcgtaggcga
tcgtgtgact attacctgta aaagctccca gtccctggtg ggtgcaagcg
gcaaaaccta cctgtactgg ctcttccaga aaccgggcaa agctccgaaa
cgcctgatct atctggtgtc taccctggat agcggtattc cgtctcgttt
ctccggtagc ggtagcggta ccgaattcac gctgaccatt agctccctcc
agccggagga ctttgctacc tattactgcc tccagggcac tcattttccg
cacactttcg gccagggtac caaactggaa atcaaacgta cggtagcggc
cccatctgtc ttcatcttcc cgccatctga tgagcagttg aaatctggaa
ctgcctctgt tgtgtgcctg ctgaataact tctatcccag agaggccaaa
gtacagtgga aggtggataa cgccctccaa tcgggtaact cccaggagag
tgtcacagag caggacagca aggacagcac ctacagcctc agcagcaccc
tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa
gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgt

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

*MKKTAIAIAV ALAGFATVAQ A*DIQMTQSPS SLSASVGDRV TITCKSSQSL
VGASGKTYLY WLFQKPGKAP KRLIYLVSTL DSGIPSRFSG SGSGTEFTLT
ISSLQPEDFA TYYCLQGTHF PHTFGQGTKL EIKRTVAAPS VFIFPPSDEQ
LKSGTASVVC LLNNFYPREA KVQWKVDNAL QSGNSQESVT EQDSKDSTYS
LSSTLTLSKA DYEKHKVYAC EVTHQGLSSP VTKSFNRGEC

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

*atgaaaaaga cagctatcgc aattgcagtg gccttggctg gtttcgctac*
*cgtagcgcaa gct*gatatcc agatgaccca gagtccaagc agtctctccg
ccagcgtagg cgatcgtgtg actattacct gtaaaagctc ccagtccctg
gtgggtgcaa gcggcaaaac ctacctgtac tggctcttcc agaaaccggg
caaagctccg aaacgcctga tctatctggt gtctaccctg gatagcggta
ttccgtctcg tttctccggt agcggtagcg gtaccgaatt cacgctgacc
attagctccc tccagccgga ggactttgct acctattact gcctccaggg
cactcatttt ccgcacactt tcggccaggg taccaaactg gaaatcaaac
gtacggtagc ggccccatct gtcttcatct tcccgccatc tgatgagcag
ttgaaatctg gaactgcctc tgttgtgtgc ctgctgaata acttctatcc
cagagaggcc aaagtacagt ggaaggtgga taacgccctc caatcgggta
actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc
ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaagt
ctacgcctgc gaagtcaccc atcagggcct gagctcacca gtaacaaaaa
gttttaatag aggggagtgt

## FIGURE 1E

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

*MSVPTQVLGL LLLWLTDARC* DIQMTQSPSS LSASVGDRVT ITCKSSQSLV
GASGKTYLYW LFQKPGKAPK RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI
SSLQPEDFAT YYCLQGTHFP HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL
KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

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

*atgtctgtcc ccacccaagt cctcggactc ctgctactct ggcttacaga*
*tgccagatgc* gatatccaga tgacccagag cccatctagc ttatccgctt
ccgttggtga tcgcgtgaca attacgtgta agagctccca atctctcgtg
ggtgcaagtg gcaagaccta tctgtactgg ctctttcaga agcctggcaa
ggcaccaaaa cggctgatct atctggtgtc taccettgac tctgggatac
cgtcacgatt ttccggatct gggagcggaa ctgagttcac actcacgatt
tcatcgctgc aacccgagga ctttgctacc tactactgcc tgcaaggcac
tcatttccct cacactttcg gccaggggac aaaactcgaa atcaaacgta
cggtagcggc cccatctgtc ttcatcttcc cgccatctga tgagcagttg
aaatctggaa ctgcctctgt tgtgtgcctg ctgaataact tctatcccag
agaggccaaa gtacagtgga aggtggataa cgccctccaa tcgggtaact
cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc
agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta
cgcctgcgaa gtcacccatc agggcctgag ctcgcccgtc acaaagagct
tcaacagggg agagtgt

1519 gH20 V-region SEQ ID NO: 29

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI
VRPFLYWGQG TLVTVS

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

gaggttccgc tggtcgagtc tggaggcggg cttgtccagc ctggagggag
cctgcgtctc tcttgtgcag tatctggctt cacgttctcc aactacggta
tggtgtgggt tcgtcaggct ccaggtaaag gtctggaatg ggtggcgtat
attgactccg acggcgacaa cacctactat cgcgactctg tgaaaggtcg
cttcaccatt tcccgcgata acgccaaatc cagcctgtac ctgcagatga
acagcctgcg tgctgaagat actgcggtgt actattgcac cactggcatc
gtgcgtccgt ttctgtattg gggtcagggt accctcgtta ctgtctcg

**FIGURE 1F** (signal sequences underlined and italicized)

1519 gH20 V-region (mammalian expression) SEQ ID NO: 31
gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcg

1519 gH20 V-region (*E. coli* expression) SEQ ID NO: 32
*MKKTAIAIAV ALAGFATVAQ A*EVPLVESGG GLVQPGGSLR LSCAVSGFTF
SNYGMVWVRQ APGKGLEWVA YIDSDGDNTY YRDSVKGRFT ISRDNAKSSL
YLQMNSLRAE DTAVYYCTTG IVRPFLYWGQ GTLVTVS

1519 gH20 V-region (*E. coli* expression) SEQ ID NO: 33
*atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac*
*cgtggcgcaa gct*gaggttc cgctggtcga gtctggaggc gggcttgtcc
agcctggagg gagcctgcgt ctctcttgtg cagtatctgg cttcacgttc
tccaactacg gtatggtgtg ggttcgtcag gctccaggta aaggtctgga
atgggtggcg tatattgact ccgacggcga caacacctac tatcgcgact
ctgtgaaagg tcgcttcacc atttcccgcg ataacgccaa atccagcctg
tacctgcaga tgaacagcct gcgtgctgaa gatactgcgg tgtactattg
caccactggc atcgtgcgtc cgtttctgta ttggggtcag ggtaccctcg
ttactgtctc g

1519 gH20 V-region (mammalian expression) SEQ ID NO: 34
*MEWSWVFLFF LSVTTGVHS*E VPLVESGGGL VQPGGSLRLS CAVSGFTFSN
YGMVWVRQAP GKGLEWVAYI DSDGDNTYYR DSVKGRFTIS RDNAKSSLYL
QMNSLRAEDT AVYYCTTGIV RPFLYWGQGT LVTVS

1519 gH20 V-region with signal sequence underlined and italicized (mammalian expression)
SEQ ID NO: 35
*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg tctcg

## FIGURE 1G

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge) SEQ ID NO: 36

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI
VRPFLYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF
PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC
NVNHKPSNTK VDKKVEPKSC DKTHTCAA

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge, *E.coli* expression) SEQ ID NO: 37

gaggttccgc tggtcgagtc tggaggcggg cttgtccagc ctggagggag
cctgcgtctc tcttgtgcag tatctggctt cacgttctcc aactacggta
tggtgtgggt tcgtcaggct ccaggtaaag gtctggaatg ggtggcgtat
attgactccg acggcgacaa cacctactat cgcgactctg tgaaaggtcg
cttcaccatt tcccgcgata acgccaaatc cagcctgtac ctgcagatga
acagcctgcg tgctgaagat actgcggtgt actattgcac cactggcatc
gtgcgtccgt ttctgtattg gggtcagggt accctcgtta ctgtctcgag
cgcttctaca aagggcccat cggtcttccc cctggcaccc tcctccaaga
gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc aggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtcgacaaga aagttgagcc
caaatcttgt gacaaaactc acacatgcgc cgcg

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

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag
cgcttctaca aagggcccat cggtcttccc cctggcaccc tcctccaaga
gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc aggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtcgacaaga aagttgagcc
caaatcttgt gacaaaactc acacatgcgc cgcg

## FIGURE 1H

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

*MKKTAIAIAV ALAGFATVAQ A*EVPLVESGG GLVQPGGSLR LSCAVSGFTF
SNYGMVWVRQ APGKGLEWVA YIDSDGDNTY YRDSVKGRFT ISRDNAKSSL
YLQMNSLRAE DTAVYYCTTG IVRPFLYWGQ GTLVTVSSAS TKGPSVFPLA
PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCAA

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

*atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac*
*cgtggcgcaa gct*gaggttc cgctggtcga gtctggaggc gggcttgtcc
agcctggagg gagcctgcgt ctctcttgtg cagtatctgg cttcacgttc
tccaactacg gtatggtgtg ggttcgtcag gctccaggta aaggtctgga
atgggtggcg tatattgact ccgacggcga caacacctac tatcgcgact
ctgtgaaagg tcgcttcacc atttcccgcg ataacgccaa atccagcctg
tacctgcaga tgaacagcct gcgtgctgaa gatactgcgg tgtactattg
caccactggc atcgtgcgtc cgtttctgta ttggggtcag ggtaccctcg
ttactgtctc gagcgcttct acaaagggcc catcggtctt ccccctggca
ccctcctcca agagcacctc tgggggcaca gcggccctgg gctgcctggt
caaggactac ttccccgaac cggtgacggt gtcgtggaac tcaggcgccc
tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc ctcaggactc
tactccctca gcagcgtggt gaccgtgccc tccagcagct tgggcaccca
gacctacatc tgcaacgtga atcacaagcc cagcaacacc aaggtcgaca
agaaagttga gcccaaatct tgtgacaaaa ctcacacatg cgccgcg

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

*MEWSWVFLFF LSVTTGVHS*E VPLVESGGGL VQPGGSLRLS CAVSGFTFSN
YGMVWVRQAP GKGLEWVAYI DSDGDNTYYR DSVKGRFTIS RDNAKSSLYL
QMNSLRAEDT AVYYCTTGIV RPFLYWGQGT LVTVSSASTK GPSVFPLAPS
SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCAA

# FIGURE 1I

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

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg
tctcgagcgc ttctacaaag ggcccatcgg tcttcccct ggcaccctcc
tccaagagca cctctggggg cacagcggcc ctgggctgcc tggtcaagga
ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc gccctgacca
gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta
catctgcaac gtgaatcaca agcccagcaa caccaaggtc gacaagaaag
ttgagcccaa atcttgtgac aaaactcaca catgcgccgc g

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

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI
VRPFLYWGQG TLVTVSSAST KGPSVFPLAP CSRSTSESTA ALGCLVKDYF
PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTKTYTC
NVDHKPSNTK VDKRVESKYG PPCPPCPAPE FLGGPSVFLF PPKPKDTLMI
SRTPEVTCVV VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV
SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP
SQEEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NHYTQKSLSL SLGK

## FIGURE 1J

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

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag
cgcttctaca aagggcccat ccgtcttccc cctggcgccc tgctccagga
gcacctccga gagcacagcc gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc aggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacgaagac ctacacctgc
aacgtagatc acaagcccag caacaccaag gtggacaaga gagttggtga
gaggccagca cagggaggga gggtgtctgc tggaagccag gctcagccct
cctgcctgga cgcaccccgg ctgtgcagcc ccagcccagg gcagcaaggc
atgccccatc tgtctcctca cccggaggcc tctgaccacc ccactcatgc
ccagggagag ggtcttctgg atttttccac caggctccgg gcagccacag
gctggatgcc cctaccccag gccctgcgca tacaggggca ggtgctgcgc
tcagacctgc caagagccat atccgggagg accctgcccc tgacctaagc
ccaccccaaa ggccaaactc tccactccct cagctcagac accttctctc
ctcccagatc tgagtaactc ccaatcttct ctctgcagag tccaaatatg
gtcccccatg cccaccatgc ccaggtaagc caacccaggc ctcgccctcc
agctcaaggc gggacaggtg ccctagagta gcctgcatcc agggacaggc
cccagccggg tgctgacgca tccacctcca tctcttcctc agcacctgag
ttcctggggg gaccatcagt cttcctgttc cccccaaaac ccaaggacac
tctcatgatc tcccggaccc ctgaggtcac gtgcgtggtg gtggacgtga
gccaggaaga ccccgaggtc cagttcaact ggtacgtgga tggcgtggag
gtgcataatg ccaagacaaa gccgcgggag gagcagttca acagcacgta
ccgtgtggtc agcgtcctca ccgtcctgca ccaggactgg ctgaacggca
aggagtacaa gtgcaaggtc tccaacaaag gcctcccgtc ctccatcgag
aaaaccatct ccaaagccaa aggtgggacc cacggggtgc gagggccaca
tggacagagg tcagctcggc ccaccctctg ccctgggagt gaccgctgtg
ccaacctctg tccctacagg gcagccccga gagccacagg tgtacaccct
gcccccatcc caggaggaga tgaccaagaa ccaggtcagc ctgacctgcc
tggtcaaagg cttctacccc agcgacatcg ccgtggagtg ggagagcaat
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga
cggctccttc ttcctctaca gcaggctaac cgtggacaag agcaggtggc
aggaggggaa tgtcttctca tgctccgtga tgcatgaggc tctgcacaac
cactacacac agaagagcct ctccctgtct ctgggtaaa

# FIGURE 1K

1519gH20 IgG4 heavy chain (V + human gamma-4P constant) with signal sequence underlined and italicised SEQ ID NO: 45

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg
tctcgagcgc ttctacaaag ggcccatccg tcttccccct ggcgccctgc
tccaggagca cctccgagag cacagccgcc ctgggctgcc tggtcaagga
ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc gccctgacca
gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cgaagaccta
cacctgcaac gtagatcaca agcccagcaa caccaaggtg gacaagagag
ttggtgagag gccagcacag ggagggaggg tgtctgctgg aagccaggct
cagccctcct gcctggacgc accccggctg tgcagcccca gcccagggca
gcaaggcatg ccccatctgt ctcctcaccc ggaggcctct gaccacccca
ctcatgccca gggagagggt cttctggatt tttccaccag gctccgggca
gccacaggct ggatgcccct accccaggcc ctgcgcatac aggggcaggt
gctgcgctca gacctgccaa gagccatatc cgggaggacc ctgcccctga
cctaagccca ccccaaaggc caaactctcc actccctcag ctcagacacc
ttctctcctc ccagatctga gtaactccca atcttctctc tgcagagtcc
aaatatggtc ccccatgccc accatgccca ggtaagccaa cccaggcctc
gccctccagc tcaaggcggg acaggtgccc tagagtagcc tgcatccagg
gacaggcccc agccgggtgc tgacgcatcc acctccatct cttcctcagc
acctgagttc ctggggggac catcagtctt cctgttcccc ccaaaaccca
aggacactct catgatctcc cggacccctg aggtcacgtg cgtggtggtg
gacgtgagcc aggaagaccc cgaggtccag ttcaactggt acgtggatgg
cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagttcaaca
gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg
aacggcaagg agtacaagtg caaggtctcc aacaaaggcc tcccgtcctc
catcgagaaa accatctcca aagccaaagg tgggacccac ggggtgcgag
ggccacatgg acagaggtca gctcggccca ccctctgccc tgggagtgac
cgctgtgcca acctctgtcc ctacagggca gccccgagag ccacaggtgt
acaccctgcc cccatcccag gaggagatga ccaagaacca ggtcagcctg
acctgcctgg tcaaaggctt ctaccccagc gacatcgccg tggagtggga
gagcaatggg cagccggaga acaactacaa gaccacgcct cccgtgctgg
actccgacgg ctccttcttc ctctacagca ggctaaccgt ggacaagagc
aggtggcagg aggggaatgt cttctcatgc tccgtgatgc atgaggctct
gcacaaccac tacacacaga agagcctctc cctgtctctg ggtaaa

# FIGURE 1L

1519gL20 FabFv light chain SEQ ID NO: 46
DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK
RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP
HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGECS GGGGSGGGGS GGGGSDIQMT QSPSSVSASV
GDRVTITCQS SPSVWSNFLS WYQQKPGKAP KLLIYEASKL TSGVPSRFSG
SGSGTDFTLT ISSLQPEDFA TYYCGGGYSS ISDTTFGCGT KVEIKRT

1519gL20 FabFv light chain SEQ ID NO: 47
gatatccaga tgacccagag cccatctagc ttatccgctt ccgttggtga
tcgcgtgaca attacgtgta agagctccca atctctcgtg ggtgcaagtg
gcaagaccta tctgtactgg ctctttcaga agcctggcaa ggcaccaaaa
cggctgatct atctggtgtc tacccttgac tctgggatac cgtcacgatt
ttccggatct gggagcggaa ctgagttcac actcacgatt tcatcgctgc
aacccgagga ctttgctacc tactactgcc tgcaaggcac tcatttccct
cacactttcg gccaggggac aaaactcgaa atcaaacgta cggtagcggc
cccatctgtc ttcatcttcc cgccatctga tgagcagttg aaatctggaa
ctgcctctgt tgtgtgcctg ctgaataact tctatcccag agaggccaaa
gtacagtgga aggtggataa cgccctccaa tcgggtaact cccaggagag
tgtcacagag caggacagca aggacagcac ctacagcctg agcagcaccc
tgacgctgtc taaagcagac tacgagaaac acaaagtgta cgcctgcgaa
gtcacccatc agggcctgag ctcaccagta acaaaaagtt ttaatagagg
ggagtgtagc ggtggcggtg gcagtggtgg gggaggctcc ggaggtggcg
gttcagacat acaaatgacc cagagtcctt catcggtatc cgcgtccgtt
ggcgataggg tgactattac atgtcaaagc tctcctagcg tctggagcaa
ttttctatcc tggtatcaac agaaaccggg gaaggctcca aaacttctga
tttatgaagc ctcgaaactc accagtggag ttccgtcaag attcagtggc
tctggatcag ggacagactt cacgttgaca atcagttcgc tgcaaccaga
ggactttgcg acctactatt gtggtggagg ttacagtagc ataagtgata
cgacatttgg gtgcggtact aaggtggaaa tcaaacgtac c

1519gL20 FabFv light chain with signal sequence underlined & italicised SEQ ID NO: 48
*MSVPTQVLGL LLLWLTDARC* DIQMTQSPSS LSASVGDRVT ITCKSSQSLV
GASGKTYLYW LFQKPGKAPK RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI
SSLQPEDFAT YYCLQGTHFP HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL
KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGECS GGGGSGGGGS
GGGGSDIQMT QSPSSVSASV GDRVTITCQS SPSVWSNFLS WYQQKPGKAP
KLLIYEASKL TSGVPSRFSG SGSGTDFTLT ISSLQPEDFA TYYCGGGYSS
ISDTTFGCGT KVEIKRT

# FIGURE 1M

1519gL20 FabFv light chain with signal sequence underlined and italicised SEQ ID NO: 49

*atgtctgtcc ccacccaagt cctcggactc ctgctactct ggcttacaga*
*tgccagatgc* gatatccaga tgacccagag cccatctagc ttatccgctt
ccgttggtga tcgcgtgaca attacgtgta agagctccca atctctcgtg
ggtgcaagtg gcaagaccta tctgtactgg ctctttcaga agcctggcaa
ggcaccaaaa cggctgatct atctggtgtc taccettgac tctgggatac
cgtcacgatt ttccggatct gggagcggaa ctgagttcac actcacgatt
tcatcgctgc aacccgagga ctttgctacc tactactgcc tgcaaggcac
tcatttccct cacactttcg gccaggggac aaaactcgaa atcaaacgta
cggtagcggc cccatctgtc ttcatcttcc cgccatctga tgagcagttg
aaatctggaa ctgcctctgt tgtgtgcctg ctgaataact tctatcccag
agaggccaaa gtacagtgga aggtggataa cgccctccaa tcgggtaact
cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctg
agcagcaccc tgacgctgtc taaagcagac tacgagaaac acaaagtgta
cgcctgcgaa gtcacccatc agggcctgag ctcaccagta acaaaaagtt
ttaatagagg ggagtgtagc ggtggcggtg gcagtggtgg gggaggctcc
ggaggtggcg gttcagacat acaaatgacc cagagtcctt catcggtatc
cgcgtccgtt ggcgataggg tgactattac atgtcaaagc tctcctagcg
tctggagcaa ttttctatcc tggtatcaac agaaaccggg gaaggctcca
aaacttctga tttatgaagc ctcgaaactc accagtggag ttccgtcaag
attcagtggc tctggatcag ggacagactt cacgttgaca atcagttcgc
tgcaaccaga ggactttgcg acctactatt gtggtggagg ttacagtagc
ataagtgata cgacatttgg gtgcggtact aaggtggaaa tcaaacgtac
c

1519gH20 FabFv heavy chain SEQ ID NO: 50

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI
VRPFLYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF
PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC
NVNHKPSNTK VDKKVEPKSC SGGGGSGGGG TGGGGSEVQL LESGGGLVQP
GGSLRLSCAV SGIDLSNYAI NWVRQAPGKC LEWIGIIWAS GTTFYATWAK
GRFTISRDNS KNTVYLQMNS LRAEDTAVYY CARTVPGYST APYFDLWGQG TLVTVSS

## FIGURE 1N

1519gH20 FabFv heavy chain SEQ ID NO: 51

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag
cgcgtccaca aagggcccat cggtcttccc cctggcaccc tcctccaaga
gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc
cccgaaccag tgacggtgtc gtggaactca ggtgccctga ccagcggcgt
tcacaccttc ccggctgtcc tacagtcttc aggactctac tccctgagca
gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtcgataaga aagttgagcc
caaatcttgt agtggaggtg gggctcagg tggaggcggg accggtggag
gtggcagcga ggttcaactg cttgagtctg gaggaggcct agtccagcct
ggagggagcc tgcgtctctc ttgtgcagta agcggcatcg acctgagcaa
ttacgccatc aactgggtga gacaagctcc ggggaagtgt ttagaatgga
tcggtataat atgggccagt gggacgacct tttatgctac atgggcgaaa
ggaaggttta caattagccg ggacaatagc aaaaacaccg tgtatctcca
aatgaactcc ttgcgagcag aggacacggc ggtgtactat tgtgctcgca
ctgtcccagg ttatagcact gcaccctact tcgatctgtg gggacaaggg
accctggtga ctgtttcaag t

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

*MEWSWVFLFF LSVTTGVHS*E VPLVESGGGL VQPGGSLRLS CAVSGFTFSN
YGMVWVRQAP GKGLEWVAYI DSDGDNTYYR DSVKGRFTIS RDNAKSSLYL
QMNSLRAEDT AVYYCTTGIV RPFLYWGQGT LVTVSSASTK GPSVFPLAPS
SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCS GGGGSGGGGT
GGGGSEVQLL ESGGGLVQPG GSLRLSCAVS GIDLSNYAIN WVRQAPGKCL
EWIGIIWASG TTFYATWAKG RFTISRDNSK NTVYLQMNSL RAEDTAVYYC
ARTVPGYSTA PYFDLWGQGT LVTVSS

## FIGURE 1P

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

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg
tctcgagcgc gtccacaaag ggcccatcgg tcttccccct ggcaccctcc
tccaagagca cctctggggg cacagcggcc ctgggctgcc tggtcaagga
ctacttcccc gaaccagtga cggtgtcgtg gaactcaggt gccctgacca
gcggcgttca caccttcccg gctgtcctac agtcttcagg actctactcc
ctgagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta
catctgcaac gtgaatcaca agcccagcaa caccaaggtc gataagaaag
ttgagcccaa atcttgtagt ggaggtgggg gctcaggtgg aggcgggacc
ggtggaggtg gcagcgaggt tcaactgctt gagtctggag gaggcctagt
ccagcctgga gggagcctgc gtctctcttg tgcagtaagc ggcatcgacc
tgagcaatta cgccatcaac tgggtgagac aagctccggg gaagtgttta
gaatggatcg gtataatatg ggccagtggg acgacctttt atgctacatg
ggcgaaagga aggtttacaa ttagccggga caatagcaaa aacaccgtgt
atctccaaat gaactccttg cgagcagagg acacggcggt gtactattgt
gctcgcactg tcccaggtta tagcactgca ccctacttcg atctgtgggg
acaagggacc ctggtgactg tttcaagt

Human VK1 2-1-(1) A30 JK2 acceptor framework SEQ ID NO: 54
DIQMTQSPSS LSASVGDRVT ITCRASQGIR NDLGWYQQKP GKAPKRLIYA
ASSLQSGVPS RFSGSGSGTE FTLTISSLQP EDFATYYCLQ HNSYPYTFGQ GTKLEIK

Human VK1 2-1-(1) A30 JK2 acceptor framework SEQ ID NO: 55
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga
cagagtcacc atcacttgcc gggcaagtca gggcattaga aatgatttag
gctggtatca gcagaaacca gggaaagccc ctaagcgcct gatctatgct
gcatccagtt tgcaaagtgg ggtcccatca aggttcagcg gcagtggatc
tgggacagaa ttcactctca caatcagcag cctgcagcct gaagattttg
caacttatta ctgtctacag cataatagtt acccttacac ttttggccag
gggaccaagc tggagatcaa a

# FIGURE 1Q

Human VH3 1-3 3-07 JH4 acceptor framework SEQ ID NO: 56

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMSWVRQA PGKGLEWVAN
IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARYF
DYWGQGTLVT VS

Human VH3 1-3 3-07 JH4 acceptor framework SEQ ID NO: 57

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctggggggtc
cctgagactc tcctgtgcag cctctggatt cacctttagt agctattgga
tgagctgggt ccgccaggct ccagggaagg ggctggagtg ggtggccaac
ataaagcaag atggaagtga gaaatactat gtggactctg tgaagggccg
attcaccatc tccagagaca acgccaagaa ctcactgtat ctgcaaatga
acagcctgag agccgaggac acggctgtgt attactgtgc gagatacttt
gactactggg gccagggaac cctggtcacc gtctcc

Rat Ab 1548 VL region SEQ ID NO: 58

DVVMTQTPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE IK

Rat Ab 1548 VL region SEQ ID NO: 59

gatgttgtga tgacccagac tccactgtct ttgtcggttg cccttggaca
accagcctcc atctcttgca agtcaagtca gagcctcgta ggtgctagtg
gaaagacata tttgtattgg ttatttcaga ggtccggcca gtctccaaag
cgactaatct atctggtgtc cacactggac tctggaattc ctgataggtt
cagtggcagt ggagcagaga cagattttac tcttaaaatc cgcagagtgg
aagccgatga tttgggagtt tattactgct tgcaaggtac acattttcct
cacacgtttg gagctgggac caagctggaa ataaaa

Rat Ab 1548 VH region SEQ ID NO: 60

EVPLVESGGG SVQPGRSMKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGQG VMVTVS

## FIGURE 1R

Rat Ab 1548 VH region SEQ ID NO: 61

gaggtgccgc tggtggagtc tgggggcggc tcagtgcagc ctgggaggtc
catgaaactc tcctgtgtag tctcaggatt cactttcagt aattatggca
tggtctgggt ccgccaggct ccaaagaagg gtctggagtg ggtcgcatat
attgattctg atggtgataa tacttactac cgagattccg tgaagggccg
attcactatc tccagaaata atgcaaaaag caccctatat ttgcaaatgg
acagtctgag gtctgaggac acggccactt attactgtac aacagggatt
gtccggccct ttctctattg gggccaagga gtcatggtca cagtctcg

Rat Ab 1644 VL region SEQ ID NO: 62

DVVMTQTPLS LSVAIGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1644 VL region SEQ ID NO: 63

gatgttgtga tgacccagac tccactgtct ttgtcggttg ccattggaca
accagcctcc atctcttgca agtcaagtca gagcctcgta ggtgctagtg
gaaagacata tttgtattgg ttatttcaga ggtccggcca gtctccaaag
cgactaatct atctggtgtc cacactggac tctggaattc ctgataggtt
cagtggcagt ggagcagaga cagattttac tcttaaaatc cgcagagtgg
aagccgatga tttgggagtt tattactgct tgcaaggtac acattttcct
cacacgtttg gagctgggac caagctggaa ctgaaa

Rat Ab 1644 VH region SEQ ID NO: 64

EVPLVESGGG SVQPGRSTKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IGSDGDNIYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGQG TTVTVS

Rat Ab 1644 VH region SEQ ID NO: 65

gaggtgccgc tggtggagtc tgggggcggc tcagtgcagc ctgggaggtc
cacgaaactc tcctgtgtag tctcaggatt cactttcagt aactatggca
tggtctgggt ccgccaggct ccaaagaagg gtctggagtg ggtcgcatat
attggttctg atggtgataa tatttactac cgagattccg tgaagggtcg
attcactatc tccagaaata atgcaaaaag caccctatat ttgcaaatgg
acagtctgag gtctgaggac acggccactt attactgtac aacagggatt
gtccggccct ttctctactg gggccaagga accacggtca ccgtctcg

## Figure 1S

Rat Ab 1496 VK region SEQ ID NO: 66

DVVMTQTPLS LSVALGQPAS ISCKSSQSLV GASGKTYLYW LFQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTKLE LK

Rat Ab 1496 VK region SEQ ID NO: 67

gatgttgtga tgacccagac tccactgtct ttgtcggttg cccttggaca
accagcctcc atctcttgca agtcaagtca gagcctcgta ggtgctagtg
gaaagacata tttgtattgg ttatttcaga ggtccggcca gtctccaaag
cgactaatct atctggtgtc cacactggac tctggaattc ctgataggtt
cagtggcagt ggagcagaga cagattttac tcttaaaatc cgcagagtgg
aagccgatga tttgggagtt tattactgct tgcaaggtac acattttcct
cacacgtttg gagctgggac caagctggaa ctgaaa

Rat Ab 1496 VH region SEQ ID NO: 68

EVLLVESGGG SVQPGRSMKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGQG TMVTVS

Rat Ab 1496 VH region SEQ ID NO: 69

gaggtgctgc tggtggagtc tgggggcggc tcagtgcagc ctgggaggtc
catgaaactc tcctgtgtag tctcaggatt cactttcagt aattatggca
tggtctgggt ccgccaggct ccaaagaagg gtctggagtg ggtcgcatat
attgattctg atggtgataa tacttactac cgagattccg tgaagggccg
attcactatc tccagaaata atgcaaaaag caccctatat ttgcaaatgg
acagtctgag gtctgaggac acggccactt attactgtac aacagggatt
gtccggccct ttctctattg gggccaagga accatggtca ccgtctcg

1519gH20 IgG1 heavy chain (V + human gamma-1 constant) SEQ ID NO:72

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY IDSDGDNTYY
RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI VRPFLYWGQG TLVTVSSAST
KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY
SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSPGK

## Figure 1T

1519gH20 IgG1 heavy chain (V + human gamma-1 constant, exons underlined) SEQ ID NO:73

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag tttacgtctc
tcttgtgctg tgtctggctt caccttctcc aattacggaa tggtctgggt cagacaagca
cctggaaagg gtcttgaatg ggtggcctat attgactctg acggggacaa cacctactat
cgggattccg tgaaaggacg cttcacaatc tcccgagata acgccaagag ctcactgtac
ctgcagatga atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag cgcttctaca
aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtcgacaaga aagttggtga gaggccagca
cagggaggga gggtgtctgc tggaagccag gctcagcgct cctgcctgga cgcatcccgg
ctatgcagcc ccagtccagg gcagcaaggc aggccccgtc tgcctcttca cccggaggcc
tctgcccgcc ccactcatgc tcagggagag ggtcttctgg ctttttcccc aggctctggg
caggcacagg ctaggtgccc ctaacccagg ccctgcacac aaaggggcag gtgctgggct
cagacctgcc aagagccata tccgggagga ccctgcccct gacctaagcc caccccaaag
gccaaactct ccactccctc agctcggaca ccttctctcc tcccagatct gagtaactcc
caatcttctc tctgcagagc ccaaatcttg tgacaaaact cacacatgcc caccgtgccc
aggtaagcca gcccaggcct cgccctccag ctcaaggcgg gacaggtgcc ctagagtagc
ctgcatccag ggacaggccc cagccgggtg ctgacacgtc cacctccatc tcttcctcag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc
tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc
ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc aagacaaagc
cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc
aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag gtgggacccg tggggtgcga gggccacatg
gacagaggcc ggctcggccc accctctgcc ctgagagtga ccgctgtacc aacctctgtc
cctacagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggatgagctg
accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg
gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag
aagagcctct ccctgtctcc gggtaaa

## Figure 1U

1519gH20 IgG1 heavy chain (V + human gamma-1 constant) with signal sequence underlined and italicized SEQ ID NO:74

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt ccattct*gag
gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg gaggaagttt acgtctctct
tgtgctgtgt ctggcttcac cttctccaat tacggaatgg tctgggtcag acaagcacct
ggaaagggtc ttgaatgggt ggcctatatt gactctgacg gggacaacac ctactatcgg
gattccgtga aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac gggaatcgtt
aggccttttc tgtactgggg acagggcacc ttggttactg tctcgagcgc ttctacaaag
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc
ctgggctgcc tggtcaagga ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc
gccctgacca gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta catctgcaac
gtgaatcaca agcccagcaa caccaaggtc gacaagaaag ttggtgagag gccagcacag
ggagggaggg tgtctgctgg aagccaggct cagcgctcct gcctggacgc atcccggcta
tgcagcccca gtccagggca gcaaggcagg ccccgtctgc ctcttcaccc ggaggcctct
gcccgcccca ctcatgctca gggagagggt cttctggctt tttccccagg ctctgggcag
gcacaggcta ggtgccccta acccaggccc tgcacacaaa ggggcaggtg ctgggctcag
acctgccaag agccatatcc gggaggaccc tgcccctgac ctaagcccac cccaaaggcc
aaactctcca ctccctcagc tcggacacct tctctcctcc cagatctgag taactcccaa
tcttctctct gcagagccca aatcttgtga caaaactcac acatgcccac cgtgcccagg
taagccagcc caggcctcgc cctccagctc aaggcgggac aggtgcccta gagtagcctg
catccaggga caggccccag ccgggtgctg acacgtccac ctccatctct tcctcagcac
ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaacccaag gacaccctca
tgatctcccg gacccctgag gtcacatgcg tggtggtgga cgtgagccac gaagaccctg
aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag acaaagccgc
gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg
actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagccccca
tcgagaaaac catctccaaa gccaaaggtg ggacccgtgg ggtgcgaggg ccacatggac
agaggccggc tcggcccacc ctctgccctg agagtgaccg ctgtaccaac ctctgtccct
acagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc
aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg
gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac
tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag
gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag
agcctctccc tgtctccggg taaa

## Figure 1V

1519 gL20 light chain (V + constant, mammalian expression alternative) SEQ ID NO: 75
gatatccaga tgacccagag cccatctagc ttatccgctt ccgttggtga
tcgcgtgaca attacgtgta agagctccca atctctcgtg ggtgcaagtg
gcaagaccta tctgtactgg ctctttcaga agcctggcaa ggcaccaaaa
cggctgatct atctggtgtc tacccttgac tctgggatac cgtcacgatt
ttccggatct gggagcggaa ctgagttcac actcacgatt tcatcgctgc
aacccgagga ctttgctacc tactactgcc tgcaaggcac tcatttccct
cacactttcg gccaggggac aaaactcgaa atcaaacgta cggtagcggc
cccatctgtc ttcatcttcc cgccatctga tgagcagttg aaatctggaa
ctgcctctgt tgtgtgcctg ctgaataact tctatcccag agaggccaaa
gtacagtgga aggtggataa cgccctccaa tcgggtaact cccaggagag
tgtcacagag caggacagca aggacagcac ctacagcctc agcagcaccc
tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa
gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgt

1519gH20 Fab' heavy chain (V + human gamma-1 CH1 + hinge, mammalian expression one base change from SEQ ID NO: 38) SEQ ID NO: 76
gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg ggacagggc accttggtta ctgtctcgag
cgcttctaca aagggcccat cggtcttccc cctggcaccc tcctccaaga
gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc aggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc
caaatcttgt gacaaaactc acacatgcgc cgcg

1519 gH20 Fab' heavy chain with signal sequence underlined and italicized (mammalian expression one base changed from SEQ ID NO: 42) SEQ ID NO: 77
*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg
tctcgagcgc ttctacaaag ggcccatcgg tcttccccct ggcaccctcc
tccaagagca cctctggggg cacagcggcc ctgggctgcc tggtcaagga
ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc gccctgacca
gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta
catctgcaac gtgaatcaca agcccagcaa caccaaggtg gacaagaaag
ttgagcccaa atcttgtgac aaaactcaca catgcgccgc g

## Figure 1W

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

```
DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK
RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP
HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
VTHQGLSSPV TKSFNRGECG GGGSGGGGSG GGGSDIQMTQ SPSSVSASVG
DRVTITCQSS PSVWSNFLSW YQQKPGKAPK LLIYEASKLT SGVPSRFSGS
GSGTDFTLTI SSLQPEDFAT YYCGGGYSSI SDTTFGCGTK VEIKRT
```

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

```
gacatccaga tgacccagtc cccctccagc ctgtccgcct ccgtgggcga
cagagtgacc atcacatgca agtcctccca gtccctggtc ggagcctccg
gcaagaccta cctgtactgg ctgttccaga agcccggcaa ggcccccaag
cggctgatct acctggtgtc taccctggac tccggcatcc cctcccggtt
ctccggctct ggctctggca ccgagttcac cctgaccatc tccagcctgc
agcccgagga cttcgccacc tactactgtc tgcaaggcac ccacttcccc
cacaccttcg gccagggcac caagctggaa atcaagcgga ccgtagcggc
cccatctgtc ttcatcttcc cgccatctga tgagcagttg aaatctggaa
ctgcctctgt tgtgtgcctg ctgaataact tctatcccag agaggccaaa
gtacagtgga aggtggataa cgccctccaa tcgggtaact cccaggagag
tgtcacagag caggacagca aggacagcac ctacagcctc agcagcaccc
tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa
gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg
agagtgtggt ggaggtggct ctggcggtgg tggctccgga ggcggaggaa
gcgacatcca gatgacccag agcccttcct ctgtaagcgc cagtgtcgga
gacagagtga ctattacctg ccaaagctcc ccttcagtct ggtccaattt
tctatcctgg tatcagcaaa agcccggaaa ggctcctaaa ttgctgatct
acgaagcaag caaactcacc agcggcgtgc ccagcaggtt cagcggcagt
gggtctggaa ctgactttac cctgacaatc tcctcactcc agcccgagga
cttcgccacc tattactgcg gtggaggtta cagtagcata agtgatacga
catttggatg cggcactaaa gtggaaatca agcgtacc
```

# FIGURE 1X

1519gH20 FabFv heavy chain (alternative sequence to SEQ ID NO: 51) SEQ ID NO: 80
gaggtgcccc tggtggaatc tggcggcgga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg tgtccggctt caccttctcc aactacggca
tggtctgggt ccgacaggct cctggcaagg gactggaatg ggtggcctac
atcgactccg acggcgacaa cacctactac cgggactccg tgaagggccg
gttcaccatc tcccgggaca acgccaagtc ctccctgtac ctgcagatga
actccctgcg ggccgaggac accgccgtgt actactgcac caccggcatc
gtgcggccct ttctgtactg gggccagggc accctggtca ccgtgtcctc
tgcttctaca aagggcccat cggtcttccc cctggcaccc tcctccaaga
gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc tggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc
caaatcttgt tccggaggtg gcggttccgg aggtggcggt acaggtggcg
gtgggtccga agtccagctg cttgaatccg gaggcggact cgtgcagccc
ggaggcagtc ttcgcttgtc ctgcgctgta tctggaatcg acctgagcaa
ttacgccatc aactgggtga gacaggcacc tgggaaatgc ctcgaatgga
tcggcattat atgggctagt gggacgacct tttatgctac atgggcgaag
ggtagattca caatctcacg ggataatagt aagaacacag tgtacctgca
gatgaactcc ctgcgagcag aggataccgc cgtttactat tgtgctcgca
ctgtcccagg ttatagcact gcaccctact ttgatctgtg gggcagggc
actctggtca ccgtctcgtc c

**Figure 1Y** (signal sequences underlined and italicised)

Rat Ab 1548 VL region (alternative sequence to SEQ ID NO: 58) SEQ ID NO: 81
DVVMTQTPLS LSVAIGQPAS ISSKSSQSLV GAGGKTYLYW LLQRSGQSPK
RLIYLVSTLD SGIPDRFSGS GAETDFTLKI RRVEADDLGV YYCLQGTHFP
HTFGAGTNLE IK

Rat Ab 1548 VL region (alternative sequence to SEQ ID NO: 59) SEQ ID NO: 82
gatgttgtga tgacccagac tccactgtct ttgtcggttg ccattggaca
accagcctcc atctcttcta agtcaagtca gagcctcgta ggtgctggtg
gaaagacata tttgtattgg ttattacaga ggtccggcca gtctccaaag
cgactaatct atctggtgtc cacactggac tctggaattc ctgataggtt
cagtggcagt ggagcagaga cagattttac tcttaaaatc cgcagagtgg
aagccgatga tttgggagtt tattactgct tgcaaggtac acattttcct
cacacgtttg gagctgggac caacctggaa ataaaa

Rat Ab 1548 VH region (alternative sequence to SEQ ID NO: 60) SEQ ID NO: 83
EVPLVESGGG SVQPGRSMKL SCVVSGFTFS NYGMVWVRQA PKKGLEWVAY
IGSDGDNTYY RDSVKGRFTI SRNNAKSTLY LQMDSLRSED TATYYCTTGI
VRPFLYWGQG VMVTVS

Rat Ab 1548 VH region (alternative sequence to SEQ IS NO: 61) SEQ ID NO: 84
gaggtgccgc tggtggagtc tgggggcggc tcagtgcagc ctgggaggtc
catgaaactc tcctgtgtag tctcaggatt cactttcagt aactatggca
tggtctgggt ccgccaggct ccaaagaagg gtctggagtg ggtcgcatat
attggttctg atggtgataa tacttactac cgagattccg tgaagggccg
attcactatc tccagaaata atgcaaaaag caccctatat ttgcaaatgg
acagtctgag gtctgaggac acggccactt attactgtac aacagggatt
gtccggccct ttctctactg gggccaagga gtcatggtca cagtctcg

## 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 ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag tttacgtctc
tcttgtgctg tgtctggctt caccttctcc aattacggaa tggtctgggt cagacaagca
cctggaaagg gtcttgaatg ggtggcctat attgactctg acggggacaa cacctactat
cgggattccg tgaaaggacg cttcacaatc tcccgagata acgccaagag ctcactgtac
ctgcagatga atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag cgcttctaca
aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttggtga gaggccagca
cagggaggga gggtgtctgc tggaagccag gctcagcgct cctgcctgga cgcatcccgg
ctatgcagcc ccagtccagg gcagcaaggc aggccccgtc tgcctcttca cccggaggcc
tctgcccgcc ccactcatgc tcagggagag ggtcttctgg ctttttcccc aggctctggg
caggcacagg ctaggtgccc ctaacccagg ccctgcacac aaaggggcag gtgctgggct
cagacctgcc aagagccata tccgggagga ccctgcccct gacctaagcc caccccaaag
gccaaactct ccactccctc agctcggaca ccttctctcc tcccagatct gagtaactcc
caatcttctc tctgcagagc ccaaatcttg tgacaaaact cacacatgcc caccgtgccc
aggtaagcca gcccaggcct cgccctccag ctcaaggcgg gacaggtgcc ctagagtagc
ctgcatccag ggacaggccc cagccgggtg ctgacacgtc cacctccatc tcttcctcag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc
tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc
ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc aagacaaagc
cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc
aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag gtgggacccg tggggtgcga gggccacatg
gacagaggcc ggctcggccc accctctgcc ctgagagtga ccgctgtacc aacctctgtc
cctacagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggatgagctg
accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg
gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag
aagagcctct ccctgtctcc gggtaaa

## Figure 1AA

1519gH20 IgG1 heavy chain (V + human gamma-1 constant) with signal sequence underlined and italicized (one base change from SEQ ID NO:72) SEQ ID NO:86

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt ccattct*gag
gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg gaggaagttt acgtctctct
tgtgctgtgt ctggcttcac cttctccaat tacggaatgg tctgggtcag acaagcacct
ggaaagggtc ttgaatgggt ggcctatatt gactctgacg gggacaacac ctactatcgg
gattccgtga aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac gggaatcgtt
aggccttttc tgtactgggg acagggcacc ttggttactg tctcgagcgc ttctacaaag
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc
ctgggctgcc tggtcaagga ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc
gccctgacca gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta catctgcaac
gtgaatcaca agcccagcaa caccaaggtg gacaagaaag ttggtgagag gccagcacag
ggagggaggg tgtctgctgg aagccaggct cagcgctcct gcctggacgc atcccggcta
tgcagcccca gtccagggca gcaaggcagg ccccgtctgc ctcttcaccc ggaggcctct
gcccgcccca ctcatgctca gggagagggt cttctggctt tttccccagg ctctgggcag
gcacaggcta ggtgccccta acccaggccc tgcacacaaa ggggcaggtg ctgggctcag
acctgccaag agccatatcc gggaggaccc tgcccctgac ctaagcccac cccaaaggcc
aaactctcca ctccctcagc tcggacacct tctctcctcc cagatctgag taactcccaa
tcttctctct gcagagccca aatcttgtga caaaactcac acatgcccac cgtgcccagg
taagccagcc caggcctcgc cctccagctc aaggcgggac aggtgcccta gagtagcctg
catccaggga caggccccag ccgggtgctg acacgtccac ctccatctct tcctcagcac
ctgaactcct ggggggaccg tcagtcttcc tcttcccccc aaaacccaag gacaccctca
tgatctcccg gacccctgag gtcacatgcg tggtggtgga cgtgagccac gaagaccctg
aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag acaaagccgc
gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccagg
actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc ccagccccca
tcgagaaaac catctccaaa gccaaaggtg ggacccgtgg ggtgcgaggg ccacatggac
agaggccggc tcggcccacc ctctgccctg agagtgaccg ctgtaccaac ctctgtccct
acagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc
aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg
gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac
tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag
gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag
agcctctccc tgtctccggg taaa

1519gH20 IgG4 heavy chain (V + human gamma-4 constant no P mutations) SEQ ID NO:87

EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY
IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI
VRPFLYWGQG TLVTVSSAST KGPSVFPLAP CSRSTSESTA ALGCLVKDYF
PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTKTYTC
NVDHKPSNTK VDKRVESKYG PPCPSCPAPE FLGGPSVFLF PPKPKDTLMI
SRTPEVTCVV VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV
SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP
SQEEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NHYTQKSLSL SLGK

## Figure 1BB

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

gaggtaccac ttgtggaaag cggaggaggt cttgtgcagc ctggaggaag
tttacgtctc tcttgtgctg tgtctggctt caccttctcc aattacggaa
tggtctgggt cagacaagca cctggaaagg gtcttgaatg ggtggcctat
attgactctg acggggacaa cacctactat cgggattccg tgaaaggacg
cttcacaatc tcccgagata acgccaagag ctcactgtac ctgcagatga
atagcctgag agccgaggat actgccgtgt actattgcac aacgggaatc
gttaggcctt ttctgtactg gggacagggc accttggtta ctgtctcgag
cgcttctaca aagggcccat ccgtcttccc cctggcgccc tgctccagga
gcacctccga gagcacagcc gccctgggct gcctggtcaa ggactacttc
cccgaaccgg tgacggtgtc gtggaactca ggcgccctga ccagcggcgt
gcacaccttc ccggctgtcc tacagtcctc aggactctac tccctcagca
gcgtggtgac cgtgccctcc agcagcttgg gcacgaagac ctacacctgc
aacgtagatc acaagcccag caacaccaag gtggacaaga gagttggtga
gaggccagca cagggaggga gggtgtctgc tggaagccag gctcagccct
cctgcctgga cgcaccccgg ctgtgcagcc ccagcccagg gcagcaaggc
atgccccatc tgtctcctca cccggaggcc tctgaccacc ccactcatgc
ccagggagag ggtcttctgg atttttccac caggctccgg gcagccacag
gctggatgcc cctaccccag gccctgcgca tacaggggca ggtgctgcgc
tcagacctgc caagagccat atccgggagg accctgcccc tgacctaagc
ccaccccaaa ggccaaactc tccactccct cagctcagac accttctctc
ctcccagatc tgagtaactc ccaatcttct ctctgcagag tccaaatatg
gtcccccatg cccatcatgc ccaggtaagc caacccaggc ctcgccctcc
agctcaaggc gggacaggtg ccctagagta gcctgcatcc agggacaggc
cccagccggg tgctgacgca tccacctcca tctcttcctc agcacctgag
ttcctggggg gaccatcagt cttcctgttc cccccaaaac ccaaggacac
tctcatgatc tcccggaccc ctgaggtcac gtgcgtggtg gtggacgtga
gccaggaaga ccccgaggtc cagttcaact ggtacgtgga tggcgtggag
gtgcataatg ccaagacaaa gccgcgggag gagcagttca acagcacgta
ccgtgtggtc agcgtcctca ccgtcctgca ccaggactgg ctgaacggca
aggagtacaa gtgcaaggtc tccaacaaag gcctcccgtc ctccatcgag
aaaaccatct ccaaagccaa aggtgggacc cacggggtgc gagggccaca
tggacagagg tcagctcggc ccaccctctg ccctgggagt gaccgctgtg
ccaacctctg tccctacagg gcagccccga gagccacagg tgtacaccct
gcccccatcc caggaggaga tgaccaagaa ccaggtcagc ctgacctgcc
tggtcaaagg cttctacccc agcgacatcg ccgtggagtg ggagagcaat
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga
cggctccttc ttcctctaca gcaggctaac cgtggacaag agcaggtggc
aggaggggaa tgtcttctca tgctccgtga tgcatgaggc tctgcacaac
cactacacac agaagagcct ctccctgtct ctgggtaaa

## Figure 1CC

1519gH20 IgG4 heavy chain (V + human gamma-4 constant) with signal sequence underlined and italicised– no P mutation SEQ ID NO:89

*atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggagt*
*ccattct*gag gtaccacttg tggaaagcgg aggaggtctt gtgcagcctg
gaggaagttt acgtctctct tgtgctgtgt ctggcttcac cttctccaat
tacggaatgg tctgggtcag acaagcacct ggaaagggtc ttgaatgggt
ggcctatatt gactctgacg gggacaacac ctactatcgg gattccgtga
aaggacgctt cacaatctcc cgagataacg ccaagagctc actgtacctg
cagatgaata gcctgagagc cgaggatact gccgtgtact attgcacaac
gggaatcgtt aggccttttc tgtactgggg acagggcacc ttggttactg
tctcgagcgc ttctacaaag ggcccatccg tcttccccct ggcgccctgc
tccaggagca cctccgagag cacagccgcc ctgggctgcc tggtcaagga
ctacttcccc gaaccggtga cggtgtcgtg gaactcaggc gccctgacca
gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cgaagaccta
cacctgcaac gtagatcaca agcccagcaa caccaaggtg gacaagagag
ttggtgagag gccagcacag ggagggaggg tgtctgctgg aagccaggct
cagccctcct gcctggacgc accccggctg tgcagcccca gcccagggca
gcaaggcatg ccccatctgt ctcctcaccc ggaggcctct gaccacccca
ctcatgccca gggagagggt cttctggatt tttccaccag gctccgggca
gccacaggct ggatgcccct accccaggcc ctgcgcatac aggggcaggt
gctgcgctca gacctgccaa gagccatatc cgggaggacc ctgcccctga
cctaagccca ccccaaaggc caaactctcc actccctcag ctcagacacc
ttctctcctc ccagatctga gtaactccca atcttctctc tgcagagtcc
aaatatggtc ccccatgccc atcatgccca ggtaagccaa cccaggcctc
gccctccagc tcaaggcggg acaggtgccc tagagtagcc tgcatccagg
gacaggcccc agccgggtgc tgacgcatcc acctccatct cttcctcagc
acctgagttc ctggggggac catcagtctt cctgttcccc ccaaaaccca
aggacactct catgatctcc cggacccctg aggtcacgtg cgtggtggtg
gacgtgagcc aggaagaccc cgaggtccag ttcaactggt acgtggatgg
cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagttcaaca
gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg
aacggcaagg agtacaagtg caaggtctcc aacaaaggcc tcccgtcctc
catcgagaaa accatctcca aagccaaagg tgggacccac ggggtgcgag
ggccacatgg acagaggtca gctcggccca ccctctgccc tgggagtgac
cgctgtgcca acctctgtcc ctacagggca gccccgagag ccacaggtgt
acaccctgcc cccatcccag gaggagatga ccaagaacca ggtcagcctg
acctgcctgg tcaaaggctt ctaccccagc gacatcgccg tggagtggga
gagcaatggg cagccggaga acaactacaa gaccacgcct cccgtgctgg
actccgacgg ctccttcttc ctctacagca ggctaaccgt ggacaagagc
aggtggcagg aggggaatgt cttctcatgc tccgtgatgc atgaggctct
gcacaaccac tacacacaga agagcctctc cctgtctctg ggtaaa

## Figure 1DD

1519 gL20 V-region (mammalian expression alternative to SEQ ID NO: 17) SEQ ID NO:90

gacatccaga tgacccagtc cccctccagc ctgtccgcct ccgtgggcga
cagagtgacc atcacatgca agtcctccca gtccctggtc ggagcctccg
gcaagaccta cctgtactgg ctgttccaga agcccggcaa ggcccccaag
cggctgatct acctggtgtc taccctggac tccggcatcc cctcccggtt
ctccggctct ggctctggca ccgagttcac cctgaccatc tccagcctgc
agcccgagga cttcgccacc tactactgtc tgcaaggcac ccacttcccc
cacaccttcg gccagggcac caagctggaa atcaag

1519 gL20 light chain (V + constant, mammalian expression alternative to SEQ ID NO: 24) SEQ ID NO:91

gacatccaga tgacccagtc cccctccagc ctgtccgcct ccgtgggcga
cagagtgacc atcacatgca agtcctccca gtccctggtc ggagcctccg
gcaagaccta cctgtactgg ctgttccaga agcccggcaa ggcccccaag
cggctgatct acctggtgtc taccctggac tccggcatcc cctcccggtt
ctccggctct ggctctggca ccgagttcac cctgaccatc tccagcctgc
agcccgagga cttcgccacc tactactgtc tgcaaggcac ccacttcccc
cacaccttcg gccagggcac caagctggaa atcaagcgga ccgtggccgc
tccctccgtg ttcatcttcc caccctccga cgagcagctg aagtccggca
ccgcctccgt cgtgtgcctg ctgaacaact tctacccccg cgaggccaag
gtgcagtgga aggtggacaa cgccctgcag tccggcaact cccaggaatc
cgtcaccgag caggactcca aggacagcac ctactccctg tcctccaccc
tgaccctgtc caaggccgac tacgagaagc acaaggtgta cgcctgcgaa
gtgacccacc agggcctgtc cagccccgtg accaagtcct tcaaccgggg
cgagtgc

## Figure 1EE

1519 gH20 V-region (mammalian expression alternative to SEQ ID NO: 31) SEQ ID NO:92
gaggtgcccc tggtggaatc tggcggcgga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg tgtccggctt caccttctcc aactacggca
tggtctgggt ccgacaggct cctggcaagg gactggaatg ggtggcctac
atcgactccg acggcgacaa cacctactac cgggactccg tgaagggccg
gttcaccatc tcccgggaca acgccaagtc ctccctgtac ctgcagatga
actccctgcg ggccgaggac accgccgtgt actactgcac caccggcatc
gtgcggccct ttctgtactg gggccagggc accctggtca ccgtgtcc

## Figure 1FF

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

```
gaggtgcccc tggtggaatc tggcggcgga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg tgtccggctt caccttctcc aactacggca
tggtctgggt ccgacaggct cctggcaagg gactggaatg ggtggcctac
atcgactccg acggcgacaa cacctactac cgggactccg tgaagggccg
gttcaccatc tcccgggaca acgccaagtc ctccctgtac ctgcagatga
actccctgcg ggccgaggac accgccgtgt actactgcac caccggcatc
gtgcggccct ttctgtactg gggccagggc accctggtca ccgtgtcctc
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gggcttctac ccctccgaca ttgccgtgga atgggagtcc aacggccagc
ccgagaacaa ctacaagacc acccccccctg tgctggacag cgacggctcc
ttcttcctgt actctcggct gaccgtggac aagtcccggt ggcaggaagg
caacgtcttc tcctgctccg tgatgcacga ggccctgcac aaccactaca
cccagaagtc cctgtccctg agcctgggca ag
```

Human β2M (SEQ ID NO:95)

IQKTPQIQVYSRHPPENGKPNFLNCYVSQFHPPQIEIELLKNGKKIPNIEMSDLSFSKDWSFYILAHTEFTPTETDVYA
CRVKHVTLKEPKTVTWDRDM

# 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              DVVMTQTPLSLSVALGQPASISCKSSQSLVGASGKTYLYWLFQRSGQSPKRLIYLVSTLDSGIPDRFSGSGAETDFTLKIRRVEADDLGVYYCLQGTHFPHTFGAGTKLELK
                         ||   | |   ||| |||| | ||  |||||||||| | || || ||      || | |  | |      || |   | |||||| |||     |||| |   |     |
VK1 2-1-(1) A30         DIQMTQSPSSLSASVGDRVTITCRASQGIRN-----DLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPYTFGQGTKLEIK
                         |                                      ||                    |        ||
1519 gL20               DIQMTQSPSSLSASVGDRVTITCKSSQSLVGASGKTYLYWLFQKPGKAPKRLIYLVSTLDSGIPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQGTHFPHTFGQGTKLEIK
```

**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

```
                 1   5   10   15   20   25   30   35   40   45   50  a 55   60   65   70   75   80  abc 85   90   95  100  105  110
Heavy 1519       EVPLVESGGGSVQPGRSMKLSCVVSGFTFSNYGMVWVRQAPKKGLEWVAYIDSDGDNTYYRDSVKGRFTISRNNAKSTLYLQMDSLRSEDTATYYCTTGIVRPFLYWGQGTTVTVS
                   |       |    | ||   ||      | | |      |       | ||  |||  |           |   ||     |   |    |   ||||||| |   |
VH3 1-3 3-07     EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR----YFDYWGQGTLVTVS
                   |                    |                                                    |                   ||
1519gH20         EVPLVESGGGLVQPGGSLRLSCAVSGFTFSNYGMVWVRQAPGKGLEWVAYIDSDGDNTYYRDSVKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCTTGIVRPFLYWGQGTLVTVS
```

## 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.

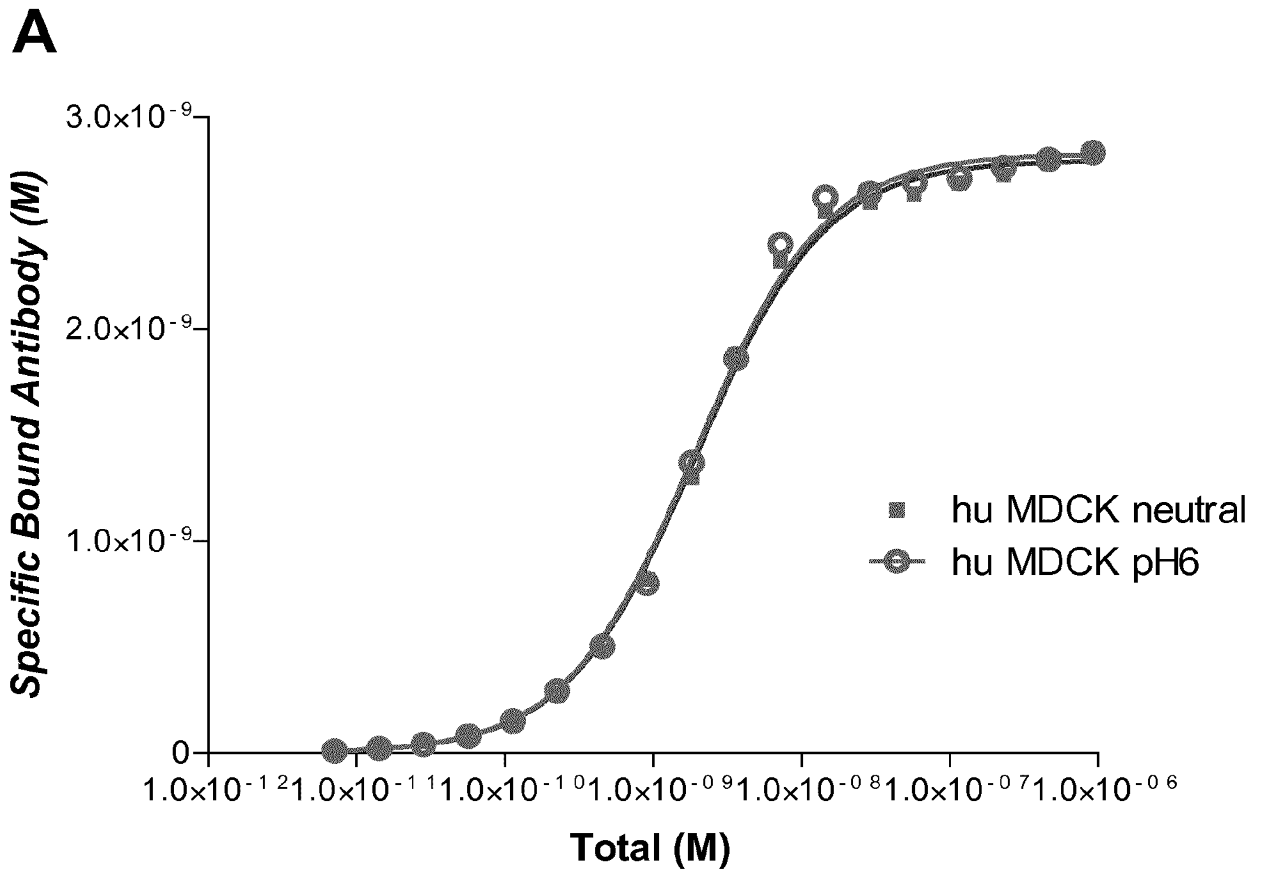


FIGURE 3B CA170_01519.g57 Fab'PEG binding on MDCK II clone 34 cells in acidic and neutral pH.

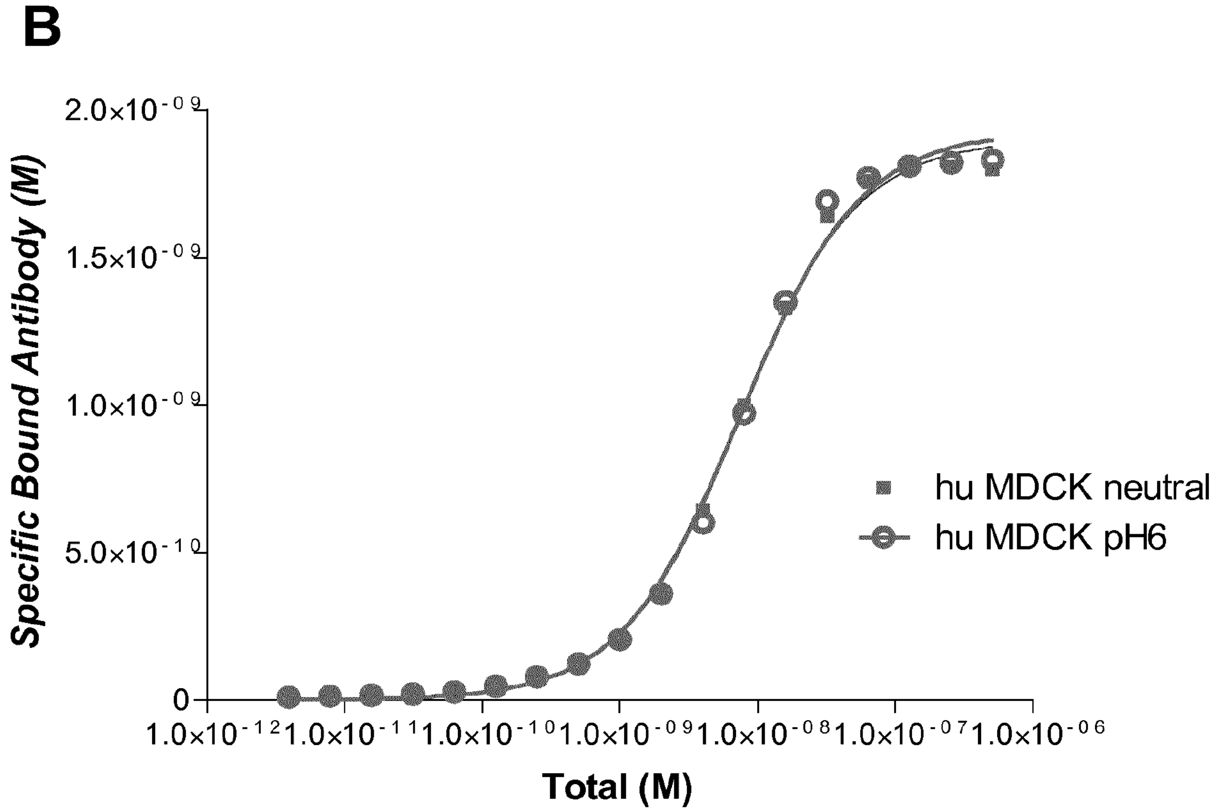
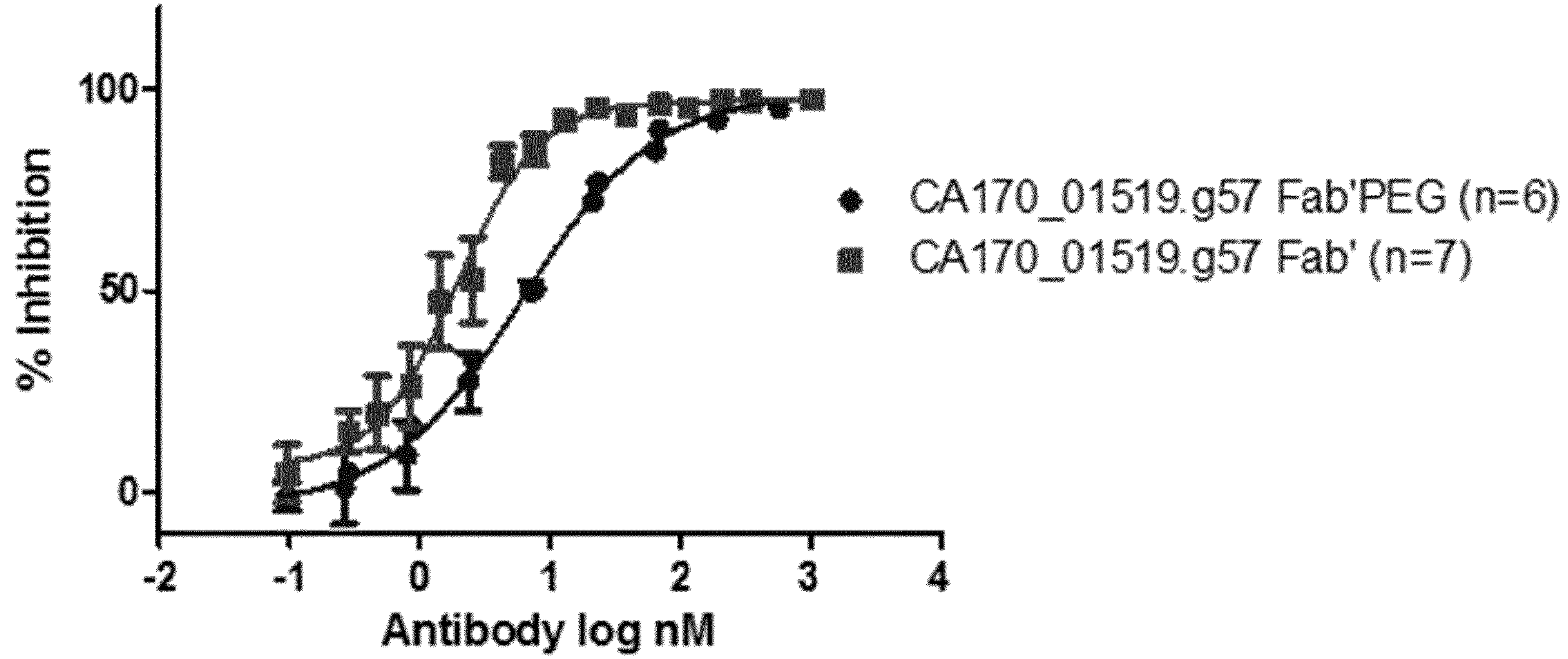


FIGURE 4 CA170_01519.g57 inhibits IgG recycling in MDCK II clone 34 cells



| | 1519.g57 Fab'PEG (n=6) | 1519.g57 Fab' (n=7) |
|---|---|---|
| EC50 | 6.034 | 1.937 |
| 95% CI (nM) | 4.614 to 7.891 | 1.426 to 2.632 |

FIGURE 5 CA170_01519.g57 Fab'PEG inhibits apical to basolateral IgG transcytosis in MDCK II clone 34 cells

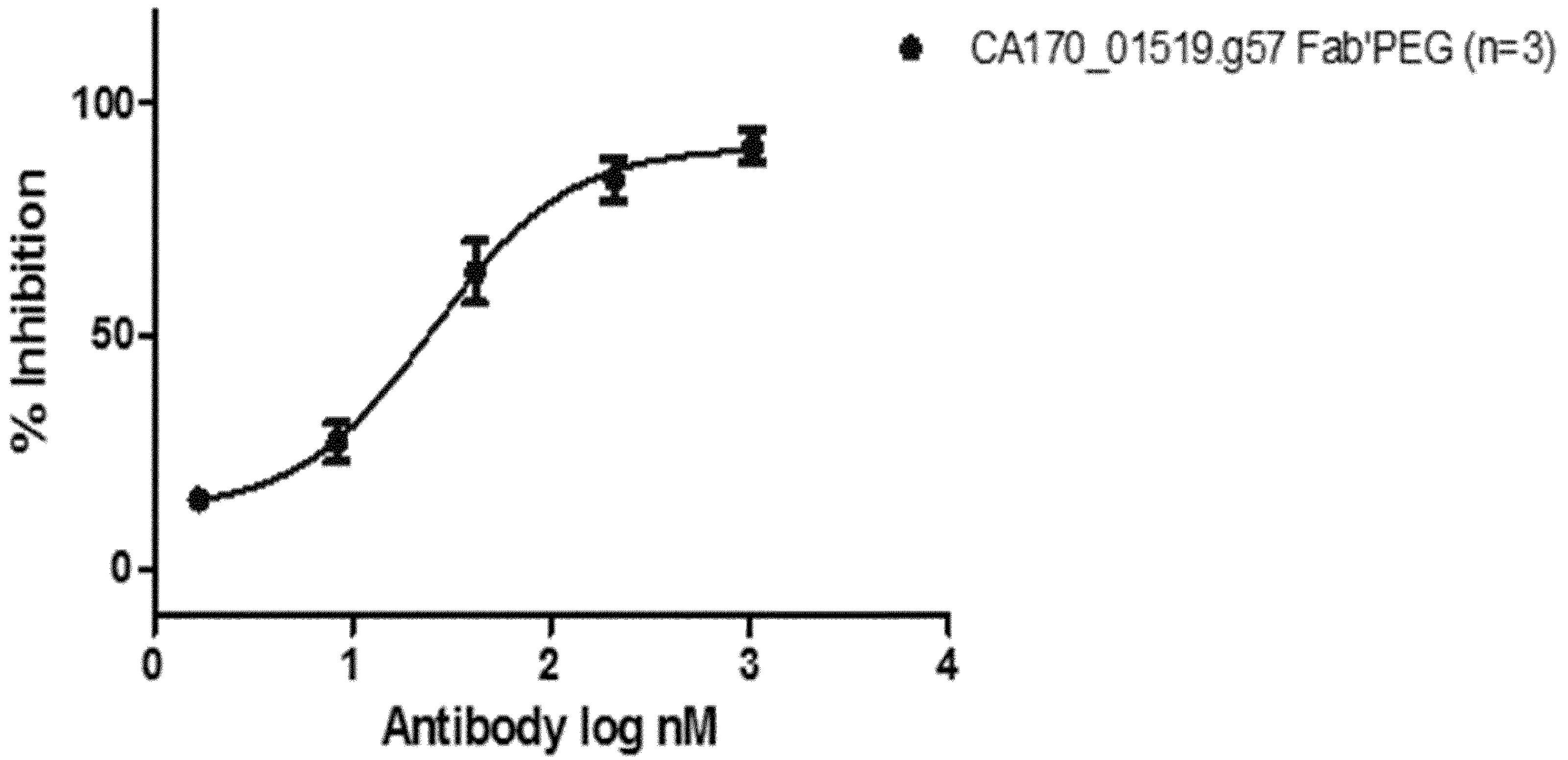


FIGURE 6A- CA170_01519.g57 Fab' binding on cynomoglus MDCK II (cm) cells in acidic and neutral pH

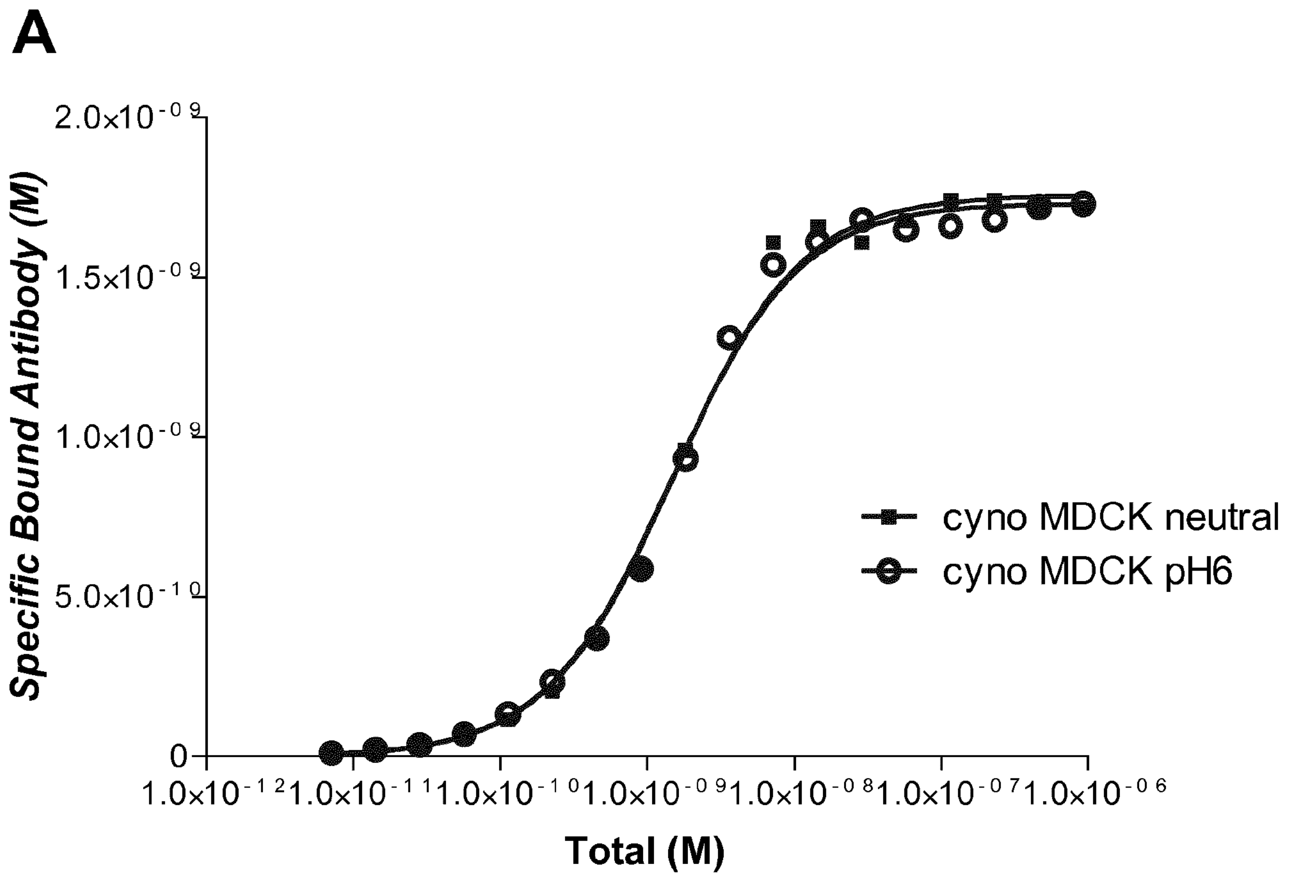


FIGURE 6B - CA170_01519.g57 Fab'PEG binding on cynomoglus MDCK II (cm) cells in acidic and neutral pH

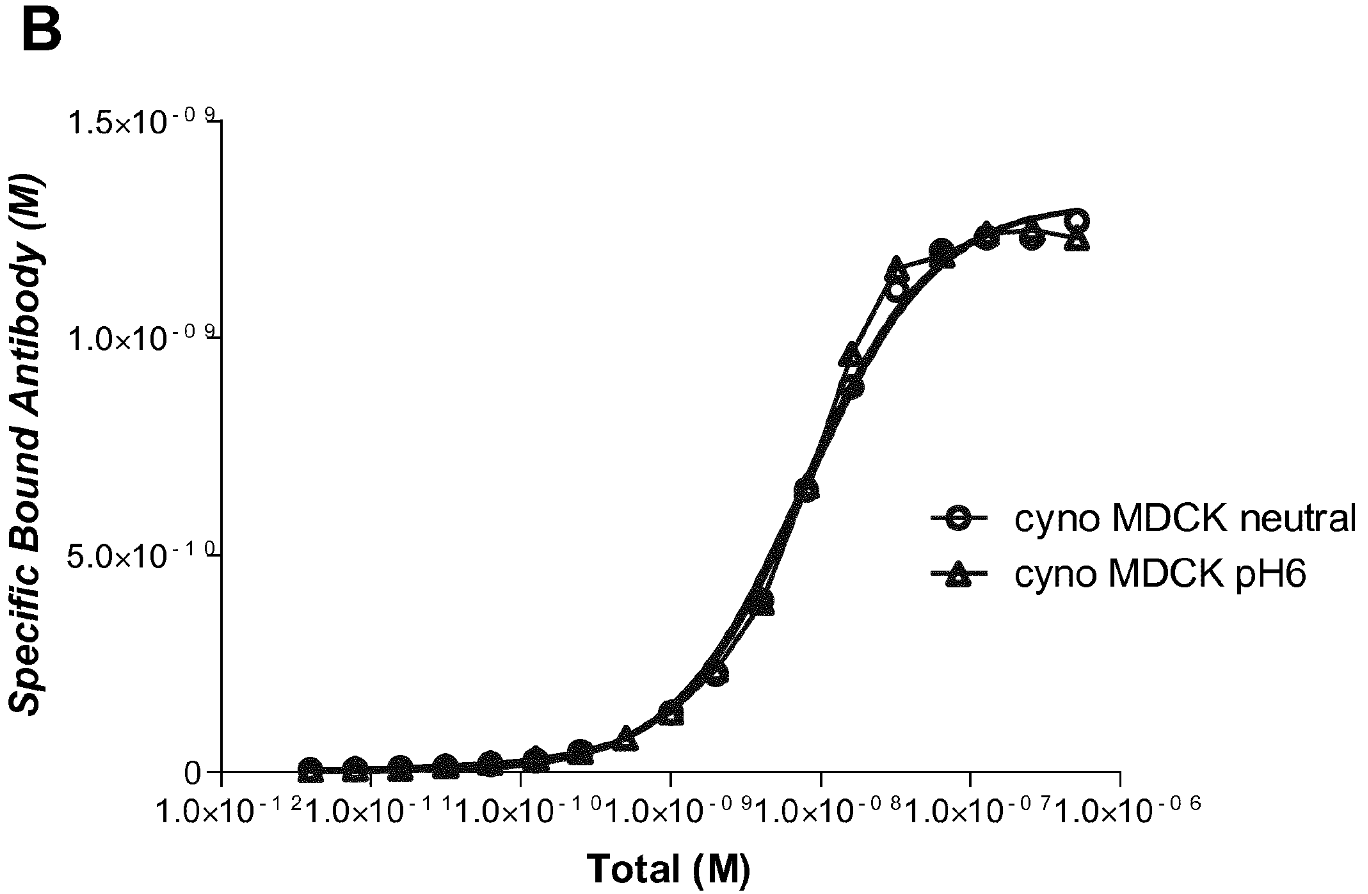


FIGURE 7 CA170_01519.g57 inhibits IgG recycling in human and cynomoglus MDCK II clone 34 cells and MDCK II (cm) cells.

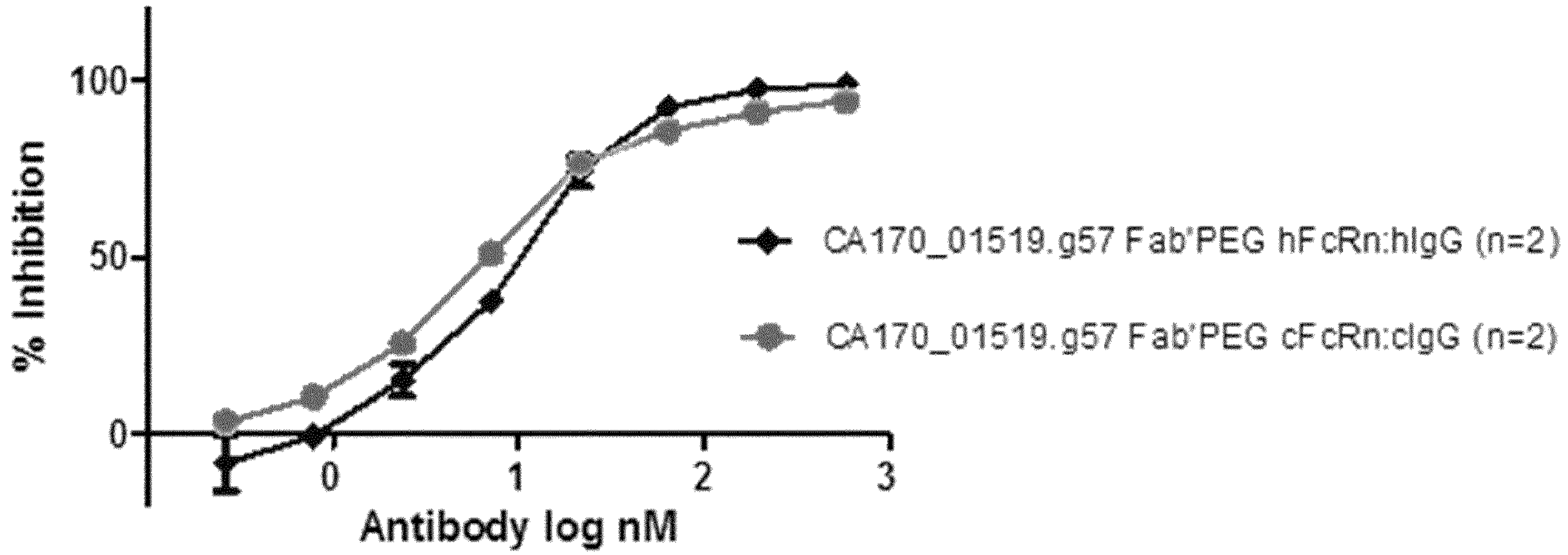


FIGURE 8 Cynomoglus Monkey- single dose of 1519 Fab'PEG on Plasma IgG levels

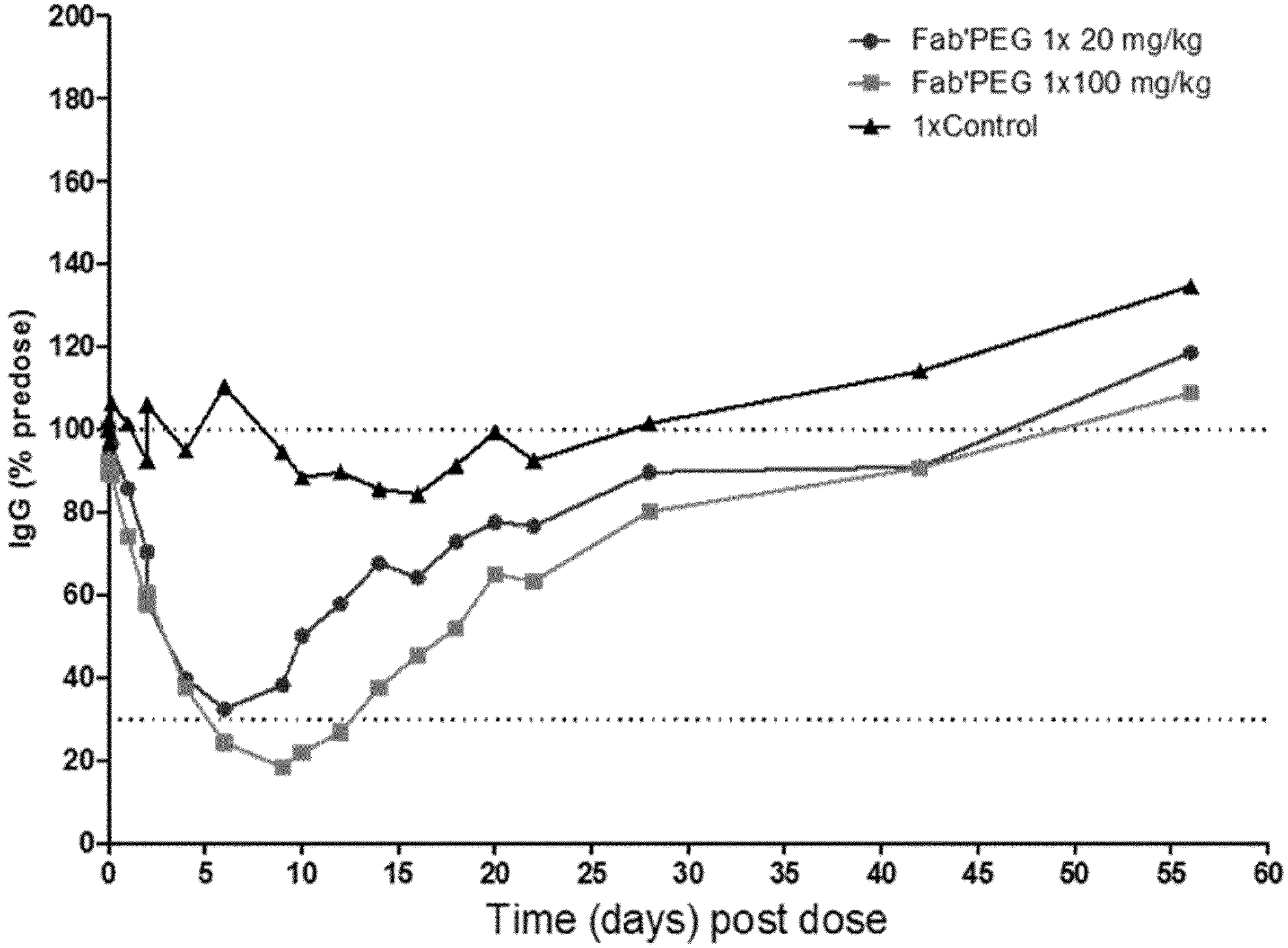


FIGURE 9 Cynomoglus Monkey 4 weekly doses of 1519 Fab'PEG on Plasma IgG Levels

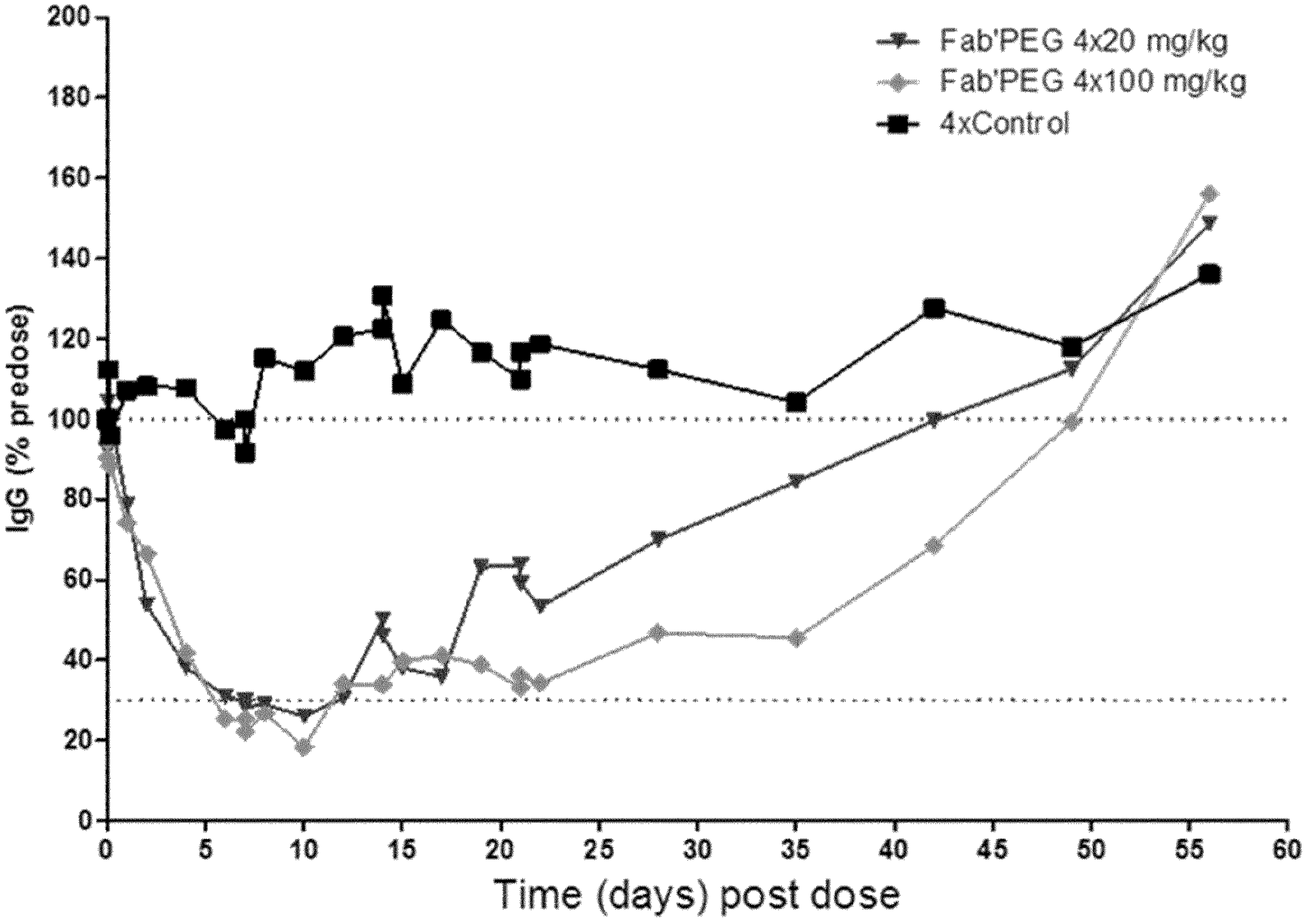


Figure 10

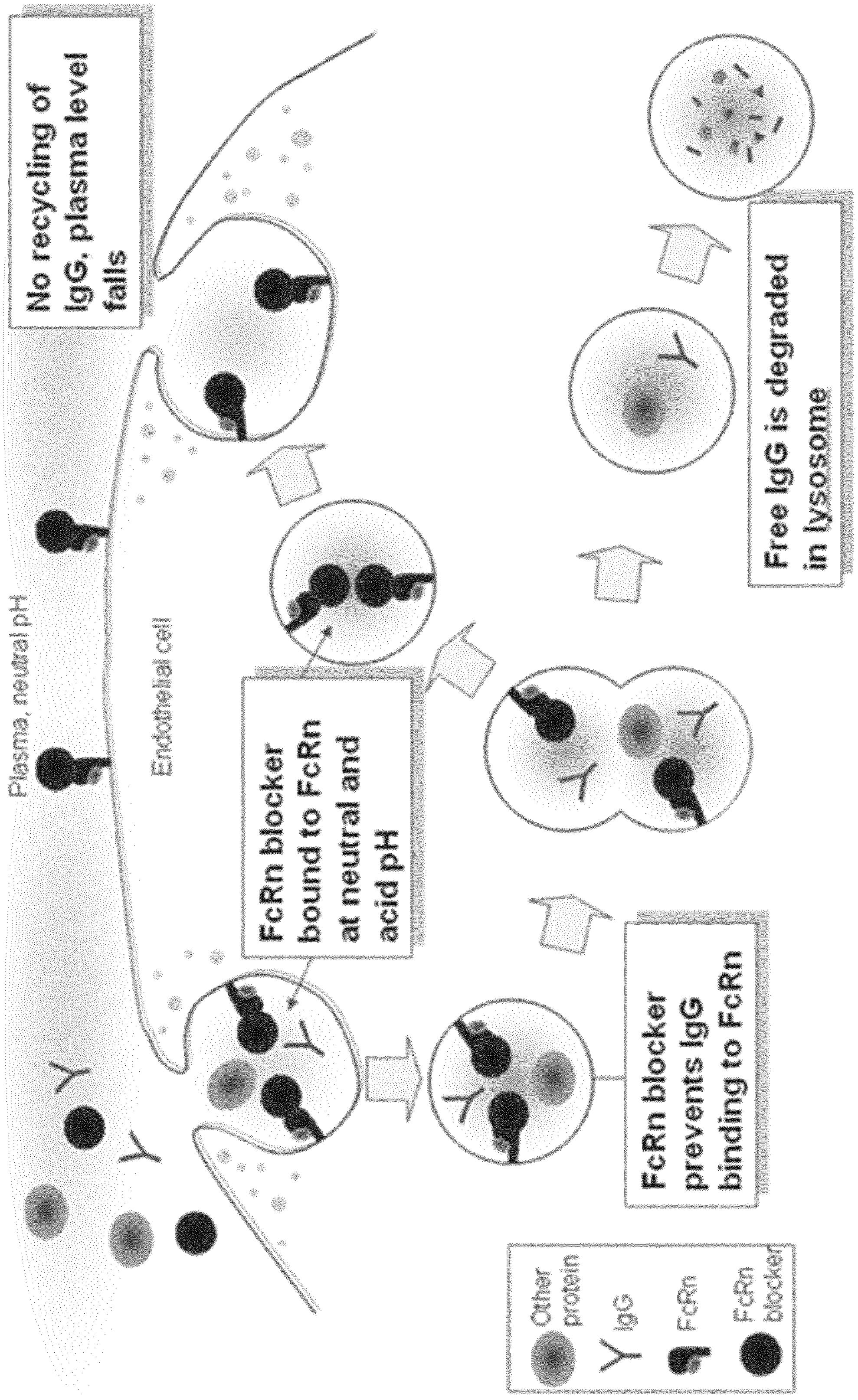

Plasma, neutral pH
Endothelial cell
FcRn blocker bound to FcRn at neutral and acid pH
No recycling of IgG, plasma level falls
FcRn blocker prevents IgG binding to FcRn
Free IgG is degraded in lysosome
Other protein
IgG
FcRn
FcRn blocker

**Figure 11** Flow Cytometry based human IgG blocking assay using purified gamma 1 IgG Antibodies

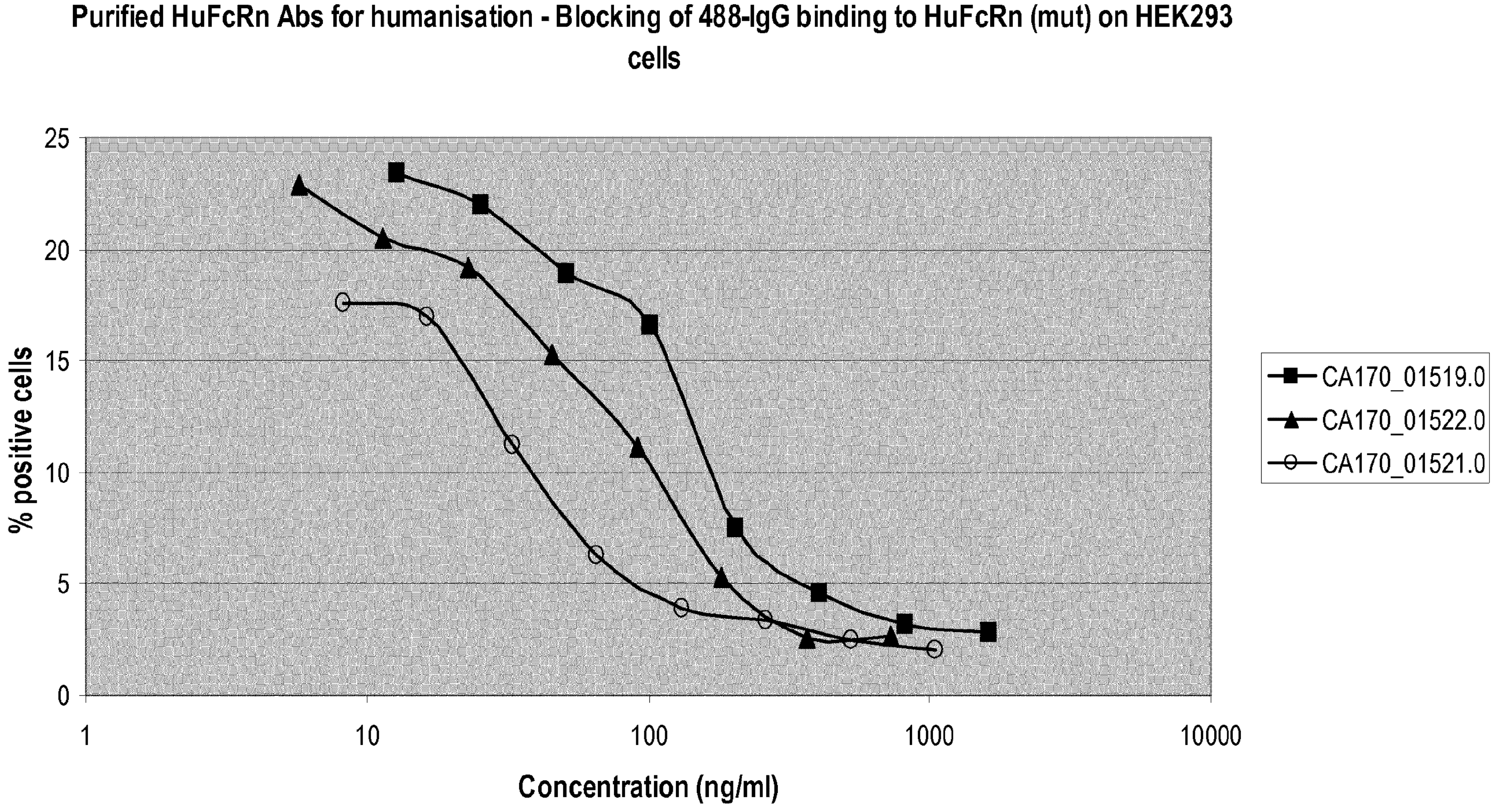


FIGURE 12 Fab'PEG single/intermittent IV doses in Normal Cyno (4 animals n: 4-7) -1519 Fab'PEG 20mg/Kg days 1 and 67 IgG pharmacodynamics

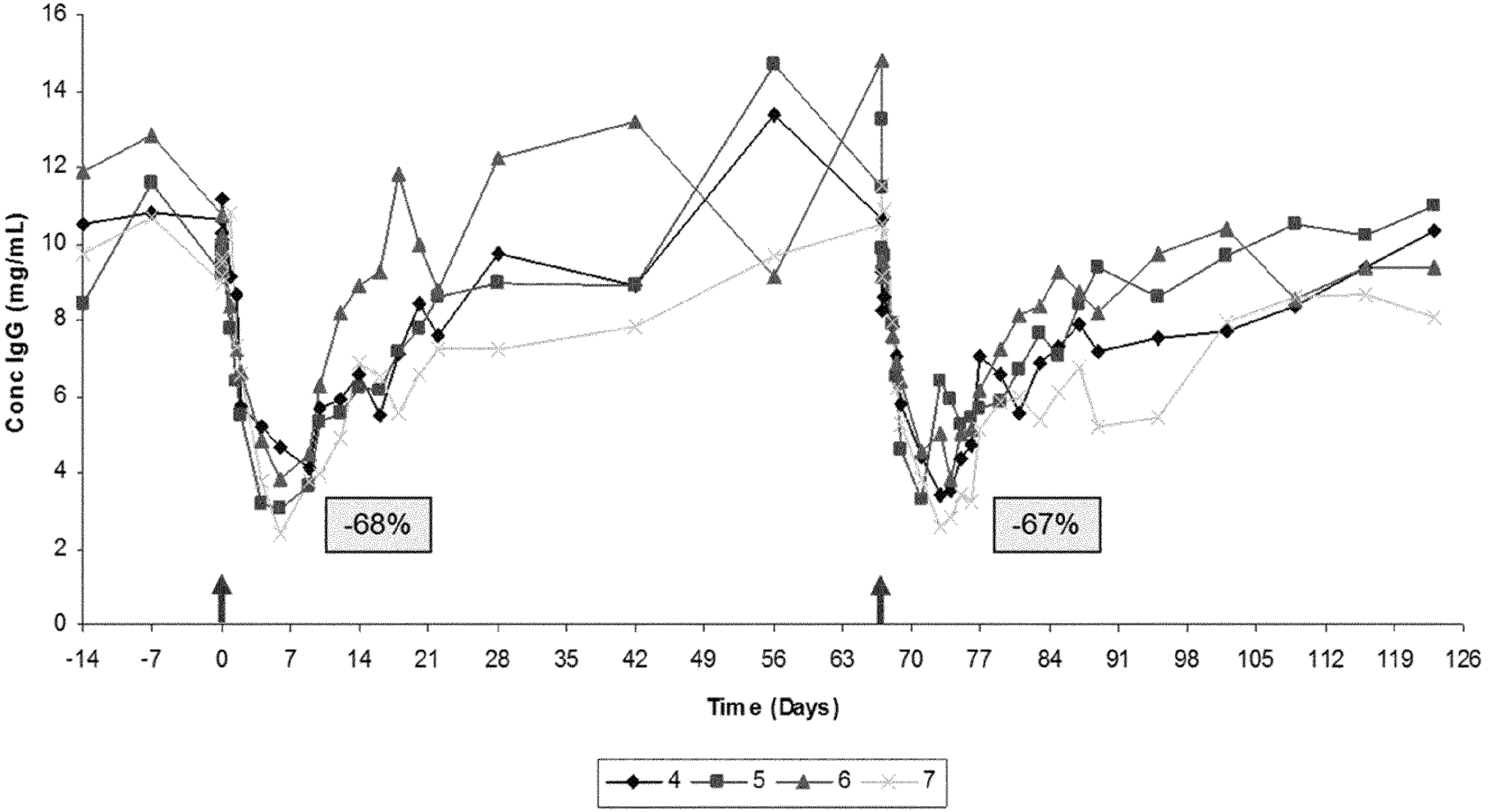


FIGURE 13 Fab'PEG: repeat IV doses in normal cyno- 4x 20 or 100 mg/Kg (top and bottom respectively) per week IgG pharmacodynamics (individual animals)

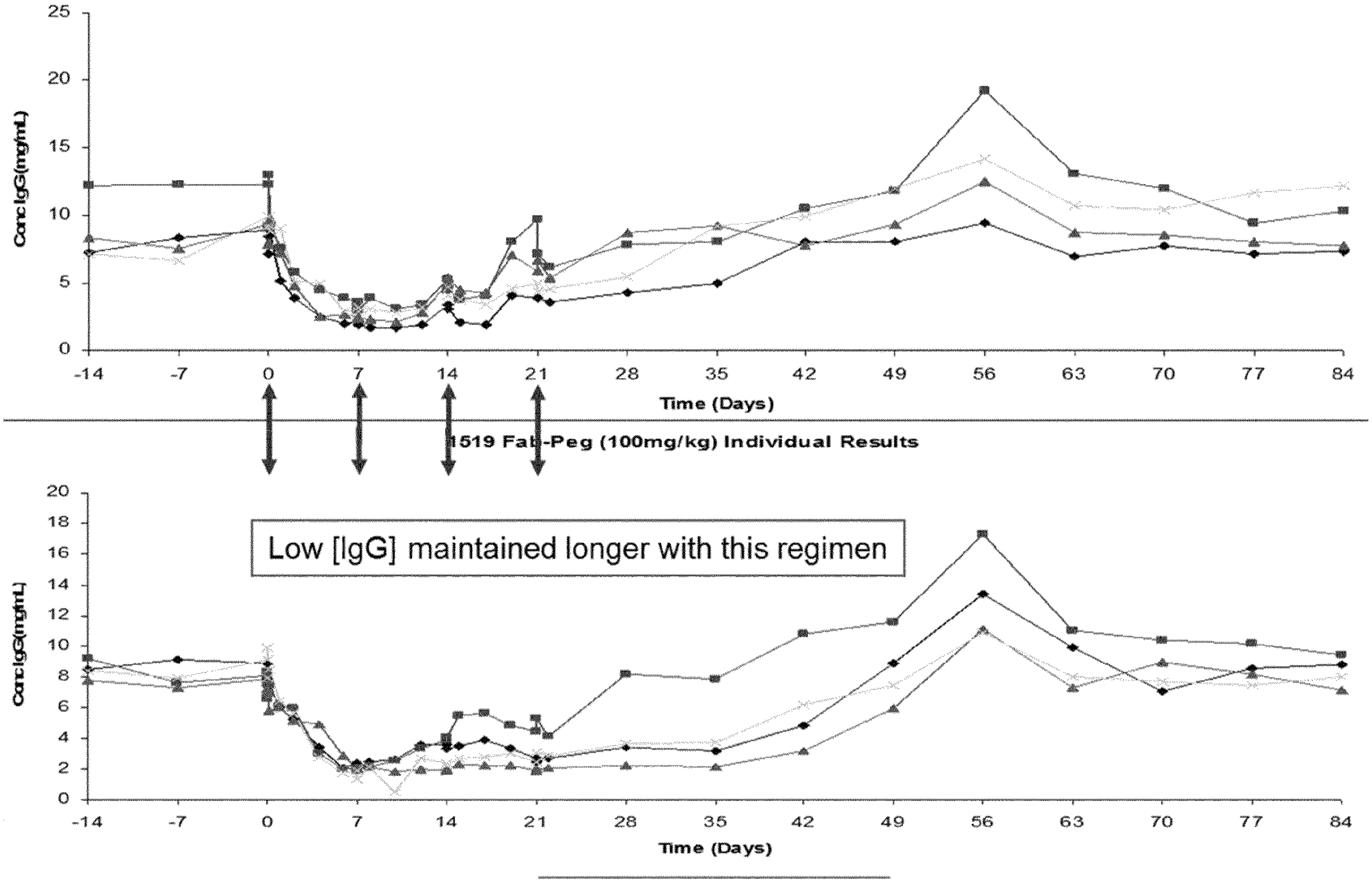


FIGURE 14 Fab'PEG single/intermittent IV doses in normal cyno 20 mg/Kg and 100 mg/Kg days 1 and 67 IgG Pharmacodynamics

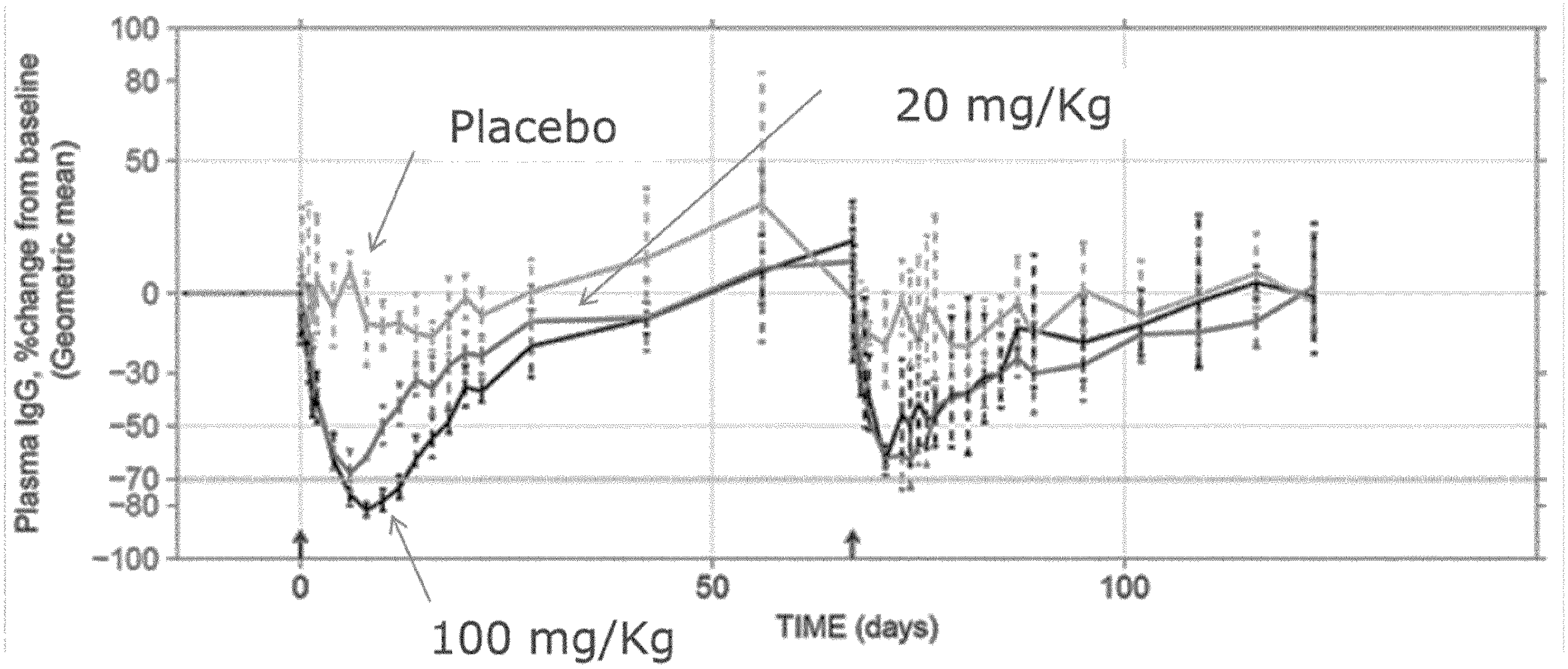


Figure 15 Change in plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 20mg/Kg 1519.g57 Fab'PEG

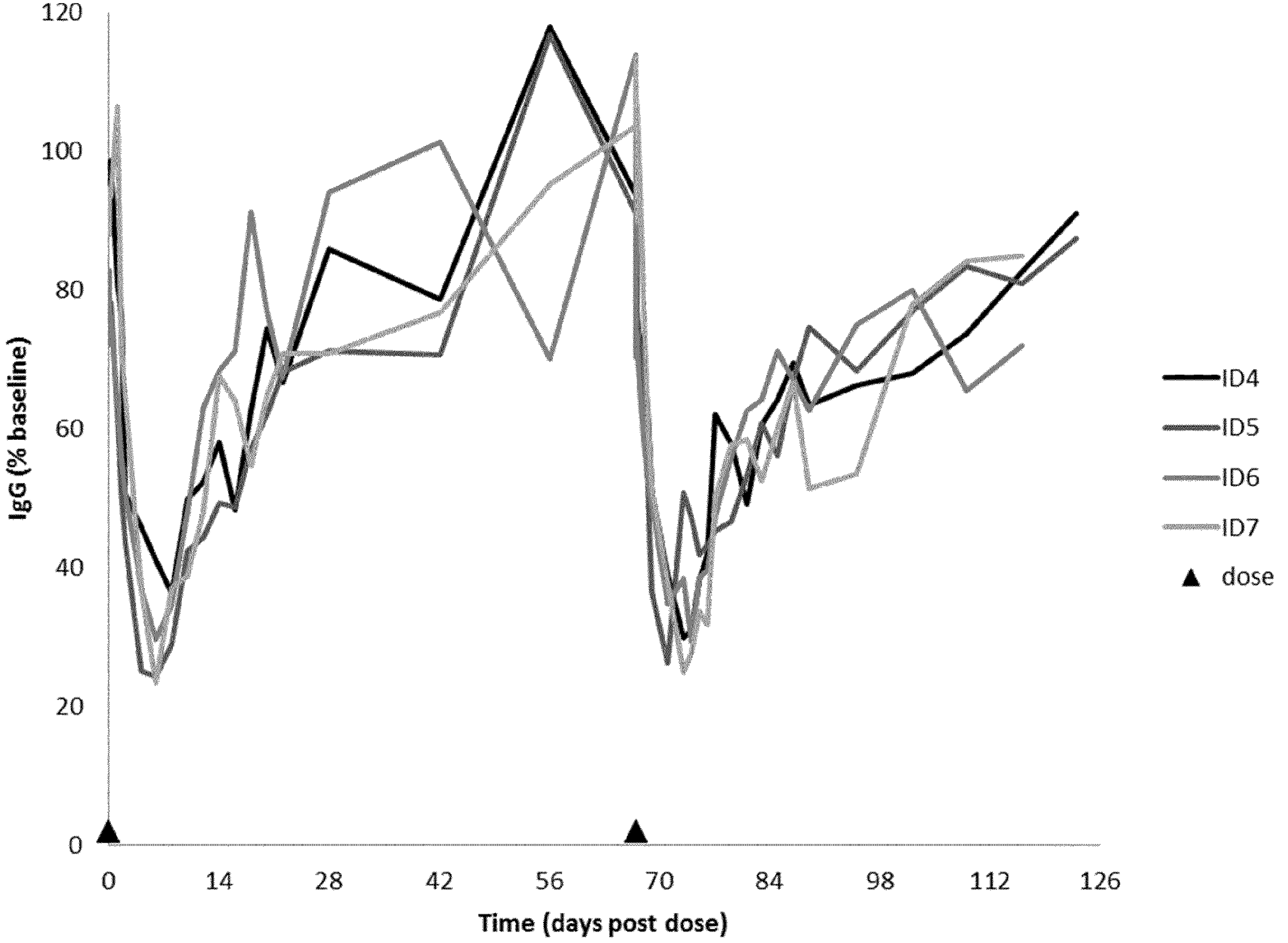


**Figure 16** Change in plasma IgG levels in 4 cynomolgus monkeys receiving 10 IV doses of 20mg/Kg 1519.g57 Fab'PEG every 3 days

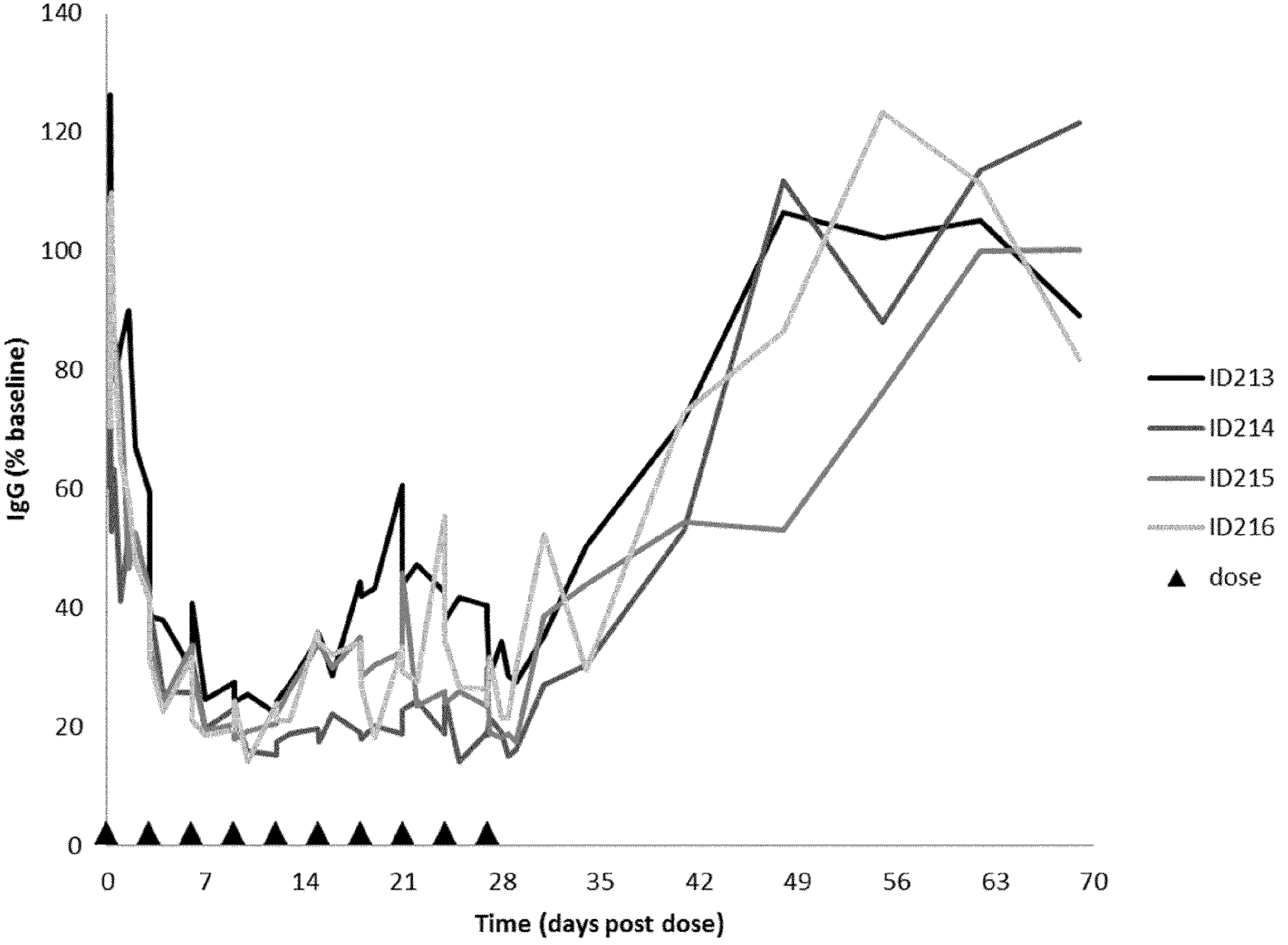


Figure 17 Change in plasma IgG levels in 4 cynomolgus monkeys after 2 IV doses of 30 mg/Kg 1519.g57 IgG4P i.v

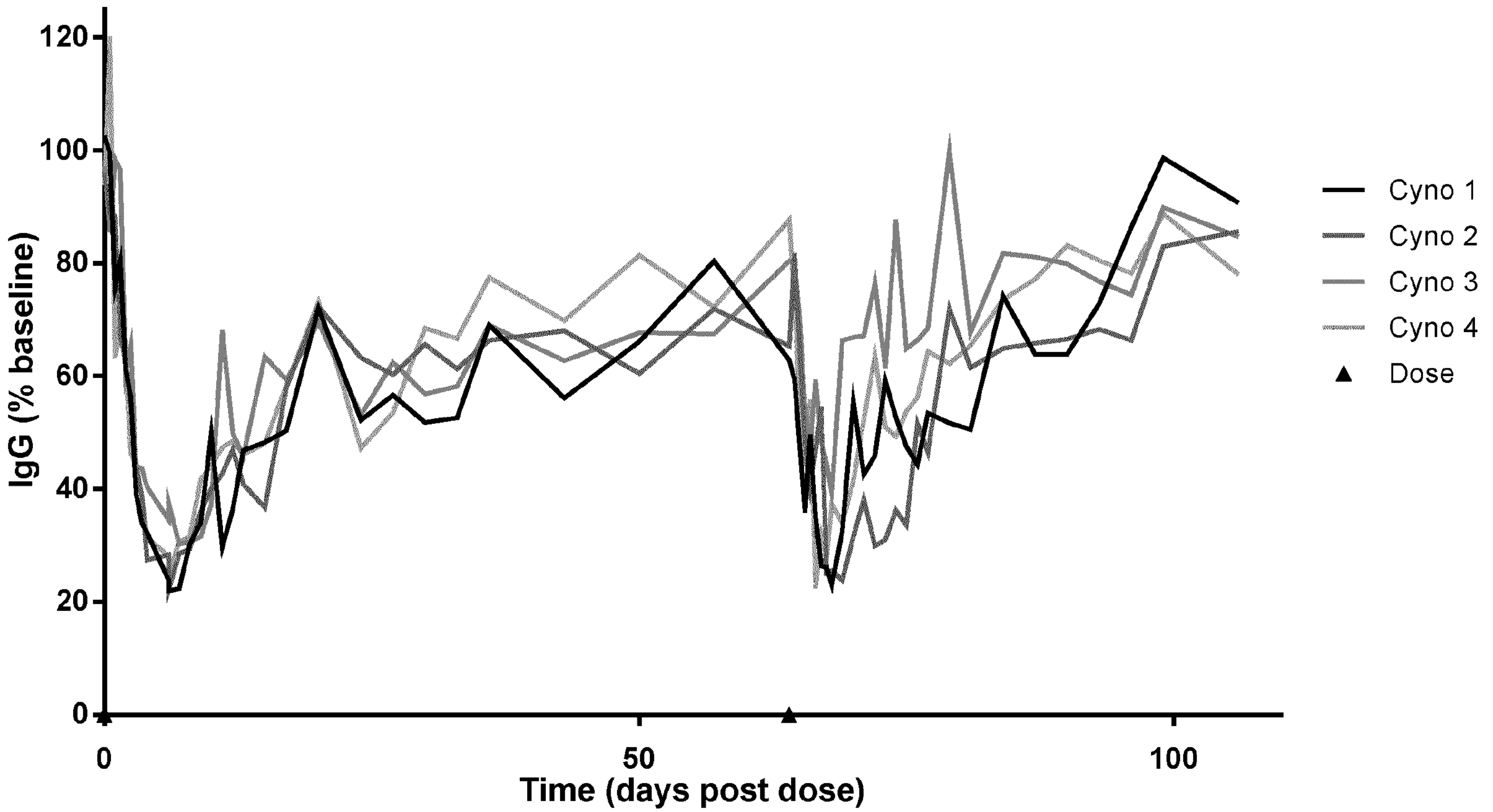


**Figure 18** Change in plasma IgG levels in cynomolgus monkeys treated with 30 mg/Kg 1519.g57 IgG4P on day 0 followed by 5mg/Kg 1519.g57 IgG4P daily for 41 days

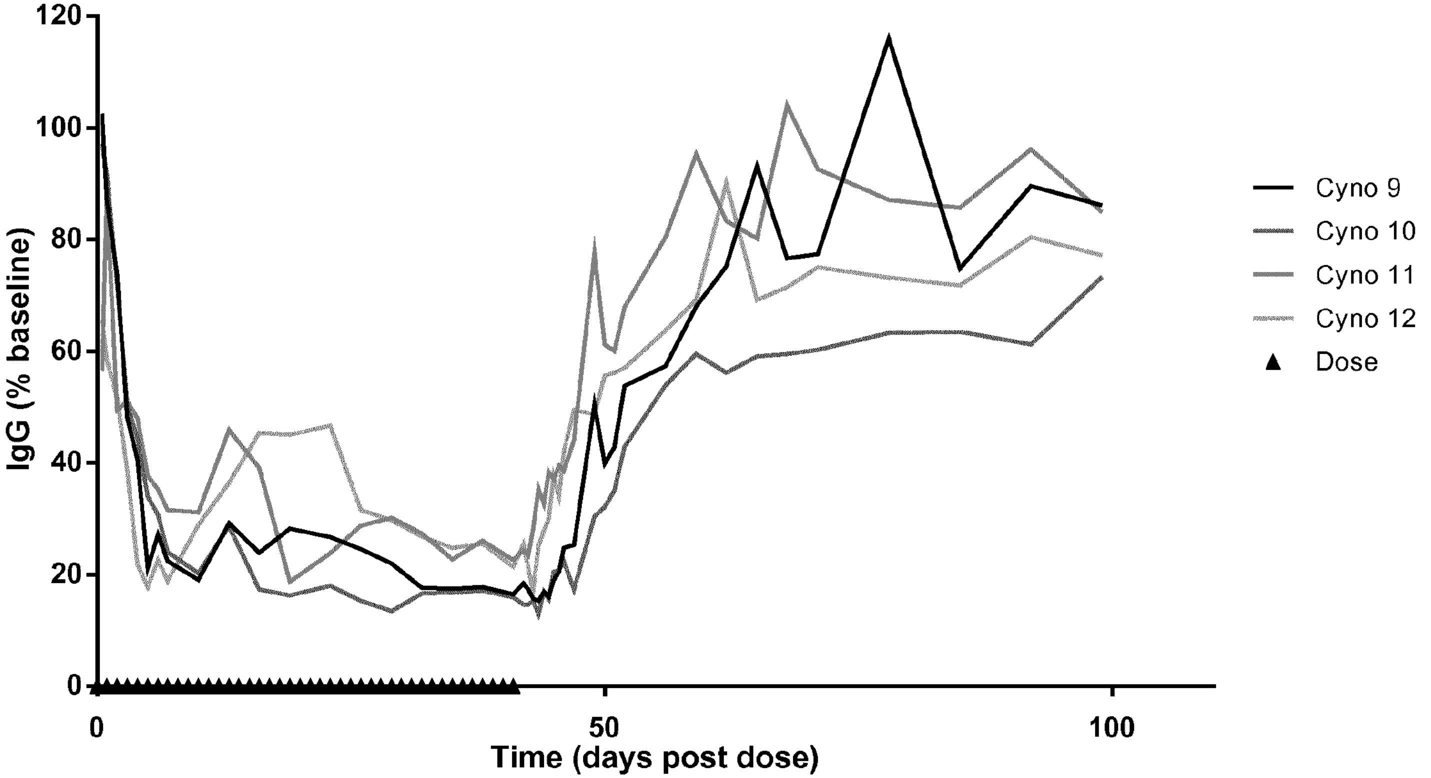


Figure 19

Change in plasma IgG levels in 4 cynomolgus monkeys receiving 42 daily doses of vehicle

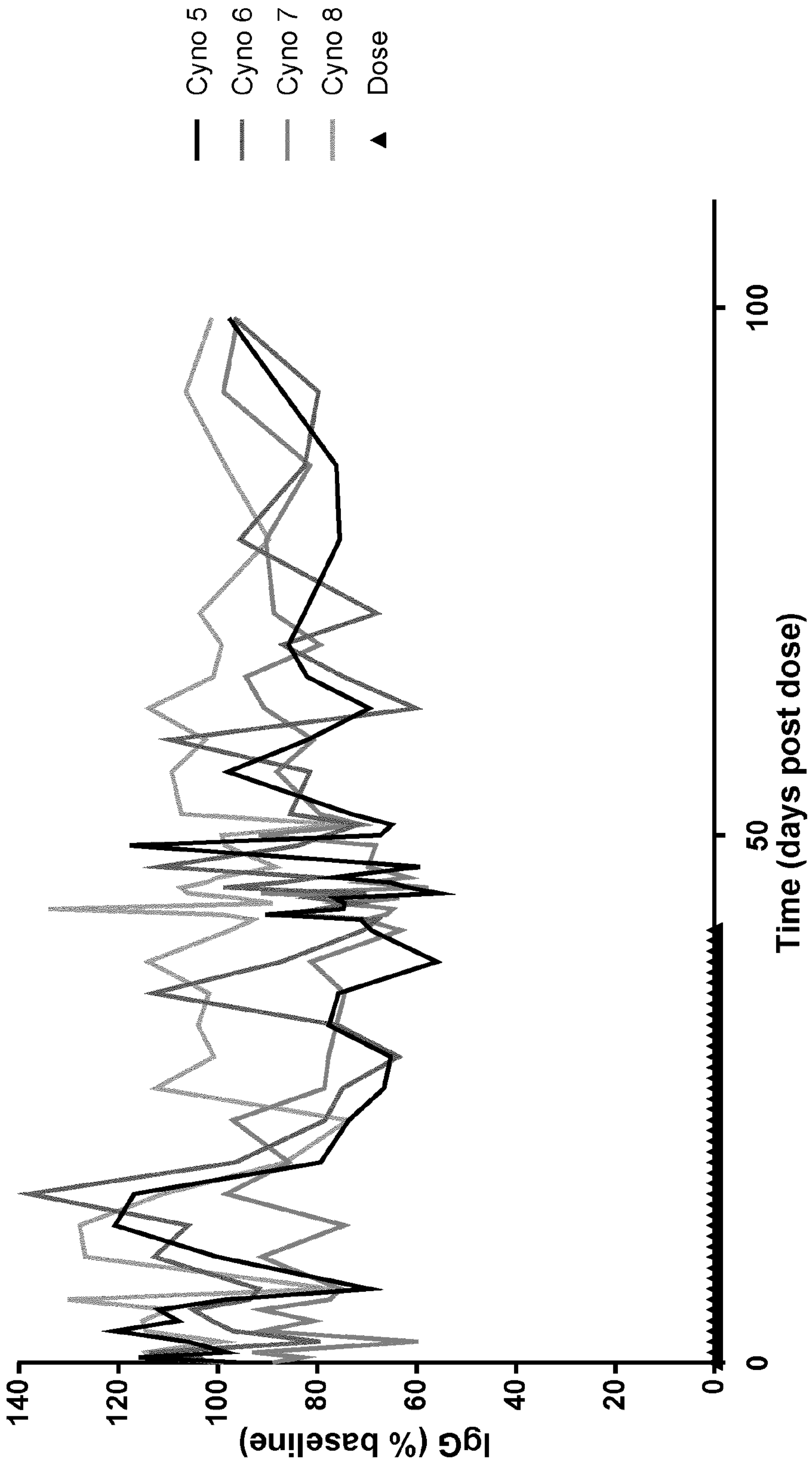


**Figure 20** Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'PEG or PBS IV

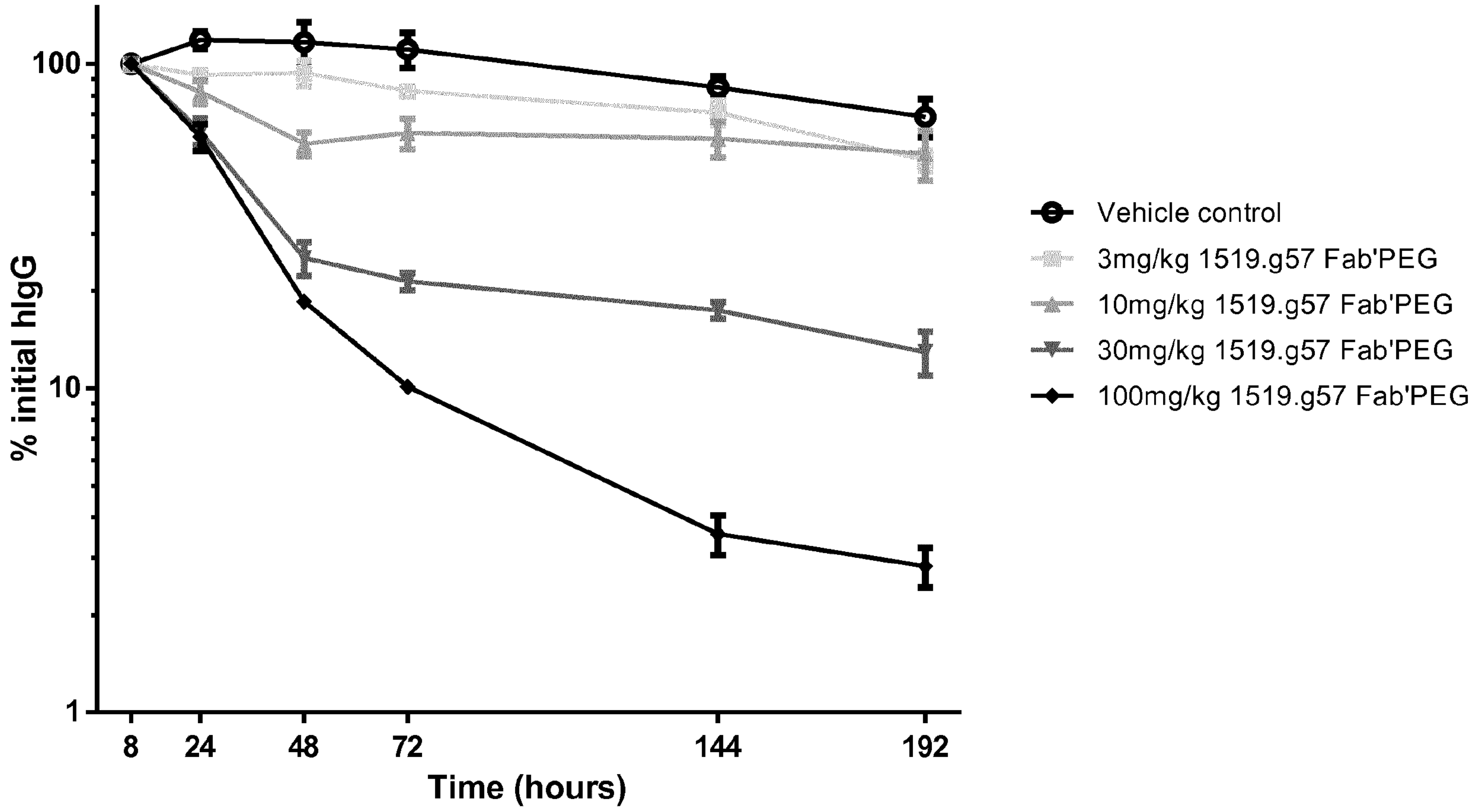


Figure 21 Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 IgG1 or IgG4 or PBS IV

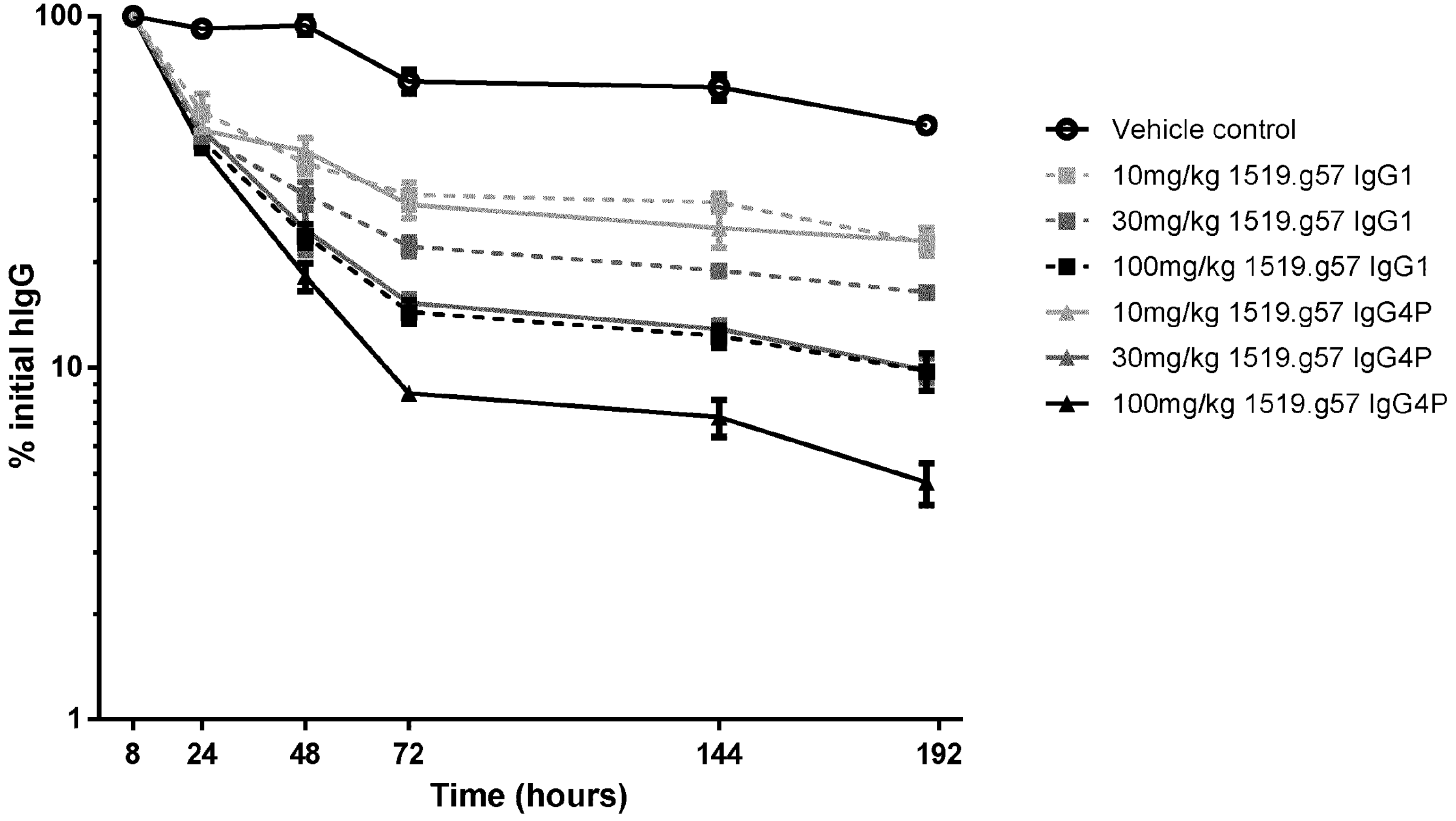


**Figure 22** Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab'-human serum albumin or PBS IV

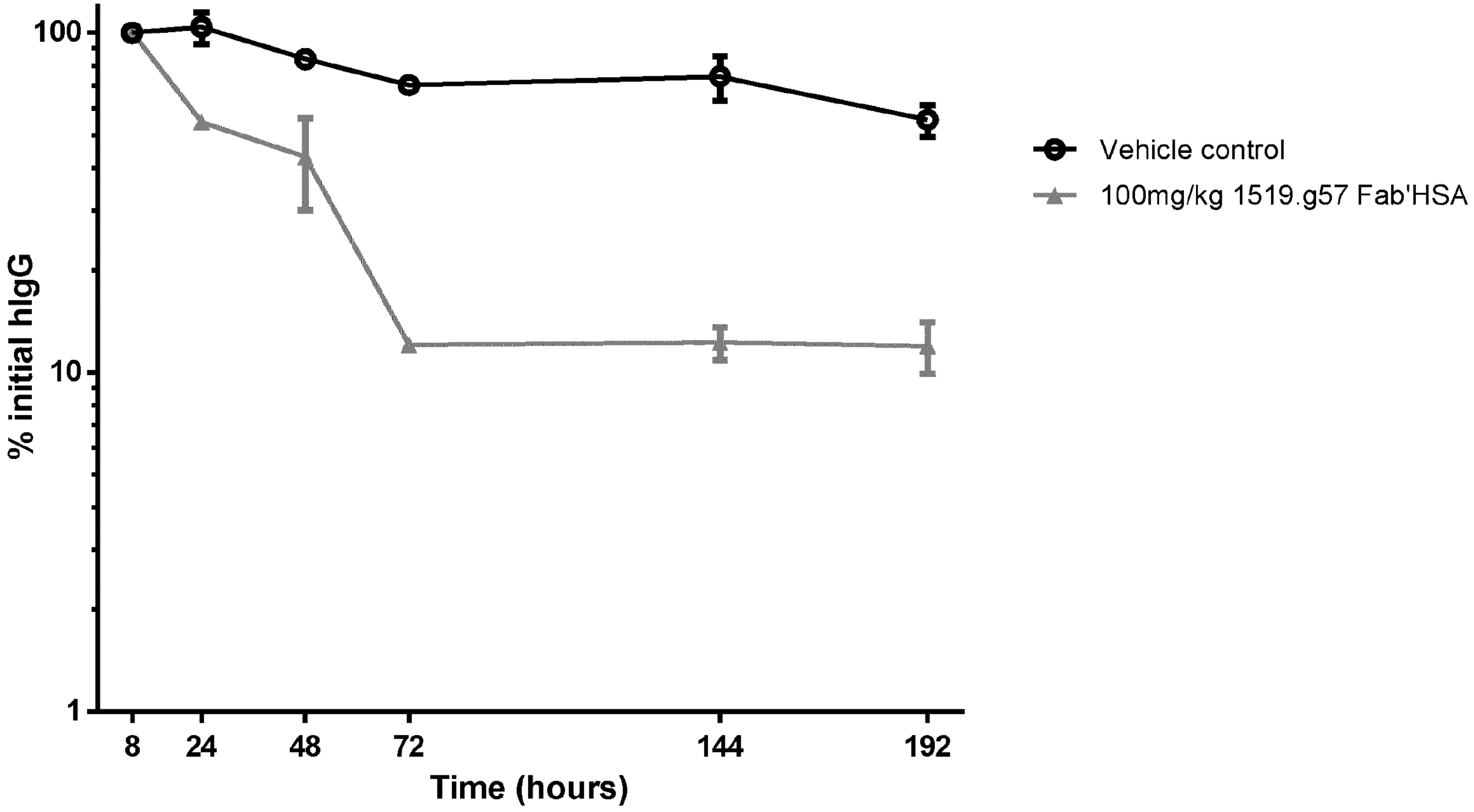


Figure 23 Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 FabFv or PBS IV

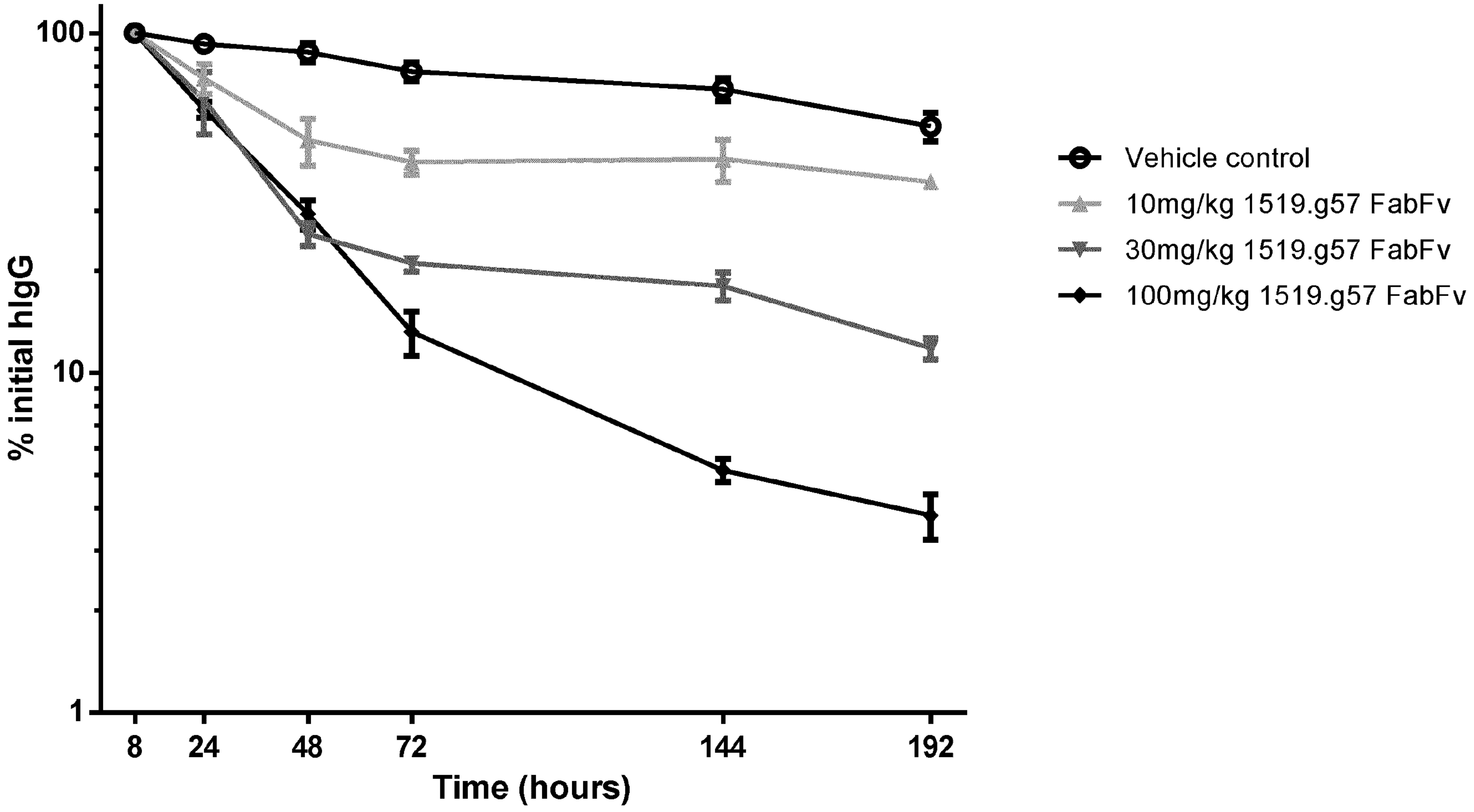


Figure 24 **Increased clearance of IV hIgG in plasma of hFcRn transgenic mice treated with CA170_01519.g57 Fab or Fab'PEG or PBS IV**

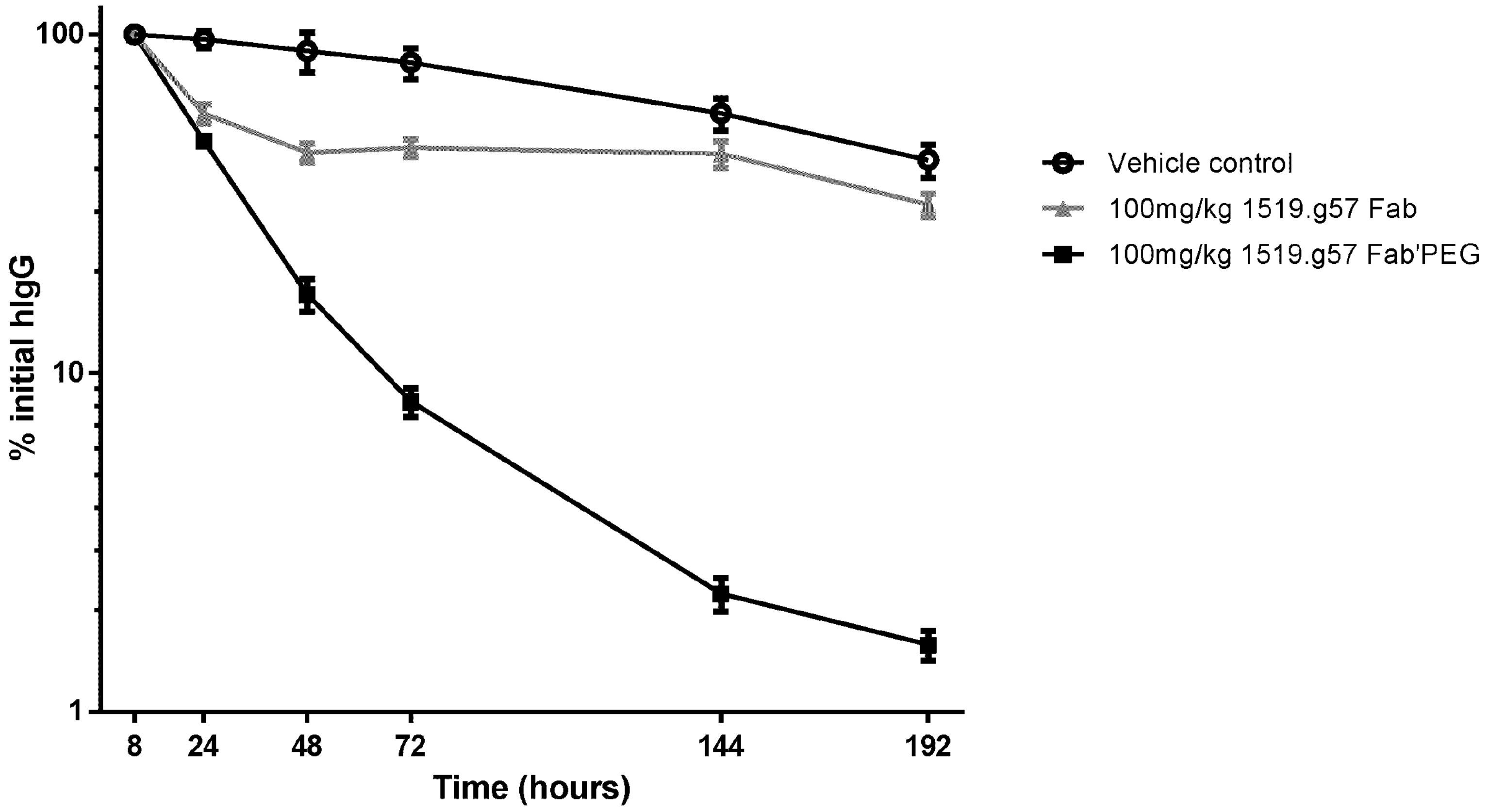
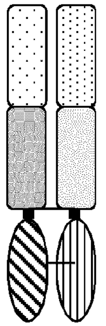


**Figure 25**



First Variable region of light chain VL1 ░

First Variable region of heavy chain VH1 chain ░

Constant regions cKappa ▩ and CH1 ▒

Second variable region of light chain VL2 ▧

Second variable region of heavy chain VH2 ▥

Disulphide bond —