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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 October 2021 (21.10.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/212081 A1

(51) International Patent Classification:
C07K 16/00 (2006.01) *C07K 16/22* (2006.01)
A61P 37/06 (2006.01) *C07K 16/24* (2006.01)

(21) International Application Number:
PCT/US2021/027836

(22) International Filing Date:
16 April 2021 (16.04.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/011,453 17 April 2020 (17.04.2020) US

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

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

(54) Title: CANINE ANTIBODY VARIANTS

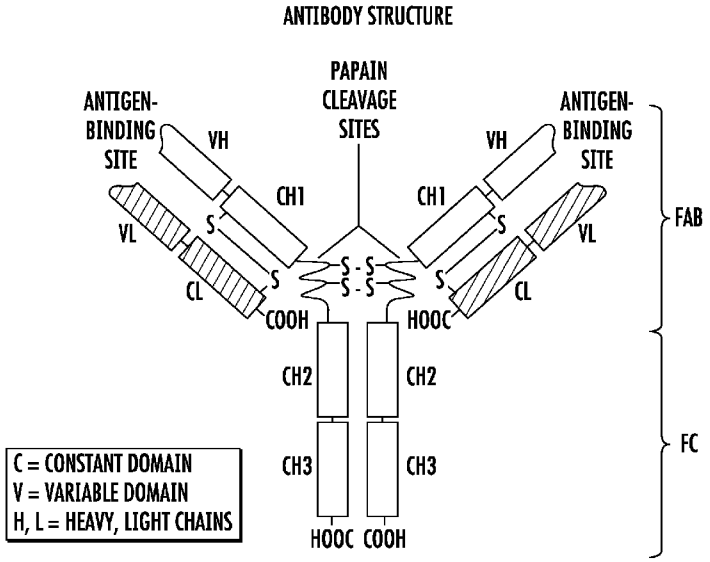

FIG. 1

(57) Abstract: The invention relates generally to canine antibody variants and uses thereof. Specifically, the invention relates to mutations in the constant region of canine antibody for improving its half-life and other characters.

[Continued on next page]

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Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

CANINE ANTIBODY VARIANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of United States Provisional Patent Application 63/011453, filed April 17, 2020, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to canine antibody variants and uses thereof. Specifically, the invention relates to a mutation in the Fc constant region of canine antibody for improving half-life.

BACKGROUND OF THE INVENTION

[0003] Canine IgG monoclonal antibodies (mAbs) are being developed as effective therapeutics in veterinary medicine. Several years ago, four canine IgG subclasses were identified and characterized (Bergeron *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 157(1-2), pages 31-41). However, not much work has been done on extending the half-life of canine IgGs.

[0004] Through a recycling mechanism, the neonatal Fc receptor (FcRn) prolongs the half-life of an IgG in a pH-dependent interaction with its fragment crystallizable (Fc) region. Specifically, the Fc region spanning the interface of CH2 and CH3 domains interacts with the FcRn on the surface of cells to regulate IgG homeostasis. This interaction is favored by an acidic interaction after IgG pinocytosis and thus IgG is protected from degradation. The endocytosed IgG is then recycled back to the cell surface and released into the blood stream at an alkaline pH thereby maintaining sufficient serum IgG for proper function. Accordingly, the pharmacokinetic profile of IgGs depend on the structural and functional properties of their Fc regions.

[0005] Three canine IgG subclasses bind canine FcRn and have been compared to human IgG analogues. Half-life of canine IgG remains to be fully studied because, without any experimental support, one cannot expect or predict whether or not they will align closely with human IgGs.

[0006] Extended half-life of IgG could allow less frequent dosing and/or lower dose of the antibody drug, which in turn reduces veterinary visits, improves patient compliance, and lowers the concentration-dependent cytotoxicity/adverse events.

[0007] Accordingly, there exists a need to identify mutations in the Fc constant regions to improve half-life.

SUMMARY OF THE INVENTION

[0008] The invention relates to mutant canine IgGs that provide higher FcRn affinity and higher half-life, relative to wild-type canine IgGs. Specifically, the inventors of the instant application have found that substituting the amino acid residue asparagine (Asn or N) at position 434 with another amino acid surprisingly and unexpectedly enhanced the affinity to FcRn, and thereby increased the half-life of IgG.

[0009] In one aspect, the invention provides a modified IgG comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat. In an exemplary embodiment, said substitution is a substitution of asparagine at position 434 with histidine.

[00010] In another aspect, the invention provides a polypeptide comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

[00011] In yet another aspect, the invention provides an antibody or a molecule comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

[00012] In a further aspect, the invention provides a method for producing or manufacturing an antibody or a molecule, the method comprising: providing a vector or a host cell having an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

[00013] In another aspect, the invention provides a method for increasing an antibody serum half-life in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat. In an exemplary embodiment, the antibody increases the half-life for about 30 days.

[00014] In another aspect, the invention provides a method for maintaining a therapeutic serum level of an antibody in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat. In an exemplary embodiment, the antibody, maintains the therapeutic serum level of said antibody in said dog over a period ranging from about 1 month to about 7 months.

[00015] Other features and advantages of the present invention will become apparent from the following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[00016] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[00017] FIG. 1 illustrates domain structure of IgG. Fc mutation N434H was made in the CH3 domain to increase IgG half-life by increasing affinity to FcRn at pH6.

[00018] FIG. 2A shows the amino acid sequences of Canine IgGB having N434H and wild-type (WT) canine IgGB.

[00019] FIG. 2B shows the alignment of the amino acid sequences of wild-type (WT) human IgG1, WT canine 1gGA, WT canine IgGB, WT canine IgGC, and WT canine IgGD. The amino acid residues are numbered according to the Eu index as in Kabat. The CH1, hinge, CH2, and CH3 amino acid residues are in red, violet, blue, and green, respectively.

[00020] FIG. 2C shows Fc nucleotide sequences of WT IgGB 65.

[00021] FIG. 3 shows individual serum concentrations for WT mAb1 IgG in 4 dogs, 2 male (01M, 02M) and 2 female (03F, 04F) after a single injection of 2mg/kg measured over a 56 day period.

[00022] FIG. 4 shows individual serum concentrations for N434H mAb1 IgG in after a 4 dogs, 2 male (17M, 18M) and 2 female (19F, 20F) single injection of 2mg/kg measured over a 56 day period.

[00023] FIG. 5 shows individual serum concentrations for WT mAb2 IgG in 8 dogs, 4 male (H03433, H03434. H03435, H03436) and 4 female (H03453, H03454, H03455, H03456) after three injections of 2mg/kg (SC/SC/IV) measured over a 98 day period.

[00024] FIG. 6 shows individual serum concentrations for N434H mAb2 IgG in 8 dogs, 4 male (H03433, H03434. H03435, H03436) and 4 female (H03453, H03454, H03455, and H03456) after three injections of 2mg/kg (SC/SC/IV) measured over a 98 day period.

[00025] FIG. 7 shows serum profiles of ZTS-00008183 in dogs following a single 4 mg/kg subcutaneous administration. The colors represent different animal identification numbers.

[00026] FIG. 8 shows mean serum profiles of ZTS-00008183 in dogs following a single 4 mg/kg subcutaneous administration.

[00027] FIG. 9. Plot of least squares means by treatment by time point (3-5 months). alpha levels: Day 84 = 0.07085, Day 112 = 0.04575, Day 140 = 0.04352.

[00028] FIG. 10. Plot of least squares means and percent change by treatment by time point (3-5 months). % change in means = 100 x [mean(T01) - mean(T02)/mean(T01)].

[00029] FIG. 11. Pruritic score box plots for all time points. T01 = Placebo 0 mg/kg, T02 = ZTS-00008183 4 mg/kg.

[00030] FIG. 12. Pruritic score plot of arithmetic means by treatment for all time points. Error bars represent standard errors.

[00031] FIG. 13. Pruritic score plot of arithmetic means and percent change by treatment for all time points. % change in means = 100 x [mean(T01) - mean(T0X)/mean(T01)], for X=2,3.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00032] SEQ ID NO.: 1 is the amino acid sequence of the mutant canine IgGB constant domain having N434H mutation;

[00033] SEQ ID NO.: 2 is the amino acid sequence of the wildtype canine IgGB constant domain;

[00034] SEQ ID NO.: 3 is the nucleic acid sequence of the wildtype canine IgG constant domain codon optimized (IgGB_65_WT);

[00035] SEQ ID NO.: 4 is the nucleic acid sequence of the wildtype canine IgGB constant domain;

[00036] SEQ ID NO.: 5 is the amino acid sequence of IgGB CH1 domain positions 118-215;

[00037] SEQ ID NO.: 6 is the amino acid sequence of IgGB hinge Domain positions 217-230;

[00038] SEQ ID NO.: 7 is the amino acid sequence of wildtype IgGB CH2 domain positions 231-340;

[00039] SEQ ID NO.: 8 is the amino acid sequence of wildtype IgGB CH3 domain positions 341-447;

[00040] SEQ ID NO.: 9 is the nucleic acid sequence of IgGB CH1 domain;

[00041] SEQ ID NO.: 10 is the nucleic acid sequence of IgGB hinge Domain;

[00042] SEQ ID NO.: 11 is the nucleic acid sequence of wildtype IgGB CH2 domain;

[00043] SEQ ID NO.: 12 is the nucleic acid sequence of wildtype IgGB CH3 domain;

[00044] SEQ ID NO: 13 is a variable heavy chain CDR1 of anti-IL31 antibody referred to herein as 11E12-VH-CDR1;

[00045] SEQ ID NO: 14 is a variable heavy chain CDR1 of anti-IL31 antibody referred to herein as 34D03-VH-CDR1;

[00046] SEQ ID NO: 15 is a variable heavy chain CDR2 of anti-IL31 antibody referred to herein as 11E12-VH-CDR2;

[00047] SEQ ID NO: 16 is a variable heavy chain CDR2 of anti-IL31 antibody referred to herein as 34D03-VH-CDR2;

[00048] SEQ ID NO: 17 is a variable heavy chain CDR3 of anti-IL31 antibody referred to herein as 11E12-VH-CDR3;

[00049] SEQ ID NO: 18 is a variable heavy chain CDR3 of anti-IL31 antibody referred to herein as 34D03-VH-CDR3;

[00050] SEQ ID NO: 19 is a variable light chain CDR1 of anti-IL31 antibody referred to herein as 11E12-VL-CDR1;

[00051] SEQ ID NO: 20 is a variable light chain CDR1 of anti-IL31 antibody referred to herein as 34D03-VL-CDR1;

[00052] SEQ ID NO: 21 is a variable light chain CDR2 of anti-IL31 antibody referred to herein as 11E12-VL-CDR2;

[00053] SEQ ID NO: 22 is a variable light chain CDR2 of anti-IL31 antibody referred to herein as 34D03-VL-CDR2;

[00054] SEQ ID NO: 23 is a variable light chain CDR3 of anti-IL31 antibody referred to herein as 11E12-VL-CDR3;

[00055] SEQ ID NO: 24 is a variable light chain CDR3 of anti-IL31 antibody referred to herein as 34D03-VL-CDR3;

[00056] SEQ ID NO: 25 is a variable light chain sequence of anti-IL31 antibody referred to herein as MU-11E12-VL;

[00057] SEQ ID NO: 26 is a variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VL-cUn-FW2;

[00058] SEQ ID NO: 27 is a variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VL-cUn-13;

[00059] SEQ ID NO: 28 is a variable light chain sequence of anti-IL31 antibody referred to herein as MU-34D03-VL;

[00060] SEQ ID NO: 29 is a variable light chain sequence of anti-IL31 antibody referred to herein as CAN-34D03-VL-998-1;

[00061] SEQ ID NO: 30 is a variable heavy chain sequence of anti-IL31 antibody referred to herein as MU-11E12-VH;

[00062] SEQ ID NO: 31 is a variable heavy chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VH-415-1;

[00063] SEQ ID NO: 32 is a variable heavy chain sequence of anti-IL31 antibody referred to herein as MU-34D03-VH;

[00064] SEQ ID NO: 33 is a variable heavy chain sequence of anti-IL31 antibody referred to herein as CAN-34D03-VH-568-1;

[00065] SEQ ID NO: 34 is the amino acid sequence corresponding to GenBank Accession No. C7G0W1 and corresponds to Canine IL-31 full-length protein;

[00066] SEQ ID NO: 35 is the nucleotide sequence corresponding to GenBank Accession No. C7G0W1 and corresponds to the nucleotide sequence encoding Canine IL-31 full-length protein;

[00067] SEQ ID NO: 36 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as MU-11E12-VL;

[00068] SEQ ID NO: 37 is the nucleotide sequence encoding the variable heavy chain sequence of anti-IL31 antibody referred to herein as MU-11E12-VH;

[00069] SEQ ID NO: 38 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as MU-34D03-VL;

[00070] SEQ ID NO: 39 is the nucleotide sequence encoding the variable heavy chain sequence of anti-IL31 antibody referred to herein as MU-34D03-VH;

[00071] SEQ ID NO: 40 is the amino acid sequence for the canine wildtype heavy chain constant region referred to herein as HC-64 (GenBank accession no. AF354264);

[00072] SEQ ID NO: 41 is the nucleotide sequence encoding the canine wildtype heavy chain constant region referred to herein as HC-64 (GenBank accession no. AF354264);

[00073] SEQ ID NO: 42 is the amino acid sequence for the canine wildtype heavy chain constant region referred to herein as HC-65 (GenBank accession no. AF354265);

[00074] SEQ ID NO: 43 is the nucleotide sequence encoding the canine wildtype heavy chain constant region referred to herein as HC-65 (GenBank accession no. AF354265);

[00075] SEQ ID NO: 44 is the amino acid sequence for the canine light chain constant region referred to herein as kappa (GenBank Accession No. XP_532962);

[00076] SEQ ID NO: 45 is the nucleotide sequence encoding the canine light chain constant region referred to as kappa (GenBank Accession No. XP_532962);

[00077] SEQ ID NO: 46 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as CAN-34D03-VL-998-1;

[00078] SEQ ID NO: 47 is the nucleotide sequence encoding the variable heavy chain sequence of anti-IL31 antibody referred to herein as CAN-34D03-VH-568-1;

[00079] SEQ ID NO: 48 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VL-cUn-FW2;

[00080] SEQ ID NO: 49 is the nucleotide sequence encoding the variable heavy chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VH-415-1;

[00081] SEQ ID NO: 50 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VL-cUn-13;

[00082] SEQ ID NO: 51 is a variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12_VL_cUn_1;

[00083] SEQ ID NO: 52 is the nucleotide sequence encoding the variable light chain sequence of anti-IL31 antibody referred to herein as CAN-11E12-VL-cUn-1;

[00084] SEQ ID NO: 53 corresponds to the amino acid sequence of the canine IL-31 full-length construct for E. coli expression;

[00085] SEQ ID NO: 54 is the nucleotide sequence corresponding to the canine IL-31 full-length construct for E. coli expression;

[00086] SEQ ID NO: 55 is the nucleotide sequence encoding the variable heavy chain sequence of the anti-NGF antibody referred to herein as ZTS-841;

[00087] SEQ ID NO: 56 is the amino acid sequence encoding the variable heavy chain sequence of the anti-NGF antibody referred to herein as ZTS-841;

[00088] SEQ ID NO: 57 is a variable heavy chain CDR1 of anti-NGF antibody referred to herein as ZTS-841;

[00089] SEQ ID NO: 58 is a variable heavy chain CDR2 of anti-NGF antibody referred to herein as ZTS-841;

[00090] SEQ ID NO: 59 is a variable heavy chain CDR3 of anti-NGF antibody referred to herein as ZTS-841;

[00091] SEQ ID NO: 60 is the nucleotide sequence encoding the variable light chain sequence of the anti-NGF antibody referred to herein as ZTS-841;

[00092] SEQ ID NO: 61 is the amino acid sequence encoding the variable light chain sequence of the anti-NGF antibody referred to herein as ZTS-841;

[00093] SEQ ID NO: 62 is a variable light chain CDR1 of anti-NGF antibody referred to herein as ZTS-841;

[00094] SEQ ID NO: 63 is a variable light chain CDR2 of anti-NGF antibody referred to herein as ZTS-841;

[00095] SEQ ID NO: 64 is a variable light chain CDR3 of anti-NGF antibody referred to herein as ZTS-841;

[00096] SEQ ID NO: 65 is the amino acid sequence of mutant CH3 domain of IgGB positions 341-447;

[00097] SEQ ID NO: 66 is the amino acid sequence of a mutant region within CH3 domain of IgGB;

[00098] SEQ ID NO: 67 is the nucleic acid sequence of a light chain of anti-NGF antibody referred to herein as ZTS-00008183;

[00099] SEQ ID NO: 68 is the amino acid sequence of a light chain of anti-NGF antibody referred to herein as ZTS-00008183;

[000100] SEQ ID NO: 69 is the nucleic acid sequence of a heavy chain of anti-NGF antibody referred to herein as ZTS-00008183;

[000101] SEQ ID NO: 70 is the amino acid sequence of a heavy chain of anti-NGF antibody referred to herein as ZTS-00008183.

DETAILED DESCRIPTION OF THE INVENTION

[000102] The present subject matter may be understood more readily by reference to the following detailed description which forms a part of this disclosure. It is to be understood that this invention is not limited to the specific products, methods, conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed invention.

[000103] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[000104] As employed above and throughout the disclosure, the following terms and abbreviations, unless otherwise indicated, shall be understood to have the following meanings.

Definitions

[000105] In the present disclosure the singular forms "a," "an," and "the" include the plural reference, and reference to a particular numerical value includes at least that particular value, unless the context clearly indicates otherwise. Thus, for example, a reference to “a molecule” or "a compound" is a reference to one or more of such molecules or compounds and equivalents thereof known to those skilled in the art, and so forth. The term "plurality", as used herein, means more than one. When a range of values is expressed, another embodiment includes from the one particular and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it is understood that the particular value forms another embodiment. All ranges are inclusive and combinable.

[000106] In the specification and claims, the numbering of the amino acid residues in an immunoglobulin heavy chain is that of the Eu index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The "Eu index as in Kabat" refers to the residue numbering of the IgG antibody and is reflected herein in FIG. 2.

[000107] The term "isolated" when used in relation to a nucleic acid is a nucleic acid that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is in a form or setting different from that

in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. An isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the polypeptide encoded therein where, for example, the nucleic acid molecule is in a plasmid or a chromosomal location different from that of natural cells. The isolated nucleic acid may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand, but may contain both the sense and anti-sense strands (i.e., may be double-stranded).

[000108] A nucleic acid molecule is "operably linked" or "operably attached" when it is placed into a functional relationship with another nucleic acid molecule. For example, a promoter or enhancer is operably linked to a coding sequence of nucleic acid if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence of nucleic acid if it is positioned so as to facilitate translation. A nucleic acid molecule encoding a variant Fc region is operably linked to a nucleic acid molecule encoding a heterologous protein (i.e., a protein or functional fragment thereof which does not, as it exists in nature, comprise an Fc region) if it is positioned such that the expressed fusion protein comprises the heterologous protein or functional fragment thereof adjoined either upstream or downstream to the variant Fc region polypeptide; the heterologous protein may by immediately adjacent to the variant Fc region polypeptide or may be separated therefrom by a linker sequence of any length and composition. Likewise, a polypeptide (used synonymously herein with "protein") molecule is "operably linked" or "operably attached" when it is placed into a functional relationship with another polypeptide.

[000109] As used herein the term "functional fragment" when in reference to a polypeptide or protein (e.g., a variant Fc region, or a monoclonal antibody) refers to fragments of that protein which retain at least one function of the full-length polypeptide. The fragments may range in size from six amino acids to the entire amino acid sequence of the full-length polypeptide minus one amino acid. A functional fragment of a variant Fc region polypeptide of the present invention retains at least one "amino acid substitution" as herein defined. A functional fragment of a variant Fc region polypeptide retains at least one function known in the art to be associated with the Fc region (e.g., ADCC, CDC, Fc receptor binding, Clq binding, down regulation of cell surface receptors or may, e.g., increase the in vivo or in vitro half-life of a polypeptide to which it is operably attached).

[000110] The term "purified" or "purify" refers to the substantial removal of at least one contaminant from a sample. For example, an antigen-specific antibody may be purified by complete or substantial removal (at least 90%, 91%, 92%, 93%, 94%, 95%, or more preferably at least 96%, 97%, 98% or 99%) of at least one contaminating non-immunoglobulin protein; it may also be purified by the removal of immunoglobulin protein that does not bind to the same antigen. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind a particular antigen results in an increase in the percent of antigen-specific immunoglobulins in the sample. In another example, a polypeptide (e.g., an immunoglobulin) expressed in bacterial host cells is purified by the complete or substantial removal of host cell proteins; the percent of the polypeptide is thereby increased in the sample.

[000111] The term "native" as it refers to a polypeptide (e.g., Fc region) is used herein to indicate that the polypeptide has an amino acid sequence consisting of the amino acid sequence of the polypeptide as it commonly occurs in nature or a naturally occurring polymorphism thereof. A native polypeptide (e.g., native Fc region) may be produced by recombinant means or may be isolated from a naturally occurring source.

[000112] The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism.

[000113] As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (e.g., bacterial cells such as E. coli, CHO cells, yeast cells, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located in vitro or in situ, or in vivo

[000114] As used herein, the term "Fc region" refers to a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the generally accepted boundaries of the Fc region of an immunoglobulin heavy chain might vary, the canine IgG heavy chain Fc region is usually defined to stretch, for example, from an amino acid residue at position 231 to the carboxyl-terminus thereof. In some embodiments, variants comprise only portions of the Fc region and can include or not include the carboxy-terminus. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. In some embodiments, variants having one or more of the constant domains are contemplated. In other embodiments, variants without such constant domains (or with only portions of such constant domains) are contemplated.

[000115] The "CH2 domain" of a canine IgG Fc region usually extends, for example, from about amino acid 231 to about amino acid 340 (see FIG. 2B). The CH2 domain is unique in that it is not closely paired with another domain. Two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule.

[000116] The "CH3 domain" of a canine IgG Fc region generally is the stretch of residues C-terminal to a CH2 domain in an Fc region extending, for example, from about amino acid residue 341 to about amino acid residue 447 (see FIG. 2B).

[000117] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. At least one effector function of a polypeptide comprising a variant Fc region of the present invention may be enhanced or diminished with respect to a polypeptide comprising a native Fc region or the parent Fc region of the variant. Examples of effector functions include, but are not limited to: Clq binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-depended cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions may require the Fc region to be operably linked to a binding domain (e.g., an antibody variable domain) and can be assessed using various assays (e.g., Fc binding assay, ADCC assays, CDC assays, target cell depletion from whole or fractionated blood samples, etc.).

[000118] A "native sequence Fc region" or "wild type Fc region" refers to an amino acid sequence that is identical to the amino acid sequence of an Fc region commonly found in nature. Exemplary native sequence canine Fc regions are shown in FIG. 2 and include a native sequence of canine IgGB_65 Fc region.

[000119] A "variant Fc region" comprises an amino acid sequence that differs from that of a native sequence Fc region (or fragment thereof) by virtue of at least one "amino acid substitution" as defined herein. In preferred embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or in the Fc region of a parent polypeptide, preferably 1, 2, 3, 4 or 5 amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. In an alternative embodiment, a variant Fc region may be generated according to the methods herein disclosed and this variant Fc region can be fused to a heterologous polypeptide of choice, such as an antibody variable domain or a non-antibody polypeptide, e.g., binding domain of a receptor or ligand.

[000120] As used herein, the term "derivative" in the context of polypeptides refers to a polypeptide that comprises and amino acid sequence which has been altered by introduction of

an amino acid residue substitution. The term "derivative" as used herein also refers to a polypeptide which has been modified by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, an antibody may be modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative polypeptide may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative polypeptide possesses a similar or identical function as the polypeptide from which it was derived. It is understood that a polypeptide comprising a variant Fc region of the present invention may be a derivative as defined herein, preferably the derivatization occurs within the Fc region.

[000121] "Substantially of canine origin" as used herein in reference to a polypeptide (e.g., an Fc region or a monoclonal antibody), indicates the polypeptide has an amino acid sequence at least 80%, at least 85%, more preferably at least 90%, 91%, 92%, 93%, 94% or even more preferably at least 95%, 95%, 97%, 98% or 99% homologous to that of a native canine amino polypeptide.

[000122] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to an Fc region (e.g., the Fc region of an antibody). The preferred FcR is a native sequence FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc gamma RI, Fc gamma RII, Fc gamma RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Another preferred FcR includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[000123] The phrase "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells (e.g., nonspecific) that express FcRs (e.g., Natural Killer ("NK") cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cells. The primary cells for mediating ADCC, NK cells, express Fc gamma RIII only, whereas monocytes express Fc gamma RI, Fc gamma RII and Fc gamma RIII.

[000124] As used herein, the phrase "effector cells" refers to leukocytes (preferably canine) which express one or more FcRs and perform effector functions. Preferably, the cells express at least Fc gamma RIII and perform ADCC effector function. Examples of leukocytes which mediate ADCC include PBMC, NK cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source (e.g., from blood or PBMCs).

[000125] A variant polypeptide with "altered" FcRn binding affinity is one which has either enhanced (i.e., increased, greater or higher) or diminished (i.e., reduced, decreased or lesser) FcRn binding affinity compared to the variant's parent polypeptide or to a polypeptide comprising a native Fc region when measured at pH 6.0. A variant polypeptide which displays increased binding or increased binding affinity to an FcRn binds FcRn with greater affinity than the parent polypeptide. A variant polypeptide which displays decreased binding or decreased binding affinity to an FcRn, binds FcRn with lower affinity than its parent polypeptide. Such variants which display decreased binding to an FcRn may possess little or no appreciable binding to an FcRn, e.g., 0-20% binding to the FcRn compared to a parent polypeptide. A variant polypeptide which binds an FcRn with "enhanced affinity" as compared to its parent polypeptide, is one which binds FcRn with higher binding affinity than the parent polypeptide, when the amounts of variant polypeptide and parent polypeptide in a binding assay are essentially the same, and all other conditions are identical. For example, a variant polypeptide with enhanced FcRn binding affinity may display from about 1.10 fold to about 100 fold (more typically from about 1.2 fold to about 50 fold) increase in FcRn binding affinity compared to the parent polypeptide, where FcRn binding affinity is determined, for example, in an ELISA assay or other method available to one of ordinary skill in the art.

[000126] As used herein, an "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a given amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (i.e., encoded by the genetic code) and selected from: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (H is); isoleucine (Ile): leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein. A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino

acid residues (s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al. Meth. Enzym. 202: 301-336 (1991).

[000127] The term "assay signal" refers to the output from any method of detecting protein-protein interactions, including but not limited to, absorbance measurements from colorimetric assays, fluorescent intensity, or disintegrations per minute. Assay formats could include ELISA, facs, or other methods. A change in the "assay signal" may reflect a change in cell viability and/or a change in the kinetic off-rate, the kinetic on-rate, or both. A "higher assay signal" refers to the measured output number being larger than another number (e.g., a variant may have a higher (larger) measured number in an ELISA assay as compared to the parent polypeptide). A "lower" assay signal refers to the measured output number being smaller than another number (e.g., a variant may have a lower (smaller) measured number in an ELISA assay as compared to the parent polypeptide).

[000128] The term "binding affinity" refers to the equilibrium dissociation constant (expressed in units of concentration) associated with each Fc receptor-Fc binding interaction. The binding affinity is directly related to the ratio of the kinetic off-rate (generally reported in units of inverse time, e.g., seconds^{-1}) divided by the kinetic on-rate (generally reported in units of concentration per unit time, e.g., molar/second). In general it is not possible to unequivocally state whether changes in equilibrium dissociation constants are due to differences in on-rates, off-rates or both unless each of these parameters are experimentally determined (e.g., by BIACORE or SAPIDYNE measurements).

[000129] As used herein, the term "hinge region" refers to the stretch of amino acids in canine IgG stretching, for example, from position 216 to position 230 of canine IgG. Hinge regions of other IgG isotypes may be aligned with the IgG sequence by placing the cysteine residues forming inter-heavy chain disulfide (S—S) bonds in the same positions.

[000130] "Clq" is a polypeptide that includes a binding site for the Fc region of an immunoglobulin. Clq together with two serine proteases, Clr and Cls, forms the complex Cl, the first component of the CDC pathway.

[000131] As used herein, the term "antibody" is used interchangeably with "immunoglobulin" or "Ig," is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological

activity or functional activity. Single chain antibodies, and chimeric, canine, or caninized antibodies, as well as chimeric or CDR-grafted single chain antibodies, and the like, comprising portions derived from different species, are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined together chemically by conventional techniques, synthetically, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or caninized chain can be expressed to produce a contiguous protein. See, e.g., U.S. Pat. No. 4,816,567; U.S. Pat. No. 4,816,397; WO 86/01533; U.S. Pat. No. 5,225,539; and U.S. Pat. Nos. 5,585,089 and 5,698,762. See also, Newman, R. et al. BioTechnology, 10: 1455-1460, 1993, regarding primatized antibody, and Ladner et al., U.S. Pat. No. 4,946,778 and Bird, R. E. et al., Science, 242:423-426, 1988, regarding single chain antibodies. It is understood that all forms of the antibodies comprising an Fc region (or portion thereof) are encompassed herein within the term "antibody." Furthermore, the antibody may be labeled with a detectable label, immobilized on a solid phase and/or conjugated with a heterologous compound (e.g., an enzyme or toxin) according to methods known in the art.

[000132] As used herein, the term "antibody fragments" refers to a portion of an intact antibody. Examples of antibody fragments include, but are not limited to, linear antibodies; single-chain antibody molecules; Fc or Fc' peptides, Fab and Fab fragments, and multispecific antibodies formed from antibody fragments. The antibody fragments preferably retain at least part of the hinge and optionally the CH1 region of an IgG heavy chain. In other preferred embodiments, the antibody fragments comprise at least a portion of the CH2 region or the entire CH2 region.

[000133] As used herein, the term "functional fragment", when used in reference to a monoclonal antibody, is intended to refer to a portion of the monoclonal antibody that still retains a functional activity. A functional activity can be, for example, antigen binding activity or specificity, receptor binding activity or specificity, effector function activity and the like. Monoclonal antibody functional fragments include, for example, individual heavy or light chains and fragments thereof, such as VL, VH and Fd; monovalent fragments, such as Fv, Fab, and Fab'; bivalent fragments such as F(ab')2; single chain Fv (scFv); and Fc fragments. Such terms are described in, for example, Harlowe and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1989); Molec. Biology and Biotechnology: A Comprehensive Desk Reference (Myers, R. A. (ed.), New York: VCH Publisher, Inc.); Huston et al., Cell Biophysics, 22:189-224 (1993); Pluckthun and Skerra, Meth. Enzymol., 178:497-515 (1989) and in Day, E. D., Advanced Immunochemistry, Second Ed., Wiley-Liss, Inc., New

York, N.Y. (1990). The term functional fragment is intended to include, for example, fragments produced by protease digestion or reduction of a monoclonal antibody and by recombinant DNA methods known to those skilled in the art.

[000134] As used herein, the term "fragment" refers to a polypeptide comprising an amino acid sequence of at least 5, 15, 20, 25, 40, 50, 70, 90, 100 or more contiguous amino acid residues of the amino acid sequence of another polypeptide. In a preferred embodiment, a fragment of a polypeptide retains at least one function of the full-length polypeptide.

[000135] As used herein, the term "chimeric antibody" includes monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer formed by a chimeric heavy chain associated through disulfide bridges with a chimeric light chain. A divalent chimeric antibody is a tetramer formed by two heavy chain-light chain dimers associated through at least one disulfide bridge. A chimeric heavy chain of an antibody for use in canine comprises an antigen-binding region derived from the heavy chain of a non-canine antibody, which is linked to at least a portion of a canine heavy chain constant region, such as CH1 or CH2. A chimeric light chain of an antibody for use in canine comprises an antigen binding region derived from the light chain of a non-canine antibody, linked to at least a portion of a canine light chain constant region (CL). Antibodies, fragments or derivatives having chimeric heavy chains and light chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to known method steps. With this approach, hosts expressing chimeric heavy chains are separately cultured from hosts expressing chimeric light chains, and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin or fragment or both the heavy and light chains can be expressed in the same host cell. Methods for producing chimeric antibodies are well known in the art (see, e.g., U.S. Pat. Nos. 6,284,471; 5,807,715; 4,816,567; and 4,816,397).

[000136] As used herein, "caninized" forms of non-canine (e.g., murine) antibodies (i.e., caninized antibodies) are antibodies that contain minimal sequence, or no sequence, derived from non-canine immunoglobulin. For the most part, caninized antibodies are canine immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-canine species (donor

antibody) such as mouse, rat, rabbit, human or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the canine immunoglobulin are replaced by corresponding non-canine residues. Furthermore, caninized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are generally made to further refine antibody performance. In general, the caninized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops (CDRs) correspond to those of a non-canine immunoglobulin and all or substantially all of the FR residues are those of a canine immunoglobulin sequence. The caninized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a canine immunoglobulin.

[000137] As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding domain of a heterologous "adhesin" protein (e.g., a receptor, ligand or enzyme) with an immunoglobulin constant domain. Structurally, immunoadhesins comprise a fusion of the adhesin amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site (antigen combining site) of an antibody (i.e., is "heterologous") with an immunoglobulin constant domain sequence.

[000138] As used herein, the term "ligand binding domain" refers to any native receptor or any region or derivative thereof retaining at least a qualitative ligand binding ability of a corresponding native receptor. In certain embodiments, the receptor is from a cell-surface polypeptide having an extracellular domain that is homologous to a member of the immunoglobulin supergenefamily. Other receptors, which are not members of the immunoglobulin supergenefamily but are nonetheless specifically covered by this definition, are receptors for cytokines, and in particular receptors with tyrosine kinase activity (receptor tyrosine kinases), members of the hematopoietin and nerve growth factor receptor superfamilies, and cell adhesion molecules (e.g., E-, L-, and P-selectins).

[000139] As used herein, the term "receptor binding domain" refers to any native ligand for a receptor, including, e.g., cell adhesion molecules, or any region or derivative of such native ligand retaining at least a qualitative receptor binding ability of a corresponding native ligand.

[000140] As used herein, an "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for

the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In certain embodiments, the isolated polypeptide is purified (1) to greater than 95% by weight of polypeptides as determined by the Lowry method, and preferably, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-page under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by a least one purification step.

[000141] As used herein, the term "disorder" and "disease" are used interchangeably to refer to any condition that would benefit from treatment with a variant polypeptide (a polypeptide comprising a variant Fc region of the invention), including chronic and acute disorders or diseases (e.g., pathological conditions that predispose a patient to a particular disorder).

[000142] As used herein, the term "receptor" refers to a polypeptide capable of binding at least one ligand. The preferred receptor is a cell-surface or soluble receptor having an extracellular ligand-binding domain and, optionally, other domains (e.g., transmembrane domain, intracellular domain and/or membrane anchor). A receptor to be evaluated in an assay described herein may be an intact receptor or a fragment or derivative thereof (e.g. a fusion protein comprising the binding domain of the receptor fused to one or more heterologous polypeptides). Moreover, the receptor to be evaluated for its binding properties may be present in a cell or isolated and optionally coated on an assay plate or some other solid phase or labeled directly and used as a probe.

Canine Wildtype IgG

[000143] Canine IgGs are well known in the art and fully described, for example, in Bergeron *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 157 (1-2), pages 31-41. In one embodiment, canine IgG is IgG_A. In another embodiment, canine IgG is IgG_B. In yet another embodiment, canine IgG is IgG_C. In further embodiment, canine IgG is IgG_D. In a particular embodiment, canine IgG is IgG_B_65.

[000144] The amino acid and nucleic acid sequences of IgG_A, IgG_B, IgG_C, and IgG_D are also well known in the art.

[000145] In one example, IgG of the invention comprises a constant domain, for example, CH1, CH2, or CH3 domains, or a combination thereof. In another example, the constant domain of

the invention comprises Fc region, including, for example, CH2 or CH3 domains or a combination thereof.

[000146] In a particular example, the wild-type constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 2. In some embodiments, the wild-type IgG constant domain is a homologue, a variant, an isomer, or a functional fragment of SEQ ID NO.: 2, but without any mutation at position 434. Each possibility represents a separate embodiment of the present invention.

[000147] IgGs contant domains also include polypeptides with amino acid sequences substantially similar to the amino acid sequence of the heavy and/or light chain. Substantially the same amino acid sequence is defined herein as a sequence with at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to a compared amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988).

[000148] The present invention also includes nucleic acid molecules that encode IgGs or portion thereof, described herein. In one embodiment, the nucleic acids may encode an antibody heavy chain comprising, for example, CH1, CH2, CH3 regions, or a combination thereof. In another embodiment, the nucleic acids may encode an antibody heavy chain comprising, for example, any one of the VH regions or a portion thereof, or any one of the VH CDRs, including any variants thereof. The invention also includes nucleic acid molecules that encode an antibody light chain comprising, for example, any one of the CL regions or a portion thereof, any one of the VL regions or a portion thereof or any one of the VL CDRs, including any variants thereof. In certain embodiments, the nucleic acid encodes both a heavy and light chain, or portions thereof.

[000149] The amino acid sequence of the wild-type constant domain set forth in SEQ ID NO.: 2 is encoded by the nucleic acid sequence set forth in in SEQ ID NO.: 4.

Modified Canine IgG

[000150] The inventors of the instant application have found that substituting the amino acid residue asparagine (Asn or N) at position 434 with another amino acid surprisingly and unexpectedly enhanced the affinity to FcRn and increased the half-life of IgG. The terms, position 434, as used herein, refers to a position numbered according to the EU index as in Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

[000151] Accordingly, in one embodiment, the invention provides a modified IgG comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat. The asparagine at position 434 can be substituted with any other amino acid. For example, the asparagine at position 434 can be substituted with with histidine (i.e., N434H), serine (i.e., N434S), alanine (i.e., N434A), phenylalanine (i.e., N434F), glycine (i.e., N434G), isoleucine (i.e., N434I), lysine (i.e., N434K), leucine (i.e., N434L), methionine (i.e., N434M), glutamine (i.e., N434Q), arginine (i.e., N434R), threonine (i.e., N434T), valine (i.e., N434V), tryptophan (i.e., N434W), tyrosine (i.e., N434Y), cysteine (i.e., N434C), aspartic acid (i.e., N434D), glutamic acid (i.e., N434E), or proline (i.e., N434P). In a particular embodiment, the substitution is a substitution with histidine (i.e., N434H).

[000152] In a particular example, the mutant constant domain of the invention comprises the amino acid sequence set forth in SEQ ID NO.: 1. In some embodiments, the mutant IgG constant domain is a homologue, a variant, an isomer, or a functional fragment of SEQ ID NO.: 1, but with mutation at position 434. Each possibility represents a separate embodiment of the present invention.

[000153] The amino acid sequence of the mutant constant domain set forth in SEQ ID NO.: 1 is encoded by its corresponding mutant nucleic acid sequence, for example, a mutant form of the nucleic acid sequence set forth in in SEQ ID NO.: 4.

[000154] In some embodiments, the mutant constant domain of the invention comprises the amino acid sequence set forth in SEQ ID NO.: 65 or 66. In some embodiments, the mutant IgG constant domain is a homologue, a variant, an isomer, or a functional fragment of SEQ ID NO.: 65 or 66, but with mutation at position 434. Each possibility represents a separate embodiment of the present invention.

[000155] The amino acid sequence of the mutant constant domain set forth in SEQ ID NO.: 65 or 66 is encoded by its corresponding mutant nucleic acid sequence.

[000156] In one aspect, the modified IgG of the invention provides the half-life for a period ranging from about 10 days to about 35 days. In one embodiment, the modified IgG of the invention provides the half-life for about 10, 12, 15, 17, 19, 20, 23, 26, 28, 30, 33, or 35 days. In a particular embodiment, the modified IgG of the invention provides the half-life for more than 30 days.

[000157] In one aspect, the modified IgG of the invention maintains a therapeutic serum level for a period ranging from about 1 month to about 7 months. In one embodiment, the modified IgG of the invention maintains a therapeutic serum level for about 7, 14, 28, 56, 84, 112, 140, 168, or 210 days. In a particular embodiment, the modified IgG of the invention maintains a therapeutic serum level for more than 3 months.

Methods for Making Antibody Molecules of the Invention

[000158] Methods for making antibody molecules are well known in the art and fully described in U.S. Patents 8,394,925; 8,088,376; 8,546,543; 10,336,818; and 9,803,023 and U.S. Patent Application Publication 20060067930, which are incorporated by reference herein in their entirety. Any suitable method, process, or technique, known to one of skilled in the art, can be used. An antibody molecule having a variant Fc region of the invention may be generated according to the methods well known in the art. In some embodiments, the variant Fc region can be fused to a heterologous polypeptide of choice, such as an antibody variable domain or binding domain of a receptor or ligand.

[000159] With the advent of methods of molecular biology and recombinant technology, a person of skilled in the art can produce antibody and antibody-like molecules by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with assembly of the synthesized chains to form active tetrameric (H2L2) structures with affinity for specific epitopes and antigenic determinants. This has permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

[000160] Regardless of the source of the antibodies, or how they are recombinantly constructed, or how they are synthesized, in vitro or in vivo, using transgenic animals, large cell cultures of laboratory or commercial size, using transgenic plants, or by direct chemical synthesis employing no living organisms at any stage of the process, all antibodies have a similar overall 3 dimensional structure. This structure is often given as H2L2 and refers to the fact that antibodies commonly comprise two light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or `V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity. The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets.

[000161] As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody binding region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues. Within the variable regions of the H or L chains that provide for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure.

[000162] The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have three CDR regions, each non-contiguous with the others. In all mammalian species, antibody peptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

[000163] The present invention further provides a vector including at least one of the nucleic acids described above. Because the genetic code is degenerate, more than one codon can be used to encode a particular amino acid. Using the genetic code, one or more different nucleotide

sequences can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an antibody or portion. Such "codon usage rules" are disclosed by Lathe, et al., 183 J. Molec. Biol. 1-12 (1985). Using the "codon usage rules" of Lathe, a single nucleotide sequence, or a set of nucleotide sequences that contains a theoretical "most probable" nucleotide sequence capable of encoding canine IgG sequences can be identified. It is also intended that the antibody coding regions for use in the present invention could also be provided by altering existing antibody genes using standard molecular biological techniques that result in variants of the antibodies and peptides described herein. Such variants include, but are not limited to deletions, additions and substitutions in the amino acid sequence of the antibodies or peptides.

[000164] For example, one class of substitutions is conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a canine antibody peptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and lie; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gin, exchange of the basic residues Lys and Arg, replacements among the aromatic residues Phe, Tyr, and the like. Guidance concerning which amino acid changes are likely to be phenotypically silent is found in Bowie *et al.*, 247 *Science* 1306-10 (1990).

[000165] Variant canine antibodies or peptides may be fully functional or may lack function in one or more activities. Fully functional variants typically contain only conservative variations or variations in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

[000166] Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. Cunningham *et al.*, 244

Science 1081-85 (1989). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as epitope binding or in vitro ADCC activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallography, nuclear magnetic resonance, or photoaffinity labeling. Smith *et al.*, 224 *J. Mol. Biol.* 899-904 (1992); de Vos *et al.*, 255 *Science* 306-12 (1992).

[000167] Moreover, polypeptides often contain amino acids other than the twenty "naturally occurring" amino acids. Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques well known in the art. Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Such modifications are well known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP ribosylation, for instance, are described in most basic texts, such as Proteins-Structure and Molecular Properties (2nd ed., T. E. Creighton, W. H. Freeman & Co., N.Y., 1993). Many detailed reviews are available on this subject, such as by Wold, Posttranslational Covalent Modification of proteins, 1-12 (Johnson, ed., Academic Press, N.Y., 1983); Seifter *et al.* 182 *Meth. Enzymol.* 626-46 (1990); and Rattan *et al.* 663 *Ann. NY Acad. Sci.* 48-62 (1992).

[000168] In another aspect, the invention provides antibody derivatives. A "derivative" of an antibody contains additional chemical moieties not normally a part of the protein. Covalent modifications of the protein are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. For example, derivatization with bifunctional agents, well-known in the art,

is useful for cross-linking the antibody or fragment to a water-insoluble support matrix or to other macromolecular carriers.

[000169] Derivatives also include radioactively labeled monoclonal antibodies that are labeled. For example, with radioactive iodine (251,1311), carbon (4C), sulfur (35S), indium, tritium (H^3) or the like; conjugates of monoclonal antibodies with biotin or avidin, with enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-D-galactosidase, glucose oxidase, glucoamylase, carboxylic acid anhydrase, acetylcholine esterase, lysozyme, malate dehydrogenase or glucose 6-phosphate dehydrogenase; and also conjugates of monoclonal antibodies with bioluminescent agents (such as luciferase), chemoluminescent agents (such as acridine esters) or fluorescent agents (such as phycobiliproteins).

[000170] Another derivative bifunctional antibody of the invention is a bispecific antibody, generated by combining parts of two separate antibodies that recognize two different antigenic groups. This may be achieved by crosslinking or recombinant techniques. Additionally, moieties may be added to the antibody or a portion thereof to increase half-life in vivo (e.g., by lengthening the time to clearance from the blood stream. Such techniques include, for example, adding PEG moieties (also termed pegylation), and are well-known in the art. See U.S. Patent. Appl. Pub. No. 20030031671.

[000171] In some embodiments, the nucleic acids encoding a subject antibody are introduced directly into a host cell, and the cell is incubated under conditions sufficient to induce expression of the encoded antibody. After the subject nucleic acids have been introduced into a cell, the cell is typically incubated, normally at 37° C., sometimes under selection, for a period of about 1-24 hours in order to allow for the expression of the antibody. In one embodiment, the antibody is secreted into the supernatant of the media in which the cell is growing. Traditionally, monoclonal antibodies have been produced as native molecules in murine hybridoma lines. In addition to that technology, the present invention provides for recombinant DNA expression of the antibodies. This allows the production of antibodies, as well as a spectrum of antibody derivatives and fusion proteins in a host species of choice.

[000172] A nucleic acid sequence encoding at least one antibody, portion or polypeptide of the invention may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for

such manipulations are disclosed, e.g., by Maniatis et al., MOLECULAR CLONING, LAB. MANUAL, (Cold Spring Harbor Lab. Press, NY, 1982 and 1989), and Ausubel et al. 1993 supra, may be used to construct nucleic acid sequences which encode an antibody molecule or antigen binding region thereof.

[000173] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as peptides or antibody portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook *et al.*, 2001 supra; Ausubel *et al.*, 1993 supra.

[000174] The present invention accordingly encompasses the expression of an antibody or peptide, in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and mammalian cells either in vivo, or in situ, or host cells of mammalian, insect, bird or yeast origin. The mammalian cell or tissue may be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin. Any other suitable mammalian cell, known in the art, may also be used.

[000175] In one embodiment, the nucleotide sequence of the invention will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. See, e.g., Ausubel *et al.*, 1993 supra. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[000176] Example prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, CoIE1, pSC101, pACYC 184, .pi.vX). Such plasmids are, for example, disclosed by Maniatis et aI., 1989 supra; Ausubel et al, 1993 supra. Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, in THE MOLEC. BIO. OF THE BACILLI 307-329 (Academic Press, NY, 1982).

Suitable Streptomyces plasmids include p1J101 (Kendall et al., 169 J. Bacteriol. 4177-83 (1987), and Streptomyces bacteriophages such as phLC31 (Chater et al., in SIXTH INT'L SYMPOSIUM ON ACTINOMYCETALES BIO. 45-54 (Akademiai Kaido, Budapest, Hungary 1986). Pseudomonas plasmids are reviewed in John et aI., 8 Rev. Infect. Dis. 693-704 (1986); lzaki, 33 Jpn. J. Bacteriol. 729-42 (1978); and Ausubel et aI., 1993 supra.

[000177] Alternatively, gene expression elements useful for the expression of cDNA encoding antibodies or peptides include, but are not limited to, (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter (Okayama et aI., 3 Mol. Cell. Biol. 280 (1983), Rous sarcoma virus LTR (Gorman et aI., 79 Proc. Natl. Acad. Sci., USA 6777 (1982), and Moloney murine leukemia virus LTR (Grosschedl et aI., 41 Cell 885 (1985); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayarea et aI., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et aI., 1983).

[000178] Immunoglobulin cDNA genes can be expressed as described by Weidle *et al.*, 51 Gene 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements. For immunoglobulin genes comprised of part cDNA, part genomic DNA (Whittle et aI., 1 Protein Engin. 499 (1987)), the transcriptional promoter can be human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions can be the native chromosomal immunoglobulin sequences.

[000179] In one embodiment, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[000180] Each fused gene can be assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the immunoglobulin chain gene product are then transfected singly with a peptide or H or L chain-encoding gene, or are co-transfected with H and L chain gene.

The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

[000181] In one embodiment, the fused genes encoding the peptide or H and L chains, or portions thereof are assembled in separate expression vectors that are then used to cotransfect a recipient cell. Alternatively the fused genes encoding the H and L chains can be assembled on the same expression vector. For transfection of the expression vectors and production of the antibody, the recipient cell line may be a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid. Other suitable recipient cells include lymphoid cells such as B lymphocytes of canine or non-canine origin, hybridoma cells of canine or non-canine origin, or interspecies heterohybridoma cells.

[000182] The expression vector carrying an antibody construct or polypeptide of the invention can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment. Johnston *et al.*, 240 *Science* 1538 (1988).

[000183] Yeast may provide substantial advantages over bacteria for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides). Hitzman *et al.*, 11th Int'l Conference on Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982).

[000184] Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of peptides, antibodies, fragments and regions thereof. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also

provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast. See Vol. II DNA Cloning, 45-66, (Glover, ed.,) IRL Press, Oxford, UK 1985).

[000185] Bacterial strains can also be utilized as hosts for the production of antibody molecules or peptides described by this invention. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of antibodies, fragments and regions or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, 1985 supra; Ausubel, 1993 supra; Sambrook, 2001 supra; Colligan et al., eds. Current Protocols in Immunology, John Wiley & Sons, NY, N.Y. (1994-2001); Colligan et al., eds. Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y. (1997-2001).

[000186] Host mammalian cells may be grown in vitro or in vivo. Mammalian cells provide posttranslational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of Hand L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein. Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61) cells. Many vector systems are available for the expression of cloned peptides Hand L chain genes in mammalian cells (see Glover, 1985 supra). Different approaches can be followed to obtain complete H2L2 antibodies. It is possible to co-express Hand L chains in the same cells to achieve intracellular association and linkage of Hand L chains into complete tetrameric H2L2 antibodies and/or peptides. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both Hand L chains and/or peptides can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an H chain plasmid containing a second selectable marker. cell lines producing peptides and/or H2L2 molecules via either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable markers to

generate cell lines with enhanced properties, such as higher production of assembled H2L2 antibody molecules or enhanced stability of the transfected cell lines.

[000187] For long-term, high-yield production of recombinant antibodies, stable expression may be used. For example, cell lines, which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with immunoglobulin expression cassettes and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and grow to form foci which in turn can be cloned and expanded into cell lines. Such engineered cell lines may be particularly useful in screening and evaluation of compounds/components that interact directly or indirectly with the antibody molecule.

[000188] Once an antibody of the invention has been produced, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In many embodiments, antibodies are secreted from the cell into culture medium and harvested from the culture medium.

[000189] In another aspect, the invention provides an antibody comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434. In one embodiment the substitution is a substitution of asparagine at position 434 with histidine (N434H).

[000190] The antibody having the substitution can be any suitable antibody known to one of skilled in the art. In one example, the antibody is an anti-IL31 antibody. In another example, the antibody is an anti-NGF antibody.

[000191] Anti-IL31 antibody, without the substitution described herein, is well known in the art and fully described in, for example, U.S. Patents 10,526,405; 10,421,807; 9,206,253; and 8,790,651. Also, anti-NGF antibody, without the substitution described herein, is also well known in the art and fully described in, for example, U.S. Patents 10,125,192; 10,093,725; 9,951,128; 9,617,334; and 9,505,829.

[000192] In one embodiment, the anti-IL31 antibody of the invention (i.e., antibody having the substitution) reduces, inhibits, or neutralizes an IL-31-mediated pruritic or allergic condition. In another embodiment, the anti-IL31 antibody of the invention reduces, inhibits, or neutralizes IL-31 activity in a dog.

[000193] In one example, the anti-IL31 antibody of the invention binds to IL-31 at a region between about amino acid residues 95 and 125 of the canine IL-31 amino acid sequence of SEQ ID NO: 44, preferably at a region between about amino acid residues 102 and 122 of the canine IL-31 sequence of SEQ ID NO: 44.

[000194] VL, VH, and CDR sequences of the anti-IL31 antibodies are well known in the art and fully described in, for example, U.S. Patents 10,526,405; 10,421,807; 9,206,253; and 8,790,651. In one example, the anti-IL31 antibody of the invention may include at least one of the following combinations of complementary determining region (CDR) sequences: (1) 11E12: variable heavy (VH)-CDR1 of SEQ ID NO: 13, VH-CDR2 of SEQ ID NO: 15, VH-CDR3 of SEQ ID NO: 17, variable light (VL)-CDR1 of SEQ ID NO: 19, VL-CDR2 of SEQ ID NO: 21, and VL-CDR3 of SEQ ID NO: 23; or (2) 34D03: VH-CDR1 of SEQ ID NO: 14, VH-CDR2 of SEQ ID NO: 16, VH-CDR3 of SEQ ID NO: 18, VL-CDR1 of SEQ ID NO: 20, VL-CDR2 of SEQ ID NO: 22, and VL-CDR3 of SEQ ID NO: 24. In some embodiments, the anti-IL31 antibody of the invention may include at least one CDR described herein.

[000195] In one embodiment, the anti-IL31 antibody of the invention may include a variable light chain comprising the amino acid sequence set forth in SEQ ID NO: 25 (MU-11E12-VL), SEQ ID NO: 26 (CAN-11E12-VL-cUn-FW2), SEQ ID NO; 27 (CAN-11E12-VL-cUn-13), SEQ ID NO: 28 (MU-34D03-VL) or SEQ ID NO: 29 (CAN-34D03-VL-998-1).

[000196] In another embodiment, the anti-IL31 antibody of the invention may include a variable heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 30 (MU-11E12-VH), SEQ ID NO: 31 (CAN-11E12-VH-415-1), SEQ ID NO: 32 (MU-34D03-VH) or SEQ ID NO: 33 (CAN-34D03-VH-568-1).

[000197] In one embodiment, the mutant anti-NGF antibody of the invention (i.e., antibody having the substitution) reduces, inhibits, or neutralizes NGF activity in an animal, and/or enhanced ability to inhibit NGF binding to Trk A and p75, in order to treat an NGF-mediated pain or condition.

[000198] VL, VH, and CDR sequences of the anti-NGF antibodies are also well known in the art and fully described in, for example, U.S. Patents 10,125,192; 10,093,725; 9,951,128;

9,617,334; and 9,505,829. In one example, the anti-NGF antibody of the invention may include at least one of the following complementary determining region (CDR) sequences: ZTS-841: variable heavy (VH)-CDR1 of SEQ ID NO: 57, VH-CDR2 of SEQ ID NO: 58, VH-CDR3 of SEQ ID NO: 59, variable light (VL)-CDR1 of SEQ ID NO: 62, VL-CDR2 of SEQ ID NO: 63, and VL-CDR3 of SEQ ID NO: 64. In some embodiments, VL-CDR2 has GNG residues of SEQ ID NO: 63.

[000199] In one embodiment, the anti-NGF antibody of the invention may include a variable light chain comprising the amino acid sequence set forth in SEQ ID NO: 61 (CAN-ZTS-841-VL).

[000200] In another embodiment, the anti-NGF antibody of the invention may include a variable heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 56 (CAN-ZTS-841-VH).

Pharmaceutical and Veterinary Applications

[000201] The invention also provides a pharmaceutical composition comprising molecules of the invention and one or more pharmaceutically acceptable carriers. More specifically, the invention provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody or peptide according to the invention.

[000202] “Pharmaceutically acceptable carriers” include any excipient which is nontoxic to the cell or animal being exposed thereto at the dosages and concentrations employed. The pharmaceutical composition may include one or additional therapeutic agents.

[000203] "Pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio.

[000204] Pharmaceutically acceptable carriers include solvents, dispersion media, buffers, coatings, antibacterial and antifungal agents, wetting agents, preservatives, buggers, chelating agents, antioxidants, isotonic agents and absorption delaying agents.

[000205] Pharmaceutically acceptable carriers include water; saline; phosphate buffered saline; dextrose; glycerol; alcohols such as ethanol and isopropanol; phosphate, citrate and other

organic acids; ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; EDTA; salt forming counterions such as sodium; and/or nonionic surfactants such as TWEEN, polyethylene glycol (PEG), and PLURONICS; isotonic agents such as sugars, polyalcohols such as mannitol and sorbitol, and sodium chloride; as well as combinations thereof.

[000206] The pharmaceutical compositions of the invention may be formulated in a variety of ways, including for example, liquid, semi-solid, or solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes, suppositories, tablets, pills, or powders. In some embodiments, the compositions are in the form of injectable or infusible solutions. The composition can be in a form suitable for intravenous, intraarterial, intramuscular, subcutaneous, parenteral, transmucosal, oral, topical, or transdermal administration. The composition may be formulated as an immediate, controlled, extended or delayed release composition.

[000207] The compositions of the invention can be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. Administration of the antibodies disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), orally, or by topical administration of the antibodies (typically carried in a pharmaceutical formulation) to an airway surface. Topical administration to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler). Topical administration of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed.

[000208] In some desired embodiments, the antibodies are administered by parenteral injection. For parenteral administration, antibodies or molecules can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. For example, the vehicle may be a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, such as an aqueous carrier such vehicles are water, saline, Ringer's solution, dextrose solution, trehalose or sucrose solution, or 5% serum albumin, 0.4% saline, 0.3% glycine and the like. Liposomes and nonaqueous vehicles such as fixed oils can also be used. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15% or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, REMINGTON'S PHARMA. SCI. (15th ed., Mack Pub. Co., Easton, Pa., 1980).

[000209] The antibodies or molecules of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss and that use levels may have to be adjusted to compensate. The compositions containing the present antibodies or a cocktail thereof can be administered for prevention of recurrence and/or therapeutic treatments for existing disease. Suitable pharmaceutical carriers are described in the most recent edition of REMINGTON'S PHARMACEUTICAL SCIENCES, a standard reference text in this field of art. In therapeutic application, compositions are administered to a subject already suffering

from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications.

[000210] Effective doses of the compositions of the present invention, for treatment of conditions or diseases as described herein vary depending upon many different factors, including, for example, but not limited to, the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; target site; physiological state of the animal; other medications administered; whether treatment is prophylactic or therapeutic; age, health, and weight of the recipient; nature and extent of symptoms kind of concurrent treatment, frequency of treatment, and the effect desired.

[000211] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating veterinarian. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the subject. In an exemplary embodiment, the composition of the invention is administered bimonthly, once-in-three months, once-in-four months, once-in-five months, once-in-six months, or once-in-seven months.

[000212] Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[000213] The pharmaceutical compositions of the invention may include a "therapeutically effective amount." A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the molecule to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the molecule are outweighed by the therapeutically beneficial effects.

[000214] In another aspect, the compositions of the invention can be used, for example, in the treatment of various diseases and disorders in dogs. As used herein, the terms "treat" and "treatment" refer to therapeutic treatment, including prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change associated with a disease or condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or condition, stabilization of a disease or condition (i.e., where the disease or condition does not worsen),

delay or slowing of the progression of a disease or condition, amelioration or palliation of the disease or condition, and remission (whether partial or total) of the disease or condition, whether detectable or undetectable. Those in need of treatment include those already with the disease or condition as well as those prone to having the disease or condition or those in which the disease or condition is to be prevented.

[000215] The mutant molecule of the invention can be used to treat any suitable disease or disorder. For example, the mutant anti-IL31 antibody of the invention can be used to treat an IL-31-mediated pruritic or allergic condition. The examples of IL-31-mediated pruritic condition include, for example, but not limited to, atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis. The examples of IL-31-mediated allergic condition include, for example, but not limited to, allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstruction pulmonary disease, and inflammatory processes resulting from autoimmunity.

[000216] The mutant anti-NGF antibody of the invention can be used to treat an NGF-mediated pain or a condition. The examples of a pain include, for example, but not limited to, a chronic pain, an inflammatory pain, a post-operative incision pain, a neuropathic pain, a fracture pain, an osteoporotic fracture pain, a post-herpetic neuralgia, a cancer pain, a pain resulting from burns, a pain associated with wounds, a pain associated with trauma, a neuropathic pain, a pain associated with a musculoskeletal disorder, a rheumatoid arthritis, an osteoarthritis, an ankylosing spondylitis, a seronegative (non-rheumatoid) an arthropathies, a non-articular rheumatism, a periarticular disorder, or a peripheral neuropathy. In a particular embodiment, the pain is an osteoarthritis pain.

[000217] All patents and literature references cited in the present specification are hereby incorporated by reference in their entirety.

[000218] The following examples are provided to supplement the prior disclosure and to provide a better understanding of the subject matter described herein. These examples should not be considered to limit the described subject matter. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be apparent to persons skilled in the art and are to be included within, and can be made without departing from, the true scope of the invention.

EXAMPLES

EXAMPLE 1

Construction of canine IgG Fc mutants

[000219] Construction of all canine IgGs (**FIG. 1**) was carried out as described by Bergeron *et al.* (Bergeron *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 157(1-2), pages 31-41), in which plasmids containing sequence encoding for canine constant regions for the IgGB (65) sub-class were utilized and VH/VL sequences for each mAb investigated herein were inserted upstream and in frame with the nucleotides encoding for the constant domains. Mutations were incorporated into position N434 of the CH3 domain (**FIG. 2**) of each plasmid using Agilent's QuikChange II Mutagenesis and associated Agilent primer design tools for single-site directed mutagenesis (https://www.agilent.com/store/primerDesignProgram.jsp).

[000220] Antibody constructs were transiently expressed either in HEK 293 cells using a standard lipofectamine transfection protocol (Invitrogen Life Technologies, Carlsbad, CA, USA) or into CHO cells using the ExpiCHO transient system (ThermoFisher Scientific) kit protocols. ExpiCHO expression followed protocols outlined by ThermoFisher for a co-transfection of plasmid containing gene sequence encoding for an IgG light and an IgG heavy chain. For HEK293 expression, equal amounts by weight of heavy chain plasmid and light chain plasmid were co-tranfected. Cells were allowed to grow for 7 days after which supernatants were collected for antibody purification. Antibodies were screened for binding to protein A or protein G sensors via Octet QKe quantitation (Pall ForteBio Corp, Menlo Park, CA, USA). Constructs which bound to protein A were purified and quantified as described in Bergeron et al. for protein quality.

EXAMPLE 2

Target binding affinity and potency assay

[000221] Affinity for each mAb was assessed by Biacore and the IC50 was determined via a suitable cell based potency assay. Surface Plasmon Resonance was performed on a Biacore T200 (GE Healthcare, Pittsburgh, PA) to measure binding affinities of each antibody to its target, 2.5 μg/ml of each target protein was immobilized by amine coupling using EDC/NHS for a final density ~250 RU (resonance unit) on CM5 sensor flow cells 2–4, respectively.

[000222] Flow cell 1 was used as an internal reference to correct running buffer effects. Antibody binding was measured at 15°C with a contact time of 250 s and flow rate of 30μl/min. The dissociation period was 300 s. Regeneration was performed with regeneration buffers (10 mM Glycine pH1.5 and 10 mM NaOH) and flow rate at 20μl/min for 60 s each. Running/dilution buffer (1X HBS-EP, GE Healthcare, BR-1006-69, 10X including 100 mM HEPES, 150 mM NaCl, 30 mM EDTA and 0.5% v/v surfactant P20, pH7.4, 1:10 in filtered MQ H2O) was used as negative control at the same assay format.

[000223] Data were analyzed with Biacore T200 Evaluation software by using the method of double referencing. The resulting curve was fitted with the 1:1 binding model. No differences in binding affinities or IC50 were observed between wild-type and N434H mutant IgGs (**Table 1**).

Table 1. Affinities and potencies of WT and N434H mutant IgGs. No differences were measured between the WT and mutant IgGs:

	Wild-Type		**N434H Mutant**	
mAb	**Affinity**	**IC50**	**Affinity**	**IC50**
mAb1	4.83E-10	1.1 nM	3.73E-10	0.99 nM
mAb2	8.51E-11	23.7 nM	5.55E-11	20.9 nM

mAb1 and mAb2 refer to caninized anti-IL31 and anti-NGF antibodies, respectively. Anti-IL31 antibody is well known in the art. *See e.g.*, U.S. Patents 10,526,405; 10,421,807; 9,206,253; 8,790,651. Anti-NGF antibody is also well known in the art. *See e.g.*, U.S. Patents 10,125,192; 10,093,725; 9,951,128; 9,617,334; and 9,505,829.

EXAMPLE 3

In vitro FcRn Binding assay

[000224] Canine FcRn was isolated, prepared and mutant Fc IgGs were assayed against canine FcRn according to Bergeron *et.al.*, discussed above. Standard PCR was used to amplify canine FcRn-α subunit and β-microglobulin using degenerate primers designed from sequence alignments from cynomolgus monkey, human, mouse and rat. Primers contained HindIII at 3 prime and BamH1 at the 5 prime ends to facilitate subcloning into pcDNA3.1(+) vectors, engineered with a c-terminal 6x His + BAP tag (AGLNDIFEAQKIEWHE). FcRn-α subunit and β-microglobulin were co-transfected into HEK 293 cells and the FcRn complex was purified by IMAC affinity purification via the c-terminal His tag. KD's were measured by Biacore 3000 or Biacore T200 (GE Healthcare, Pittsburgh, PA, USA) using a CM5 sensor chip.

[000225] FcRn was immobilized on the surface of the sensor using the standard amine immobilization method to reach the desired surface density. HBS-EP was used as the immobilization running buffer and 10mM MES; 150mM NaCl; 0.005% Tween20; 0.5 mg/mL BSA; pH6 and pH7.2 and PBS; 0.005% Tween20; 0.5 mg/mL BSA; pH7.4 were used for method running buffers and titrations. Fc mutant IgGs were flowed over receptor surfaces and affinity was determined using Scrubber2 software analysis (BioLogic Software Pty, Ltd., Campbell, Australia) or T200 evaluation software (**Table 2**). Blank runs containing buffer only were subtracted out from all runs. Flow cells were regenerated using 50 mM Tris pH8. Runs were performed at 15 °C.

[000226] Mutations made at position 434 for mAbs 1 and 2 have a marked effect on the affinity of the IgG to FcRn at pH6. The mutation N434H has a significant increase in FcRn affinity at pH6, while maintaining weak affinity at pH 7.2 for all three mAbs. Extensive mutagenesis at position 434 reveals that several other mutations have increase affinity for FcRn at pH6. This study reveals that the increase in FcRn affinity for IgG is not dependent on the VHVL domains, and is universal for any canine IgGB (65).

Table 2. Binding of wild-type (WT) and N434 mutant IgGs to Canine FcRn measured by surface plasmon resonance (Biacore):

Name	Mutation	FcRn pH6	FcRn pH7.2
mAb1	WT	15-24.4 nM	NBO

Name	Mutation	FcRn pH6	FcRn pH7.2
mAb1	N434H	0.7-1.3 nM	5 μM
mAb2	WT	57.2 nM	NBO
mAb2	N434H	7.3 nM	1.3 μM

mAb1 and mAb2 refer to caninized anti-IL31 and anti-NGF antibodies, respectively; NBO = No binding Observed.

EXAMPLE 4

Fc mutant IgG PK Studies in dogs

[000227] The objective of the study was to determine the pharmacokinetics of 2 IgG monoclonal antibodies in dogs (mAb1 and mAb2), raised against two distinct and different targets with the Fc mutant N434H incorporated into each IgG.

[000228] For the mAb1 WT and N434H mutant IgGs, groups of 4 male beagle dogs were administered a single 2 mg/kg dose intravenously. For the mAb2 WT and mutant IgGs, groups of 4 male and 4 female beagle dogs were administered three 2 mg/kg doses of one of the IgGs at 28 day intervals. The first two doses were subcutaneously administered and the last dose was intravenously administered. 'Free' IgGs in canine serum were assayed using validated ligand binding assays specific to each IgG and automated on the Gyrolab xP™ platform (**Figs. 3-6**). Pharmacokinetic calculations were performed using the noncompartmental approach (linear trapezoidal rule for AUC calculations) with the aid of Watson™ (**Table 3**). For the mAb2 IgGs, half-lives were estimated for the first and second doses using the time points from 7 to 28 days after dosing. Half-lives for the last dose were estimated using time points from 7 to 42 days after dosing. Additional calculations were performed with Excel™, including correction of the AUC for the overlap of the concentration-time profiles after the 2nd and 3rd injections of drug. Summaries of concentration-time data and pharmacokinetic data with simple statistics (mean, standard deviation, coefficient of variation) were calculated using Excel™ or Watson™. No other statistical analyses were conducted.

Table 3. Calculated Half-Life's for wild-type and N434H mutant canine IgGs:

IgG	Half-Life (days)
mAb1 WT	9.7 +/- 2.6
mAb1 N434H	17.1 +/- 5.1
mAb2 WT	9.2 +/- 1.7

IgG	Half-Life (days)
mAb2 N434H	19.3 +/- 3.1

mAb1 and mAb2 refer to caninized anti-IL31 and anti-NGF antibodies, respectively.

[000229] The canine IgGB (65) point mutation N434H has been shown to increase the half-life of two different canine IgGs in beagle dogs. For mAb1 the half-life increased from 9.7+/-2.6 days to 17.1+/- 5.1 days, and for Ma2b2 from 9.2+/- 1.7 to 19.3 +/- 3.1. The mechanism of action is via enhancing affinity to canine FcRn at pH6 and it has been demonstrated with three canine IgGs, that bind very different and distinct soluble targets. Therefore, it has been demonstrated that the half-life extension of N434 mutations of IgGB (65) is independent of the VHVL domains.

EXAMPLE 5

FcRn Binding assay

[000230] Canine FcRn was isolated, prepared and mutant Fc IgGs were assayed against canine FcRn according to Bergeron *et.al.*, discussed above. Standard PCR was used to amplify canine FcRn-α subunit and β-microglobulin. FcRn-α subunit and β-microglobulin were co-transfected into HEK 293 cells and the FcRn complex was purified by IMAC affinity purification via the c-terminal His tag. FcRn complex was biotin labeled through BirA enzymatic biotinylatoin reaction. KD's were measured by Biacore T200 (GE Healthcare, Pittsburgh, PA, USA) or Biacore 8K (Cytiva, Marlborough, MA, USA) using a SA sensor chip.

[000231] FcRn was captured on the surface of the sensor using a modified SA capture method. 10mM MES; 150mM NaCl; 0.005% Tween20; 0.5 mg/mL BSA; pH6 was used as capture, method running running buffer and titrations. 1x HBS-P, 0.5 mg/mL BSA; pH7.4 was also used for method running buffer and titrations. Fc mutant IgGs were flowed over receptor surfaces and affinity was determined using T200 evaluation software or Biacore Insight Evaluation software. Blank runs containing buffer only were subtracted out from all runs. Flow cells were regenerated using 50 mM Tris pH8 or pH9. Runs were performed at 15 °C.

[000232] Mutations made at respective positions have a marked effect on the affinity of the IgG to FcRn at pH6. Binding of wild-type (WTs) and mutant IgGs to canine FcRn were measured by surface plasmon resonance (Biacore).

[000233] The marked effect on the affinity was observed in completely different and structurally different antibodies that bind different targets (i.e., anti-IL31 and anti-NGF

antibodies) and also different versions of antibodies that bind the same target (i.e., different versions of anti-IL31 and anti-NGF antibodies) (Tables 1-4) . Therefore, the increase in FcRn affinity for IgG is not dependent on the VHVL domains or CDR regions. In addition, the marked effect on the affinity was observed in multiple IgG subclasses although there is a slight variation. Generally, the results show that the increase in FcRn affinity for IgG is independent of canine IgG subclass.

Table 4A. Binding of wild-type (WT) and N434 mutant IgGs to Canine FcRn

Mutation	canine IgG subclass	Target 1 + mAb1			Target 2 + mAb2		
		ID No.	KD pH6	KD pH7.4	ID No.	KD pH6	KD pH7.4
WT	IgGA	1	7.99E-08	NBO	39	9.55E-08	NBO
N434F	IgGA	2	1.27E-08	NBO	40	9.81E-09	NBO
N434H	IgGA	3	1.23E-08	NBO	41	1.04E-08	NBO
N434R	IgGA	4	4.38E-08	NBO	42	1.51E-08	NBO
N434S	IgGA	5	NBO	NBO	43	1.47E-07	NBO
N434W	IgGA	6	1.99E-08	NBO	44	1.06E-08	1.52E-06
N434Y	IgGA	7	2.52E-08	5.73E-07	45	1.43E-08	9.53E-06
WT	IgGB	8	1.99E-08	1.87E-05	46	9.15E-08	NBO
N434A	IgGB	9	1.39E-07	1.75E-07	47	9.81E-09	NBO
N434F	IgGB	10	3.07E-08	3.37E-05	48	1.92E-09	NBO
N434G	IgGB	11	1.18E-07	2.60E-05	49	3.43E-08	NBO
N434I	IgGB	12	7.71E-08	1.96E-05	50	2.76E-09	2.28E-05
N434K	IgGB	13	9.11E-09	7.86E-09	51	1.93E-09	5.94E-07
N434L	IgGB	14	1.43E-08	9.97E-07	52	1.83E-09	NBO
WT	IgGC	35	1.00E-07	NBO	53	5.20E-08	NBO
N434H	IgGC	36	8.79E-08	NBO	54	2.04E-08	NBO
WT	IgGD	37	3.45E-08	NBO	55	7.10E-08	NBO
N434H	IgGD	38	3.21E-09	NBO	56	1.20E-08	NBO

mAb1 and mAb2 refer to caninized anti-IL31 (34D03) and anti-NGF (ZTS-841) antibodies, respectively. mAb1 in this table and Tables 1-3 above are the same (i.e., 34D03). However, mAb2 in this table is ZTS-841 anti-NGF antibody which has different VL, VH, and CDR regions, relative to mAb2 antibody listed in Tables 1-3; NBO = No binding Observed.

Table 4B. Binding of wild-type (WT) and N434 mutant IgGs to Canine FcRn

Mutations	canine IgG subclass	Target 1 + mAb1		
		ID No.	KD pH6	KD pH7.4
N434M	IgGB	15	1.42E-08	1.46E-04
N434Q	IgGB	16	5.91E-09	2.18E-08
N434R	IgGB	17	9.06E-10	3.11E-09
N434S	IgGB	18	2.02E-08	6.96E-06
N434T	IgGB	19	3.42E-08	5.54E-07
N434V	IgGB	20	6.16E-09	2.75E-06
N434W	IgGB	21	1.98E-09	2.53E-05
N434Y	IgGB	22	2.71E-09	1.90E-06
N434H	IgGB	31	6.45E-09	NBO
WT 2	IgGB	33	4.00E-08	NBO
N434H	IgGB	34	1.00E-09	8.39E-05

mAb1 refers to caninized anti-IL31 antibody. ID Numbers 15-21 and 31 correspond to 34D03 anti-IL31 antibody. ID numbers 33 and 34 correspond to 11E12 anti-IL31 antibody.

EXAMPLE 6

Fc Mutant IgG PK Studies in dogs

The objective of the study was to determine effectiveness of a dose of 4.0 mg/kg of ZTS-00008183 based on efficacy in a canine induced-pruritus model where efficacy was measured by assessing reduction in pruritus for up to 210 days following administration of Test Article on Day 0. The term "ZTS-00008183," as used herein refers an anti-IL31 antibody having N434H mutation in its constant region. ZTS-00008183 has the variable regions (i.e., VL and VH including CDRs) of 34D03, described herein.

[000234] ZTS-00008183 or placebo were administered by single SC injection to Beagle dogs (age at ~4 years old). The treatments are summarized below.

Table 5. Treatment Summary

Treatment Group	Route	Dose (mg/kg)	Compound	Formulation Concentration (mg/mL)	N
T01	SC	0	Placebo	0	8
T02	SC	4.0	ZTS-00008183	40	8

[000235] Serum samples were collected at pre-dose (day -7) and on days 7, 14, 28, 56, 84, 112, 140, 168 and 210 following drug administration.

[000236] Specifically, beagle dogs (n=8, age about 4 years old at the dosing date) were treated with a single subcutaneous administration of 4 mg/kg ZTS-00008183 in IL-31 induced pruritus challenge study. Serum samples were collected at predose (day -7), and on days 7, 14, 28, 56, 84, 112, 140, 168 and 210 following drug administration.

[000237] The test mAbs were quantified using ligand bind assays. Anti-drug antibody (ADA) was evaluated with qualified ADA assay methods.

[000238] Bioanalytical data were received from BioAgilytix Labs as Excel™ spreadsheets. The data were imported into Watson™ v.7.4.1. Toxicokinetic and pharmacokinetic parameters (C_{max}, t_{max}, AUC, AUCextrap and $t_{1/2}$) were calculated using the non-compartmental approach with the aid of Watson™ v.7.4.1. ZTS-00008183 half-lives were estimated for groups T02 using the timepoints from 56 to 210 days post dose. Immunogenicity was evaluated.

[000239] Serum concentrations of ZTS-00008183 are listed in Table 6

Table 6. Pharmacokinetic parameters of ZTS-00008183 in dogs following a single 4 mg/kg subcutaneous administration (T02)

Parameter	Units	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Mean	S.D.	%CV
AUC	µg*Days/mL	1440	2070	1600	2190	2110	1810	2110	2160	1940	285	14.7
AUCextrap	µg*Days/mL	1450	2090	1600	2230	2140	1830	2130	2190	1960	295	15.1
C_{max}	µg/mL	25.3	34.5	28.6	29.5	29.8	28.7	41.9	35.7	31.8	5.29	16.7
t_{max}	Days	14.0	7.00	7.00	14.0	28.0	7.00	7.00	7.00	11.4	7.42	65.3
$t_{1/2}$	Days	24.9	31.6	26.3	35.2	32.0	31.6	29.2	30.8	30.2	3.31	11.0

[000240] The mean pharmacokinetic parameters of ZTS-00008183 are demonstrated in Table 7 below.

Table 7. Mean pharmacokinetic parameters of ZTS-00008183.

Parameter	Units	ZTS-00008183
AUC	µg*Days/mL	1940±285
AUC Extrap	µg*Days/mL	1960±295
C_{max}	µg/mL	31.8±5.3
t_{max}	Days	11.4±7.4
$t_{1/2}$	Days	30.2±3.3

[000241] No treatment-induced immunogenicity has been detected.

[000242] The serum profiles of ZTS-00008183 are illustrated in Figure 7. The mean serum profiles of ZTS-00008183 are illustrated in Figure 8.

[000243] In sum, the results demonstrate that a canine IgG constant domain having N434H mutation provided the half-life for about 30 days. This is more than 2-fold increase (i.e., 200% increase) in half-life, relative to the half-life of 9.2 to 9.7 days for the wild-type (*See* Table 2).

EXAMPLE 7

Long Term Therapeutic Effect of Fc Mutant IgG

[000244] A single subcutaneous dose of ZTS-00008183 at 4 mg/kg was evaluated in a canine model of IL-31 induced pruritus.

[000245] Twenty-four healthy beagle dogs were randomized to treatment using a randomized complete block design and received ZTS-00008183 at 4 mg / kg bodyweight or a placebo in a parallel-design efficacy study. Animals were blocked by historical pruritic score to form eight (8) complete blocks of size 3.

Table 8. Treatment Summary

Treatment Group	Route	Dose (mg/kg)	Compound	Formulation Concentration (mg/mL)	N
T01	SC	0	Placebo	0	8
T02	SC	4.0	ZTS-00008183	40	8

SC refers to subcutaneous; N refers to number of animals.

[000246] Each animal was administered the IL-31 challenge (2.5 µg/kg) to induce pruritus on Study Day -7 to establish a baseline pruritic score. Additional IL-31 challenges were administered on Study Days 7, 28, 56, 84, 112, 140, 168 and 210.

[000247] Animals were observed for pruritic behavior for a 120-minute period after each challenge. Observations were made over a 1-minute window and any pruritic activity in that window resulted in a "yes" response. The cumulative number of yes responses determined the Pruritus Score.

[000248] There were no adverse events noted during this study and all test and control articles and challenge materials were administered according to protocol.

[000249] The results demonstrate that a single SC dose of ZTS-00008183 at 4.0 mg/kg showed a significantly lower least squares means pruritis score (Tables 9, 11 and 13) compared with control at 3, 4 and 5 months ($P<0.0001$) in our canine model of IL-31 induced pruritus.

[000250] As shown in FIGS. 9-13, ZTS-0008183 (T02), when dosed at 4 mg/kg, showed a significantly lower total pruritus score in our canine model of IL-31 induced pruritus compared with control on Study Days 84, 112, 140, 168, and 210.

[000251] Based on the pruritic scores, the results also demonstrate that ZTS-00008183 is therapeutically effective for 84, 112, 140, 168, and 210 days (i.e., about 7 months).

[000252] The results further demonstrate that ZTS-00008183 has a long term therapeutic effect and can be administered once every 3, 4, 5, 6, or 7 months.

Table 9. Least Squares Means Pruritic Scores with 92.9% Upper and Lower Confidence Limit at Day 84 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Treatment number	number of animals	least squares mean (lsm)	standard error	92.9% lower confidence limit	92.9% upper confidence limit	range	% change in means (1)
T01	8	86	6.0	73	98	27 to 112	
T02	8	9	6.2	-4	21	0 to 24	90.0

Table 10. Difference in Means Between Treatments Scores with 92.9% Upper and Lower Confidence Limit at Day 84 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Contrast	difference in means	standard error	92.915% lower confidence limit	92.915% upper confidence limit	df	p value	significant at 0.07085 level
T01 vs T02	77.1	8.77	59.9	94.3	13.6	<.0001	*

Table 11. Least Squares Means Pruritic Scores with 95.4% Upper and Lower Confidence Limit at Day 112 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Treatment number	number of animals	least squares mean (lsm)	standard error	95.425% lower confidence limit	95.425% upper confidence limit	range	% change in means (1)
T01	8	84	6.5	70	98	25 to 117	
T02	8	11	6.6	-3	26	1 to 29	86.8

Table 12. Difference in Means Between Treatments Scores with 95.4% Upper and Lower Confidence Limit at Day 112 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Contrast	difference in means	standard error	95.425% lower confidence limit	95.425% upper confidence limit	df	p value	significant at 0.04575 level
T01 vs T02	72.8	10.63	49.5	96.1	13.8	<.0001	*

Table 13. Least Squares Means Pruritic Scores with 95.4% Upper and Lower Confidence Limit at Day 140 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Treatment number	number of animals	least squares mean (lsm)	standard error	95.468% lower confidence limit	95.468% upper confidence limit	range	% change in means (1)
T01	8	86	5.8	74	99	33 to 116	
T02	8	21	5.9	8	34	9 to 45	76.1

Table 14. Difference in Means Between Treatments Scores with 95.4% Upper and Lower Confidence Limit at Day 140 Following Treatment with Placebo (T01) or ZTS-00008183 (T02).

Contrast	difference in means	standard error	95.468% lower confidence limit	95.468% upper confidence limit	df	p value	significant at 0.04352 level
T01 vs T02	65.8	9.67	44.3	87.3	13.9	<.0001	*

[000253] In sum, the results demonstrate that a canine IgG constant domain having N434H mutation maintains a therapeutic serum level for about 210 days (i.e., 7 months). This is several folds higher, relative to the days of therapeutic level of wild-type anti-IL31 antibody reported in earlier studies.

[000254] Having described preferred embodiments of the invention, it is to be understood that the invention is not limited to the precise embodiments, and that various changes and modifications may be effected therein by those skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A modified IgG comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

2. The modified IgG of claim 1, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

3. The modified IgG of claim 1, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type canine IgG constant domain.

4. The modified IgG of claim 1, wherein the modified IgG has a higher affinity for FcRn than the IgG having the wild-type canine IgG constant domain.

5. The modified IgG of claim 1, wherein the modified IgG is a canine or caninized IgG.

6. The modified IgG of claim 1, wherein the IgG is IgG_A, IgG_B, IgG_C, or IgG_D.

7. The modified IgG of claim 1, wherein the IgG constant domain is a constant domain of IgG_A, IgG_B, IgG_C, or IgG_D.

8. The modified IgG of claim 1, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.

9. The modified IgG of claim 1, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.

10. The modified IgG of claim 1, wherein the IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1.

11. A pharmaceutical composition comprising the modified IgG of claim 1 and a pharmaceutically acceptable carrier.

12. A kit comprising the modified IgG of claim 1, in a container, and instructions for use.

13. A polypeptide comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said

substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

14. The polypeptide of claim 13, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

15. The polypeptide of claim 13, wherein the polypeptide has an increased half-life compared to the half-life of the polypeptide of the wild-type canine IgG constant domain.

16. The polypeptide of claim 13, wherein the polypeptide has a higher affinity for FcRn than the polypeptide of the IgG having the wild-type canine IgG constant domain.

17. The polypeptide of claim 13, wherein the polypeptide is a polypeptide of a canine or caninized IgG.

18. The polypeptide of claim 17, wherein the IgG is IgG_A, IgG_B, IgG_C, or IgG_D.

19. The polypeptide of claim 13, wherein the IgG constant domain is a constant domain of IgG_A, IgG_B, IgG_C, or IgG_D.

20. The polypeptide of claim 13, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.

21. The polypeptide of claim 13, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.

22. The polypeptide of claim 13, wherein the IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1.

23. A pharmaceutical composition comprising the polypeptide of claim 13 and a pharmaceutically acceptable carrier.

24. A kit comprising the polypeptide of claim 13, in a container, and instructions for use.

25. An antibody comprising: a canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said

substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

26. The antibody of claim 25, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

27. The antibody of claim 25, wherein the antibody has an increased half-life compared to the half-life of an antibody having the wild-type canine IgG constant domain.

28. The antibody of claim 25, wherein the antibody has a higher affinity for FcRn than an antibody having the wild-type canine IgG constant domain.

29. The antibody of claim 25, wherein the antibody is a canine or caninized antibody.

30. The antibody of claim 25, wherein the antibody is IgG_A, IgG_B, IgG_C, or IgG_D.

31. The antibody of claim 25, wherein the IgG constant domain is a constant domain of IgG_A, IgG_B, IgG_C, or IgG_D.

32. The antibody of claim 25, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.

33. The antibody of claim 25, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.

34. The antibody of claim 25, wherein the IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1.

35. A pharmaceutical composition comprising the antibody of claim 25 and a pharmaceutically acceptable carrier.

36. A kit comprising the antibody of claim 25, in a container, and instructions for use.

37. A vector comprising the nucleic acid sequence encoding the amino acid sequence set forth in SEQ ID NO.: 1.

38. An isolated cell comprising the vector of claim 37.

39. A method of manufacturing an antibody or a molecule, the method comprising: providing the cell of claim 38; and culturing said cell.

40. A method of manufacturing an antibody, the method comprising: providing an antibody of any one of claims 25-34.

41. A method for increasing an antibody serum half-life in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

42. The method of claim 41, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

43. The method of claim 41, wherein the antibody has an increased half-life compared to the half-life of an antibody having the wild-type canine IgG constant domain.

44. The method of claim 41, wherein the antibody has a higher affinity for FcRn than an antibody having the wild-type canine IgG constant domain.

45. The method of claim 41, wherein the antibody is a canine or caninized antibody.

46. The method of claim 41, wherein the antibody is IgG_A, IgG_B, IgG_C, or IgG_D.

47. The method of claim 41, wherein the IgG constant domain is a constant domain of IgG_A, IgG_B, IgG_C, or IgG_D.

48. The method of claim 41, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.

49. The method of claim 41, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.

50. The method of claim 41, wherein the IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1.

51. A fusion molecule comprising: a canine IgG constant domain fused to an agent, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat.

52. The molecule of claim 51, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

53. The molecule of claim 51, wherein the molecule has an increased half-life compared to the half-life of a molecule having the wild-type canine IgG constant domain.

54. The molecule of claim 51, wherein the molecule has a higher affinity for FcRn than a molecule having the wild-type canine IgG constant domain.

55. The molecule of claim 51, wherein the molecule comprises a canine or caninized antibody.

56. The molecule of claim 51, wherein the molecule comprises IgG_A, IgG_B, IgG_C, or IgG_D.

57. The molecule of claim 51, wherein the IgG constant domain is a constant domain of IgG_A, IgG_B, IgG_C, or IgG_D.

58. The molecule of claim 51, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.

59. The molecule of claim 51, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.

60. The molecule of claim 51, wherein the IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1.

61. A pharmaceutical composition comprising the molecule of claim 51 and a pharmaceutically acceptable carrier.

62. A kit comprising the molecule of claim 51, in a container, and instructions for use.

63. A modified IgG comprising: a canine IgG constant domain comprising the amino acid sequence set forth in SEQ ID NO.: 1, 65, or 66, wherein the modified IgG has an

increased half-life compared to the half-life of an IgG having the wild-type canine IgG constant domain.

64. The modified IgG of claim 63, wherein the increased half-life is for a period ranging from about 25 days to about 35 days.

65. The modified IgG of claim 63, wherein the increased half-life is for about 30 days.

66. A modified IgG comprising: a canine IgG constant domain comprising the amino acid sequence set forth in SEQ ID NO.: 1, 65, or 66, wherein the modified IgG maintains its therapeutic level for a long term.

67. The modified IgG of claim 66, wherein the modified IgG maintains its therapeutic level over a period ranging from about 1 month to about 7 months

68. The modified IgG of claim 66, wherein the modified IgG maintains its therapeutic level over said period upon subcutaneous delivery of said IgG to a canine subject.

69. An antibody comprising: a canine IgG constant domain comprising the amino acid sequence set forth in SEQ ID NO.: 1, 65, or 66, wherein the antibody has an increased half-life compared to the half-life of an antibody having the wild-type canine IgG constant domain.

70. The antibody of claim 69, wherein the increased half-life is for a period ranging from about 25 days to about 35 days.

71. The antibody of claim 69, wherein the increased half-life is for about 30 days.

72. An antibody comprising: a canine IgG constant domain comprising the amino acid sequence set forth in SEQ ID NO.: 1, 65, or 66, wherein the antibody maintains its therapeutic level for a long term.

73. The antibody of claim 66, wherein the antibody maintains its therapeutic level over a period ranging from about 1 month to about 7 months

74. The antibody of claim 66, wherein the antibody maintains its therapeutic level over said period upon subcutaneous delivery of said antibody to a canine subject.

75. The antibody of any one of claims 25-34 and 69-74, wherein said antibody is an anti-IL31 or an anti-NGF antibody.

76. A method for increasing an antibody serum half-life in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat, and wherein said antibody is anti-IL31 antibody.

77. The method of claim 76, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

78. The method of claim 76, wherein the method increases the half-life for a period ranging from about 25 days to about 35 days.

79. The antibody of claim 76, wherein the method increases the half-life for about 30 days.

80. A method for maintaining a therapeutic serum level of an antibody in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat, and wherein said antibody is anti-IL31 antibody.

81. The method of claim 80, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

82. The method of claim 80, wherein the method maintains the therapeutic serum level of said antibody in said dog over a period ranging from about 1 month to about 7 months.

83. The method of claim 80, wherein the method maintains the therapeutic serum level over said period upon subcutaneous delivery of said antibody to said dog.

84. A method for increasing an antibody serum half-life in a dog, the method comprising: administering said dog a therapeutically effective amount of an antibody comprising a canine IgG constant domain, said canine IgG constant domain comprising at least one

amino acid substitution relative to a wild-type canine IgG constant domain, wherein said substitution is at amino acid residue 434, numbered according to the EU index as in Kabat, and wherein said antibody is anti-NGF antibody.

85. The method of claim 84, wherein said substitution is a substitution of asparagine at position 434 with histidine (N434H).

86. The method of claim 84, wherein the method increases the half-life for a period ranging from about 10 days to about 35 days.

87. The antibody of claim 84, wherein the method increases the half-life for about 30 days.

88. A method of treating an IL-31-mediated pruritic or allergic condition in a canine subject, the method comprising: administering to said subject a therapeutically effective amount of the anti-IL31 antibody of claim 75, thereby treating said IL-31-mediated pruritic or allergic condition in said canine subject.

89. The method of claim 88, wherein the IL-31-mediated pruritic or allergic condition is a pruritic condition selected from the group consisting of atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis.

90. The method of claim 88, wherein the IL-31-mediated pruritic or allergic condition is an allergic condition is selected from the group consisting of allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstruction pulmonary disease, and inflammatory processes resulting from autoimmunity.

91. The method of claim 88, wherein the antibody is administered bimonthly, once-in-three months, once-in-four months, once-in-five months, once-in-six months, or once-in-seven months.

92. The method of claim 88, wherein the antibody is administered subcutaneously at a dosage less than 4.0 mg/kg of body weight.

93. A method of treating a pain in a canine subject, the method comprising: administering to said subject a therapeutically effective amount of the anti-NGF antibody of claim 75, thereby treating said pain in said canine subject.

94. The method of claim 93, wherein said pain is a chronic pain, an inflammatory pain, a post-operative incision pain, a neuropathic pain, a fracture pain, an osteoporotic fracture pain, a post-herpetic neuralgia, a cancer pain, a pain resulting from burns, a pain associated with wounds, a pain associated with trauma, a neuropathic pain, a pain associated with a musculoskeletal disorder, a rheumatoid arthritis, an osteoarthritis, an ankylosing spondylitis, a seronegative (non-rheumatoid) an arthropathies, a non-articular rheumatism, a periarticular disorder, or a peripheral neuropathy.

95. The method of claim 93, wherein said pain is an osteoarthritis pain.

96. The method of claim 93, wherein the antibody is administered bimonthly, once-in-three months, once-in-four months, once-in-five months, once-in-six months, or once-in-seven months.

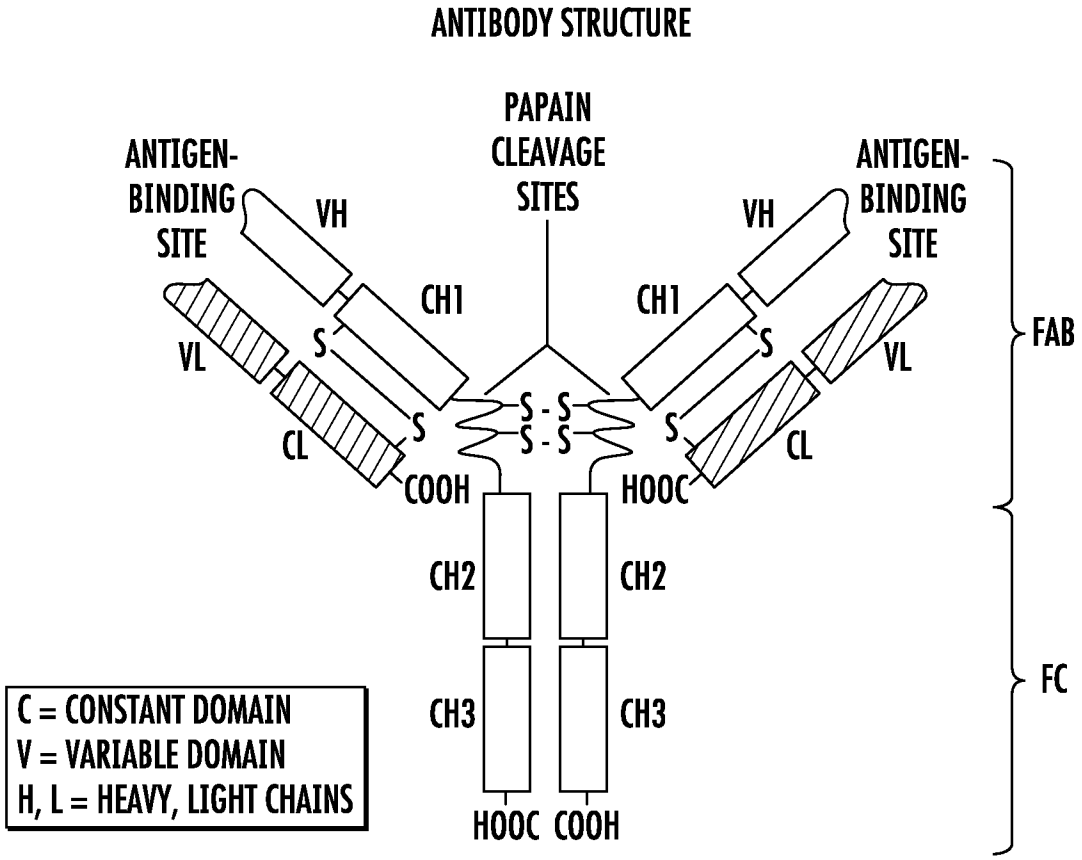
ANTIBODY STRUCTURE
PAPAIN CLEAVAGE SITES
ANTIGEN-BINDING SITE
ANTIGEN-BINDING SITE
VH
VH
CH1
CH1
VL
VL
CL
CL
S
S
S-S
S-S
COOH
HOOC
CH2
CH2
CH3
CH3
HOOC
COOH
FAB
FC
C = CONSTANT DOMAIN
V = VARIABLE DOMAIN
H, L = HEAVY, LIGHT CHAINS

FIG. 1

CANINE igGB_65_Fc_N434H
APEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIG
HQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQE
PESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHHHYTQESLSHSPGK

CANINE igGB_65_Fc_WT
APEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIG
HQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQE
PESKYRTTPPQLDEDGSYFLYSKLSVDKSRQQRGDTFICAVMHEALHNHYTQESLSHSPGK

FIG. 2A

RED=CH1 VIOLET=HINGE BLUE=CH2 GREEN=CH4

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>HUMAN igG1_EU INDEX
118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D

>CANINE igGA_EU INDEX
118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
A S T T A P S V F P L A P S C G S T S G S T V A L A C L V S G

>CANINE igGB_EU INDEX
118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
A S T T A P S V F P L A P S C G S T S G S T V A L A C L V S G

>CANINE igGC_EU INDEX
118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
A S T T A P S V F P L A P S C G S Q S G S T V A L A C L V S G

>CANINE igGD_EU INDEX
118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
A S T T A P S V F P L A P S C G S T S G S T V A L A C L V S G

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>HUMAN igG1_EU INDEX
149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L

>CANINE igGA_EU INDEX
149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L

>CANINE igGB_EU INDEX
149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
Y F P E P V T V S W N S G A L T S G V H T F P S V L Q S S G L

>CANINE igGC_EU INDEX
149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
Y I P E P V T V S W N S V S L T S G V H T F P S V L Q S S G L

>CANINE igGD_EU INDEX
149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
Y F P E P V T V S W N S G S L T S G V H T F P S V L Q S S G L

FIG. 2B

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180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K	P	S	N	T	K

>CANINE igGA_EU INDEX

180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
H	S	L	S	S	M	V	T	V	P	S	S	R	W	P	S	E	T	F	T	C	N	V	V	H	P	A	S	N	T	K

>CANINE igGB_EU INDEX

180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
Y	S	L	S	S	M	V	T	V	P	S	S	R	W	P	S	E	T	F	T	C	N	V	A	H	P	A	S	K	T	K

>CANINE igGC_EU INDEX

180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
Y	S	L	S	S	M	V	T	V	P	S	S	R	W	P	S	E	T	F	T	C	N	V	A	H	P	A	T	N	T	K

>CANINE igGD_EU INDEX

180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
Y	S	L	S	S	T	V	T	V	P	S	S	R	W	P	S	E	T	F	T	C	N	V	V	H	P	A	S	N	T	K

>HUMAN igG1_EU INDEX

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211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241
V	D	K	K	V	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P	A	P	E	L	L	G	G	P	S	V	F

>CANINE igGA_EU INDEX

211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241
V	D	K	P	V	F	-	N	E	C	R	C	T	D	T	-	P	P	C	P	V	P	E	P	L	G	G	P	S	V	L

>CANINE igGB_EU INDEX

211	212	213	214	215	216	217	218					219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237
V	D	K	P	V	-	P	K	R	E	N	G	R	V	P	R	P	P	D	C	P	K	C	P	A	P	E	M	L	G	G

>CANINE igGC_EU INDEX

211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232			233	234	235	236	237	238	239
V	D	K	P	V	-	A	K	E	C	E	C	K	C	N	C	N	N	C	P	C	P	G	C	G	L	L	G	G	P	S

>CANINE igGD_EU INDEX

211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226				227	228	229	230	231	232	233	234	235	236	237	238
V	D	K	P	V	P	K	E	S	-	-	-	-	-	T	C	K	C	I	S	P	C	P	V	P	E	S	L	G	G	P

FIG. 2B CONT'D.

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>HUMAN igG1_EU INDEX

242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272
L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E

>CANINE igGA_EU INDEX

242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272
I	F	P	P	K	P	K	D	I	L	R	I	T	R	T	P	E	V	T	C	V	V	L	D	L	G	R	E	D	P	E

>CANINE igGB_EU INDEX

238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268
P	S	V	F	I	F	P	P	K	P	K	D	T	L	L	I	A	R	T	P	E	V	T	C	V	V	V	D	L	D	P

>CANINE igGC_EU INDEX

240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
V	F	I	F	P	P	K	P	K	D	I	L	V	T	A	R	T	P	T	V	T	C	V	V	V	D	L	D	P	E	N

>CANINE igGD_EU INDEX

239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269
S	V	F	I	F	P	P	K	P	K	D	I	L	R	I	T	R	T	P	E	I	T	C	V	V	L	D	L	G	R	E

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>HUMAN igG1_EU INDEX

273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303
V	K	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V

>CANINE igGA_EU INDEX

273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303
V	Q	I	S	W	F	V	D	G	K	E	V	H	T	A	K	T	Q	S	R	E	Q	Q	F	N	G	T	Y	R	V	V

>CANINE igGB_EU INDEX

269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299
E	D	P	E	V	Q	I	S	W	F	V	D	G	K	Q	M	Q	T	A	K	T	Q	P	R	E	E	Q	F	N	G	T

>CANINE igGCC_EU INDEX

271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301
P	E	V	Q	I	S	W	F	V	D	S	K	Q	V	Q	T	A	N	T	Q	P	R	E	E	Q	S	N	G	T	Y	R

>CANINE igGCD_EU INDEX

270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
D	P	E	V	Q	I	S	W	F	V	D	G	K	E	V	H	T	A	K	T	Q	P	R	E	Q	Q	F	N	S	T	Y

FIG. 2B CONT'D.

>HUMAN IgG1_EU INDEX

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304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334
S	V	L	T	V	L	H	Q	D	W	L	N	G	K	F	Y	K	C	K	V	S	N	K	A	L	P	A	P	I	E	K

>CANINE IgGA_EU INDEX

304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334
S	V	L	P	I	E	H	Q	D	W	L	T	G	K	E	F	K	C	R	V	N	H	I	D	L	P	S	P	I	E	R

>CANINE IgGB_EU INDEX

300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
Y	R	V	V	S	V	L	P	I	G	H	Q	D	W	L	K	G	K	Q	F	T	C	K	V	N	N	K	A	L	P	S

>CANINE IgGC_EU INDEX

302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332
V	V	S	V	L	P	I	G	H	Q	D	W	L	S	G	K	Q	F	K	C	K	V	N	N	K	A	L	P	S	P	I

>CANINE IgGD_EU INDEX

301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331
R	V	V	S	V	L	P	I	E	H	Q	D	W	L	T	G	K	E	F	K	C	R	V	N	H	I	G	L	P	S	P

>HUMAN IgG1_EU INDEX

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335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365
T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L

>CANINE IgGA_EU INDEX

335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365
T	I	S	K	A	R	G	R	A	H	K	P	S	V	Y	V	L	P	P	S	P	K	E	L	S	S	S	D	T	V	S

>CANINE IgGB_EU INDEX

331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361
P	I	E	R	T	I	S	K	A	R	G	Q	A	H	Q	P	S	V	Y	V	L	P	P	S	R	E	E	L	S	K	N

>CANINE IgGC_EU INDEX

333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363
E	E	I	I	S	K	T	P	G	Q	A	H	Q	P	N	V	Y	V	L	P	P	S	R	D	E	M	S	K	N	T	V

>CANINE IgGD_EU INDEX

332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362
I	E	R	T	I	S	K	A	R	G	Q	A	H	Q	P	S	V	Y	V	L	P	P	S	P	K	E	L	S	S	S	D

FIG. 2B CONT'D.

>HUMAN IgG1_EU INDEX 9

366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396
T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P

>CANINE IgGA_EU INDEX

365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386			387	388	389	390	391	392	393
I	T	C	L	I	K	D	F	Y	P	P	D	I	D	V	E	W	Q	S	N	G	Q	Q	E	P	E	R	K	H	R	M

>CANINE IgGB_EU INDEX

362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386			387	388	389	390
T	V	S	L	T	C	L	I	K	D	F	F	P	P	D	I	D	V	E	W	Q	S	N	G	Q	Q	E	P	E	S	K

>CANINE IgGC_EU INDEX

364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386			387	388	389	390	391	392
T	L	T	C	L	V	K	D	F	F	P	P	E	I	D	V	E	W	Q	S	N	G	Q	Q	E	P	E	S	K	Y	R

>CANINE IgGD_EU INDEX

363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386				387	388	389	390
T	V	T	L	T	C	L	I	K	D	F	F	P	P	E	I	D	V	E	W	Q	S	N	G	Q	P	E	P	E	S	K

>HUMAN IgG1_EU INDEX 10

397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427
V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V

>CANINE IgGA_EU INDEX

394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424
T	P	P	Q	L	D	E	D	G	S	Y	F	L	Y	S	K	L	S	V	D	K	S	R	W	Q	Q	G	D	P	F	T

>CANINE IgGB_EU INDEX

391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
Y	R	T	T	P	P	Q	L	D	E	D	G	S	Y	F	L	Y	S	K	L	S	V	D	K	S	R	W	Q	R	G	D

>CANINE IgGC_EU INDEX

393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423
M	T	P	P	Q	L	D	E	D	G	S	Y	F	L	Y	S	K	L	S	V	D	K	S	R	W	Q	R	G	D	T	F

>CANINE IgGD_EU INDEX

391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
Y	H	T	T	A	P	Q	L	D	E	D	G	S	Y	F	L	Y	S	K	L	S	V	D	K	S	R	W	Q	Q	G	D

FIG. 2B CONT'D.

>HUMAN IgG1_EU INDEX
428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447
M H E A L H N H Y T Q K S L S L S P G K
>HUMAN IgG1_EU INDEX
425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447
C A V M H E T L Q N H Y T D L S L S H S P G K
>HUMAN IgG1_EU INDEX
422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447
T F I C A V M H E A L H N H Y T Q E S L S H S P G K
>HUMAN IgG1_EU INDEX
424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447
I C A V M H E A L H N H Y T Q I S L S H S P G K
>HUMAN IgG1_EU INDEX
422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447
T F T C A V M H E A L Q N H Y T D L S L S H S P G K

FIG. 2B
CONT'D.

CANINE_IgGB_65_CONSTANT CH2-CH3 WT
GCTCCAGAAATGCTGGGAGGACCAAGCGTGTTCATCTTTCCACCCA AGCCCAAAGACACACTGCTGATTGCTAGAACTCCCGAGGTGACCTG CGTGGTGGTGGACCTGGATCCAGAGGACCCCGAAGTGCAGATCTCC TGGTTCGTGGATGGGAAGCAGATGCAGACAGCCAAAACTCAGCCTC GGGAGGAACAGTTTAACGGAACCTATAGAGTGGTGTCTGTGCTGCC AATTGGACACCAGGACTGGCTGAAGGGCAAACAGTTTACATGCAAG GTGAACAACAAGGCCCTGCCTAGTCCAATCGAGAGGACTATTTCAA AAGCTAGGGGACAGGCTCATCAGCCTTCCGTGTATGTGCTGCCTCC ATCCCGGGAGGAACTGTCTAAGAACACAGTGAGTCTGACTTGTCTG ATCAAAGATTTCTTTCCCCCTGACATTGATGTGGAGTGGCAGAGCAA TGGGCAGCAGGAGCCAGAATCCAAGTACAGAACCACACCACCCCA GCTGGACGAAGATGGCTCCTATTTCCTGTACAGTAAGCTGTCAGTGG ACAAATCTAGGTGGCAGCGCGGGGATATCCTTTATCTGCGCCGTGAT GCACGAGGCTCTGCACAATCATTACACACAAGAAAGTCTGTCACATA GCCCCGGCAAG

FIG. 2C

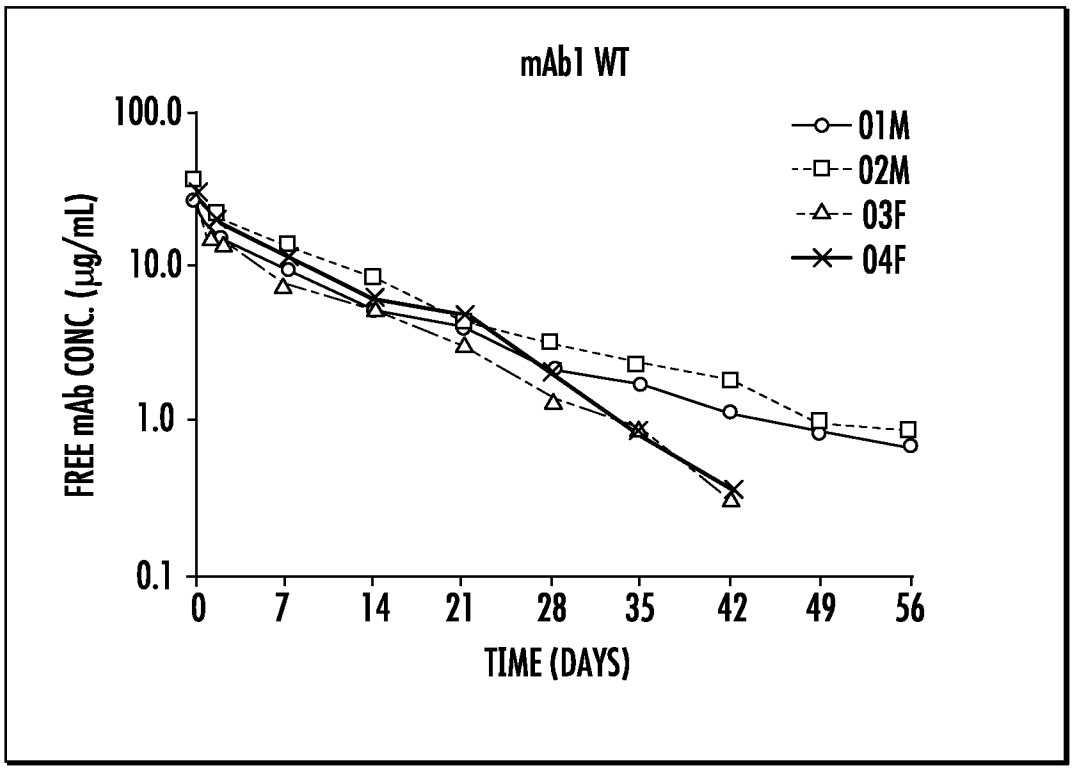
mAb1 WT
100.0
10.0
1.0
0.1
FREE mAb CONC. (µg/mL)
0
7
14
21
28
35
42
49
56
TIME (DAYS)
01M
02M
03F
04F

FIG. 3

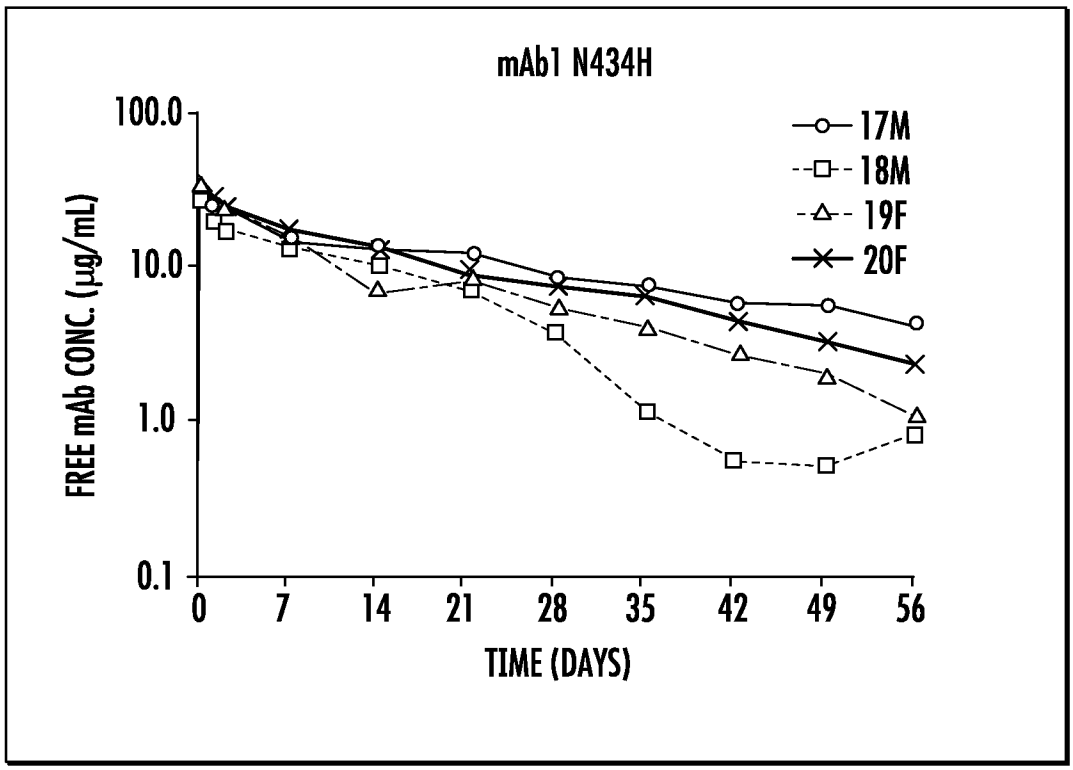
mAb1 N434H
100.0
10.0
1.0
0.1
FREE mAb CONC. (µg/mL)
0
7
14
21
28
35
42
49
56
TIME (DAYS)
17M
18M
19F
20F

FIG. 4

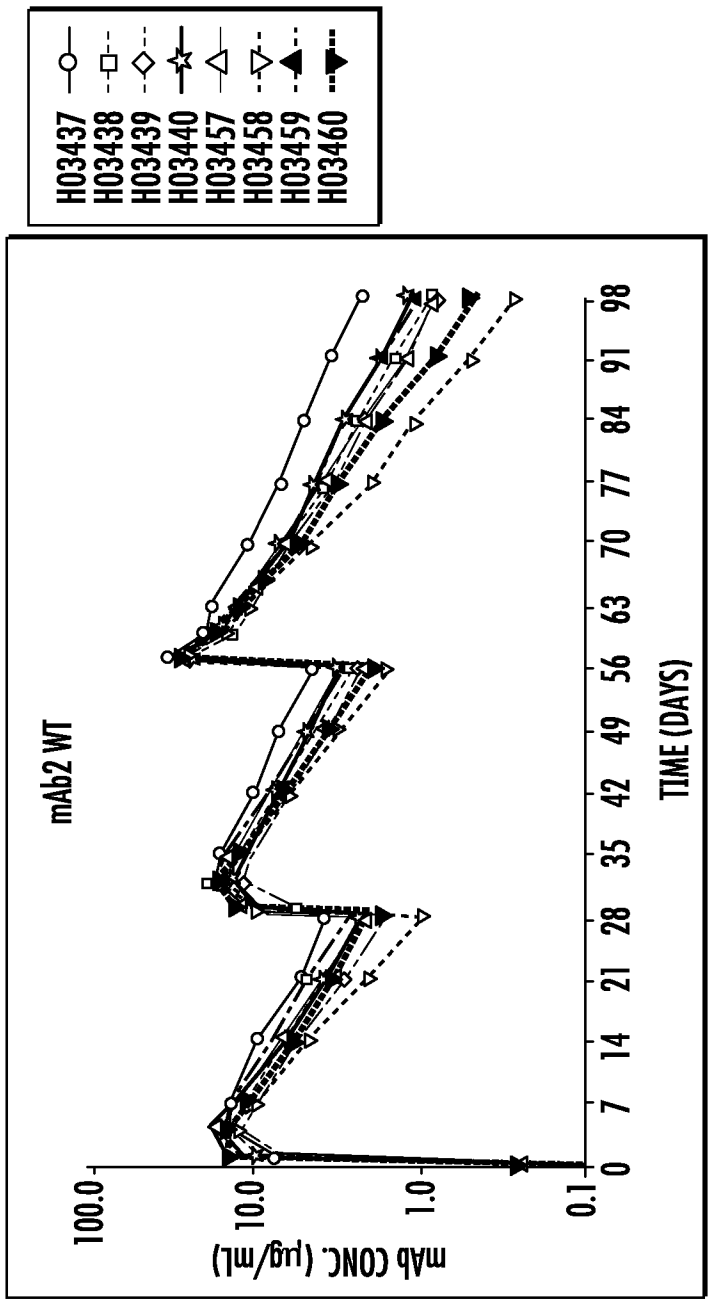
H03437
H03438
H03439
H03440
H03457
H03458
H03459
H03460
mAb2 WT
mAb CONC. (µg/mL)
100.0
10.0
1.0
0.1
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
TIME (DAYS)

Fig. 5

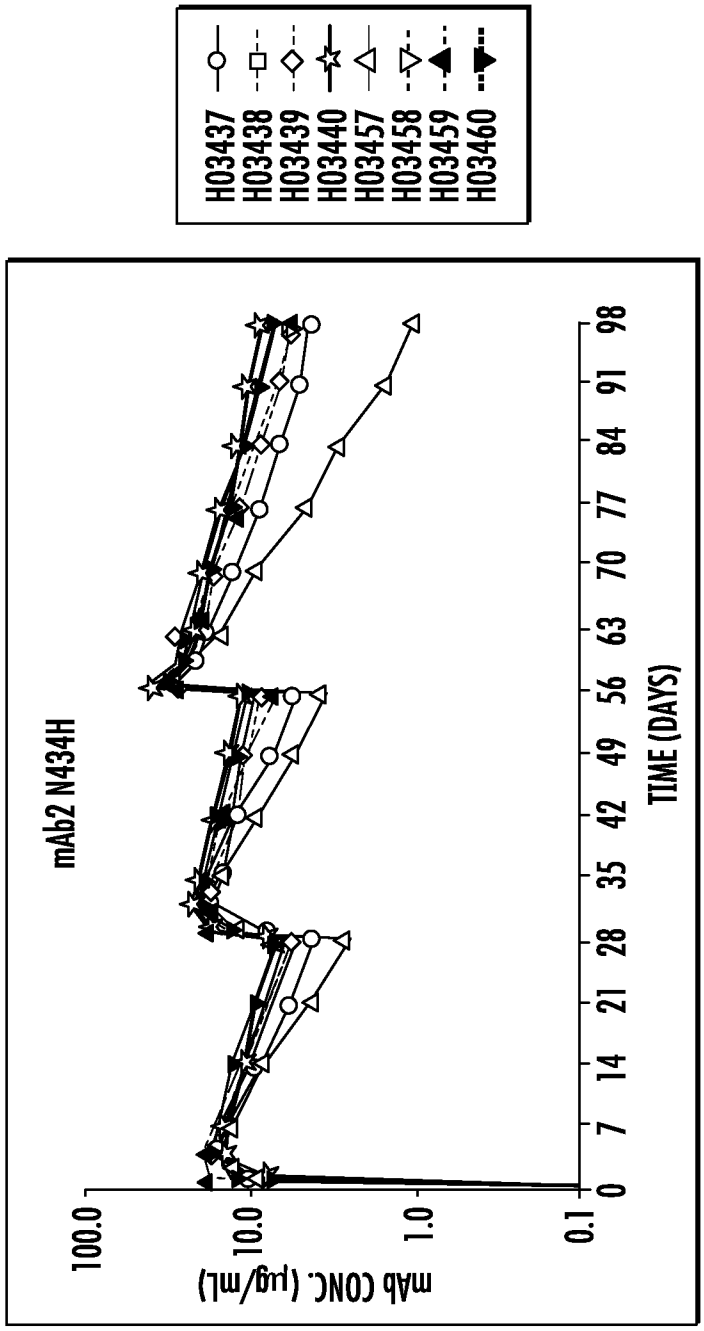
H03437
H03438
H03439
H03440
H03457
H03458
H03459
H03460
mAb2 N434H
mAb CONC. (µg/mL)
100.0
10.0
1.0
0.1
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
TIME (DAYS)

FIG. 6

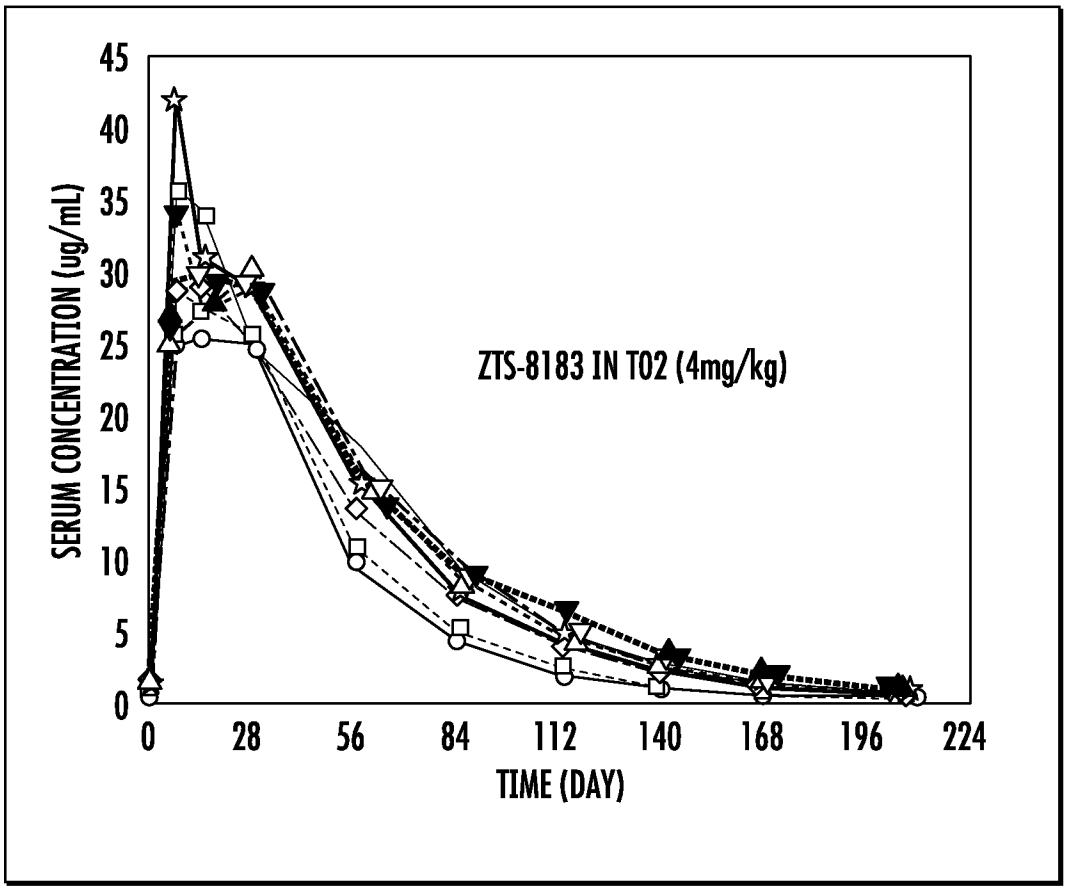
45
40
35
30
25
20
15
10
5
0
SERUM CONCENTRATION (ug/mL)
ZTS-8183 IN T02 (4mg/kg)
0
28
56
84
112
140
168
196
224
TIME (DAY)

FIG. 7

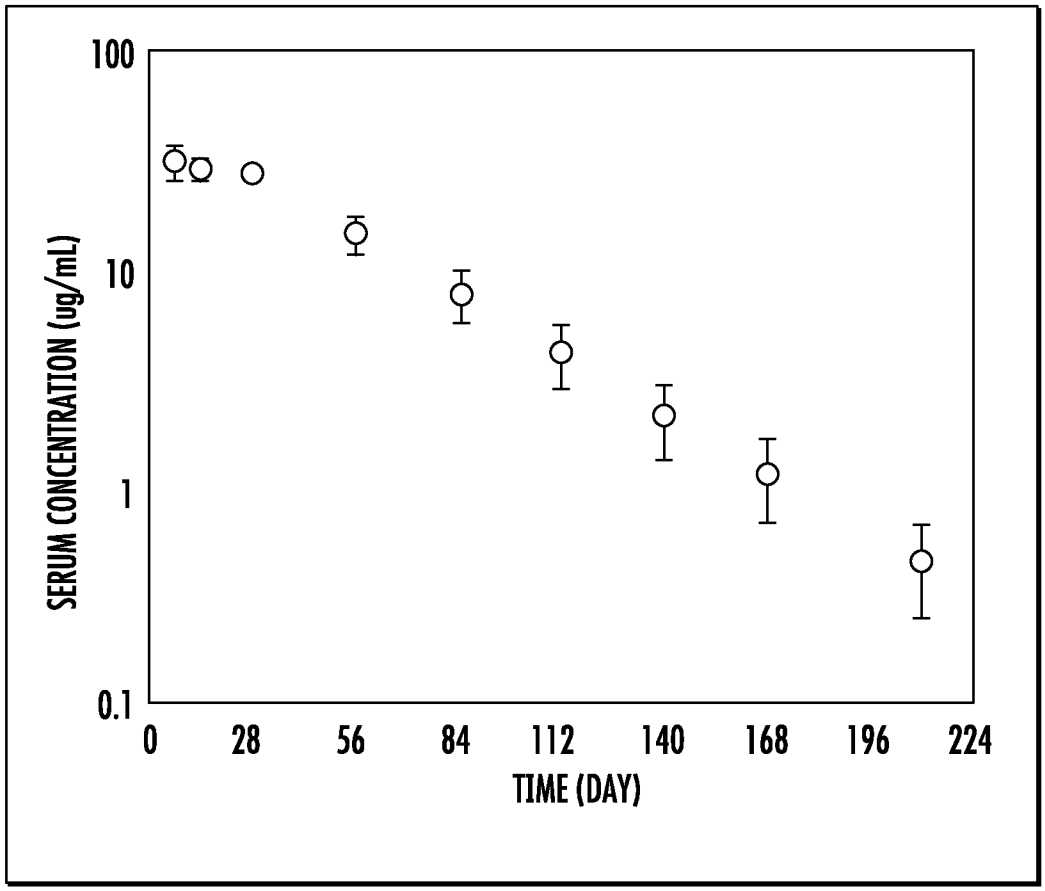
100
10
1
0.1
SERUM CONCENTRATION (ug/mL)
0
28
56
84
112
140
168
196
224
TIME (DAY)

FIG. 8

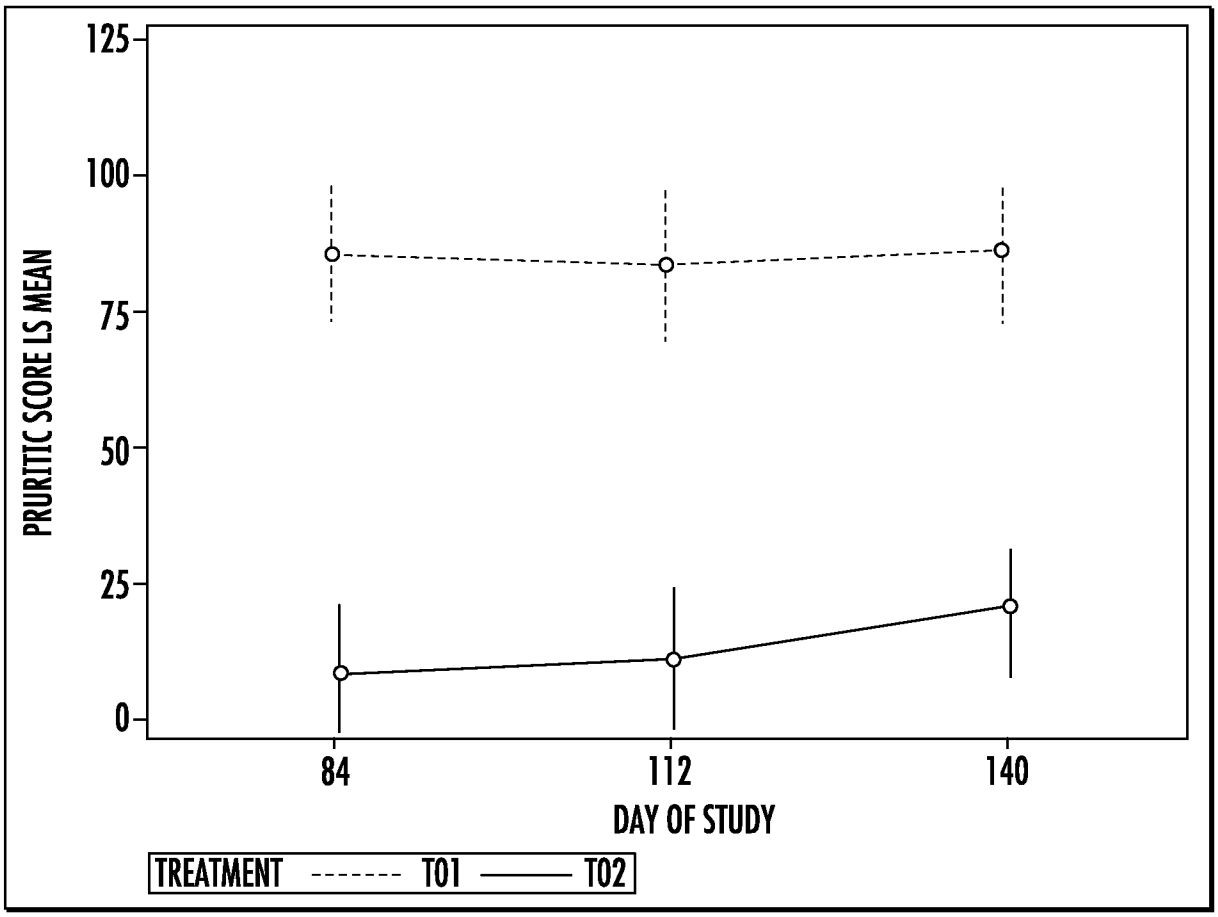
125
100
75
50
25
0
PRURITIC SCORE LS MEAN
84
112
140
DAY OF STUDY
TREATMENT
T01
T02

FIG. 9

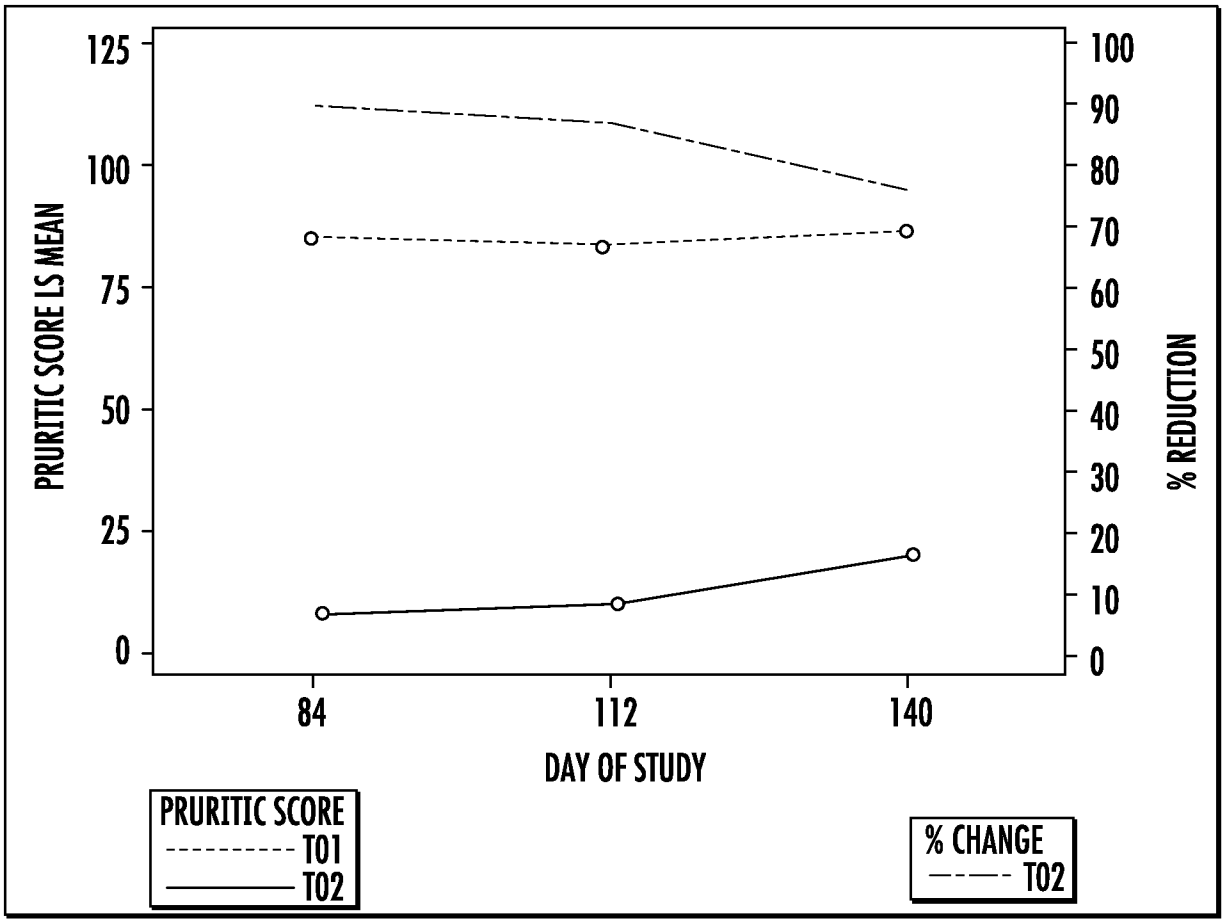
125
100
75
50
25
0
PRURITIC SCORE LS MEAN
100
90
80
70
60
50
40
30
20
10
0
% REDUCTION
84
112
140
DAY OF STUDY
PRURITIC SCORE
T01
T02
% CHANGE
T02

FIG. 10

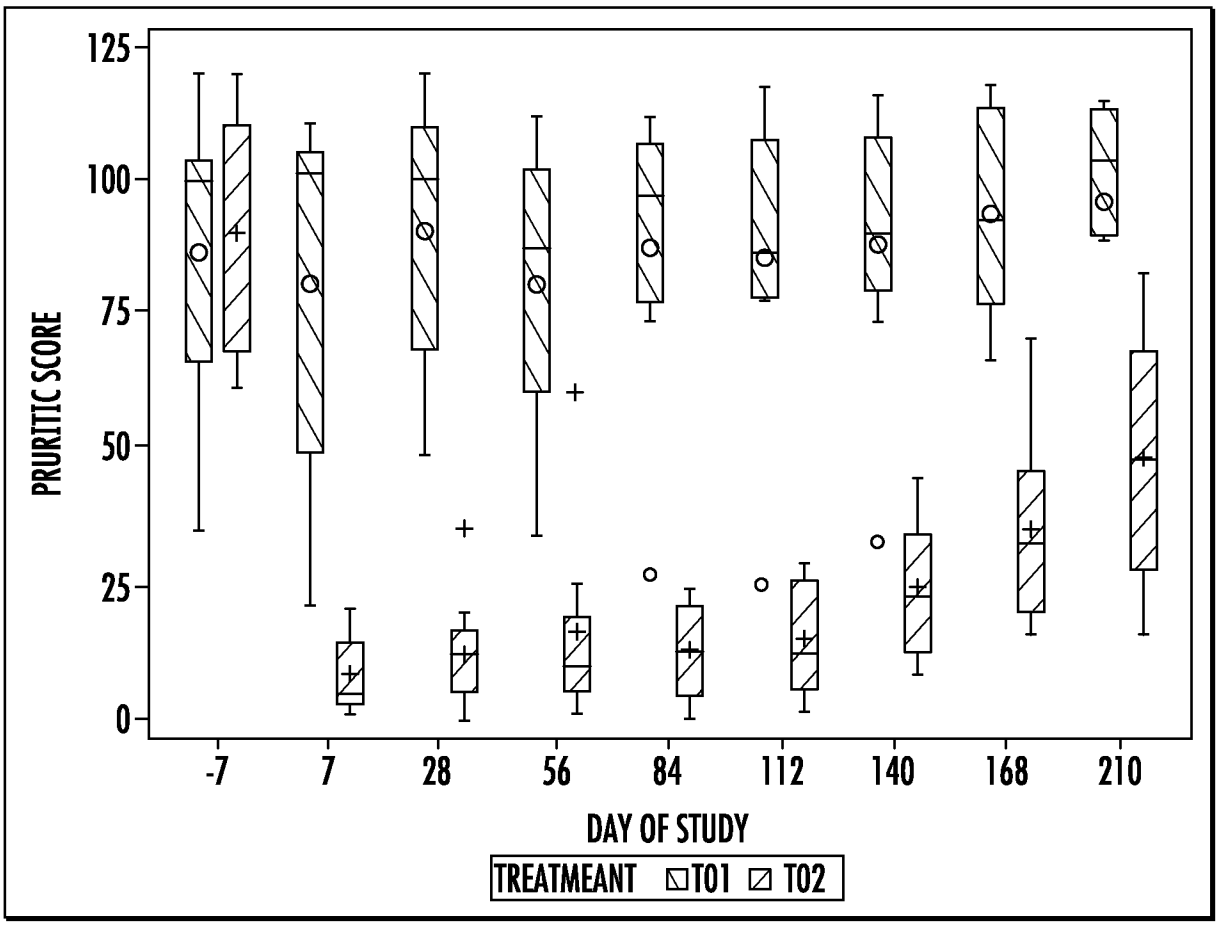
125
100
75
50
25
0
PRURITIC SCORE
-7
7
28
56
84
112
140
168
210
DAY OF STUDY
TREATMEANT
T01
T02

FIG. 11

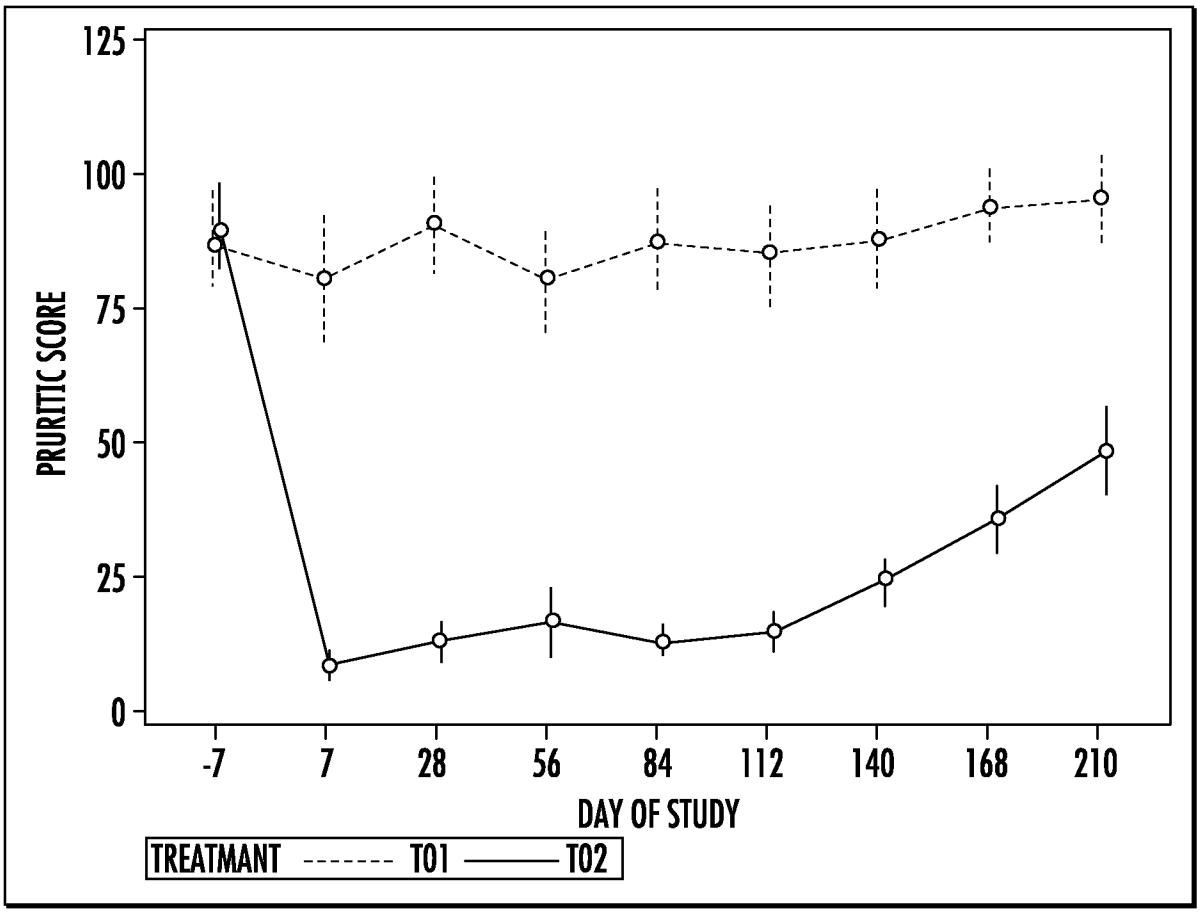
125
100
75
50
25
0
PRURITIC SCORE
-7
7
28
56
84
112
140
168
210
DAY OF STUDY
TREATMANT
T01
T02

FIG. 12

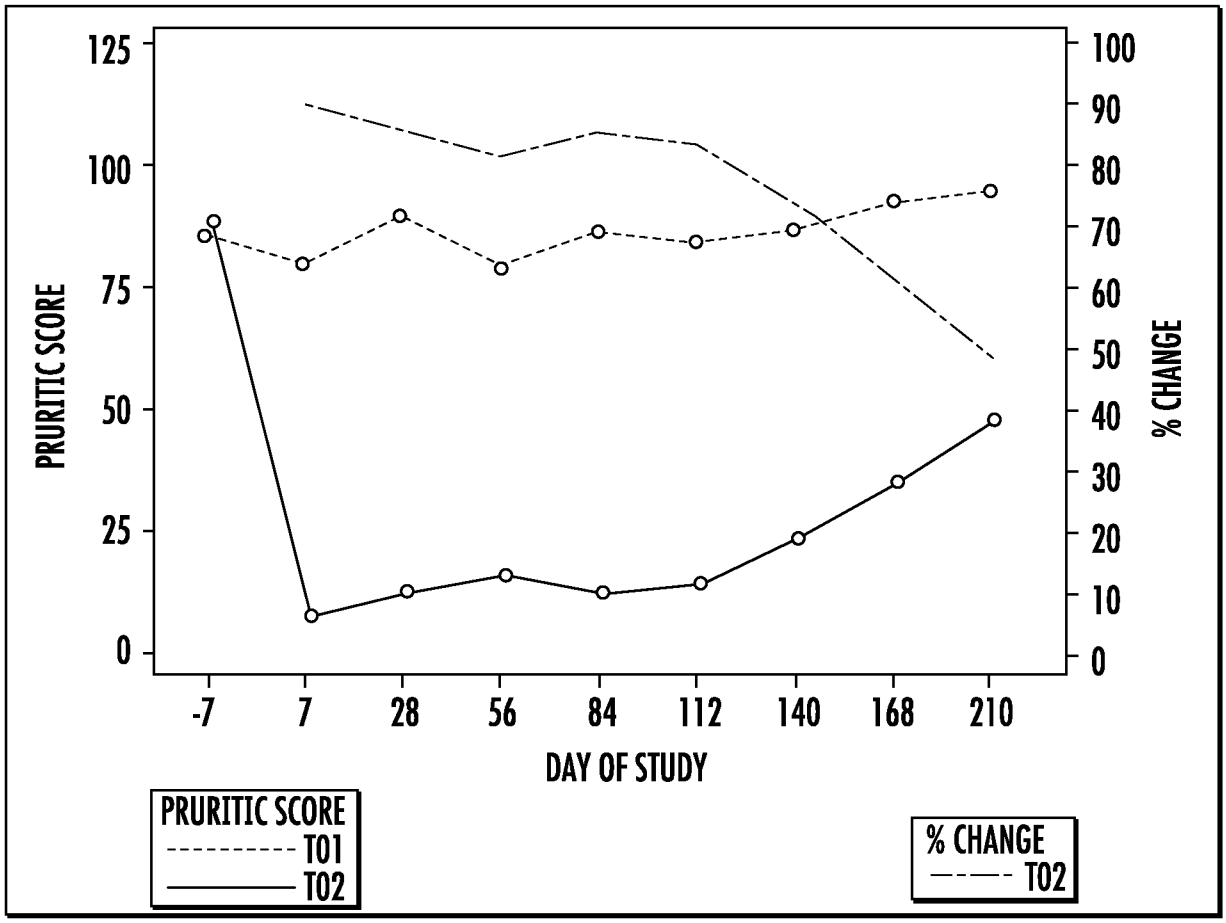
125
100
75
50
25
0
PRURITIC SCORE
100
90
80
70
60
50
40
30
20
10
0
% CHANGE
-7
7
28
56
84
112
140
168
210
DAY OF STUDY
PRURITIC SCORE
T01
T02
% CHANGE
T02

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/027836

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/00 A61P37/06 C07K16/22 C07K16/24
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NG CHEE M ET AL: "Modeling approach to investigate the effect of neonatal Fc receptor binding affinity and anti-therapeutic antibody on the pharmacokinetic of humanized monoclonal anti-tumor necrosis factor-[alpha] IgG antibody in cynomolgus mo", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, ELSEVIER AMSTERDAM, NL, vol. 51, 31 August 2013 (2013-08-31), pages 51-58, XP028772607, ISSN: 0928-0987, DOI: 10.1016/J.EJPS.2013.08.033	1-9, 12-21, 23-33, 35,36, 38-40, 51-59, 61-87
Y	table 1	41-49
	----- -/--	

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2021	09/08/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/027836

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 494 991 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]; UNIV JICHI MEDICAL [JP] ET AL.) 12 June 2019 (2019-06-12)	1-9, 11-21, 23-33, 35,36, 38-40, 51-59, 61-87
Y	paragraphs [0233], [0230] sequence 5	41-49
A	WO 2018/073185 A1 (VETOQUINOL SA [FR]) 26 April 2018 (2018-04-26) the whole document claim 42 figure 4 example 4	1-96
X,P	WO 2020/142625 A2 (INVETX INC [US]) 9 July 2020 (2020-07-09) paragraphs [0438], [0487]; table 12	1-96
X,P	WO 2020/082048 A1 (KINDRED BIOSCIENCES INC [US]) 23 April 2020 (2020-04-23) the whole document sequences 46,36,41,31,99,98	1-96
X,P	WO 2020/191289 A1 (KINDRED BIOSCIENCES INC [US]) 24 September 2020 (2020-09-24) the whole document sequence 204	1-96
A	KACSKOVICS ET AL: "Cloning and characterization of the dromedary (Camelus dromedarius) neonatal Fc receptor (drFcRn)", DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, PERGAMON PRESS, US, vol. 30, no. 12, 1 January 2006 (2006-01-01), pages 1203-1215, XP005583532, ISSN: 0145-305X, DOI: 10.1016/J.DCI.2006.02.006 bottom left; page 1213	1-96

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/027836

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TABRIZI MOHAMMAD ET AL: "Pharmacokinetic Properties of Humanized IgG1 and IgG4 Antibodies in Preclinical Species:Translational Evaluation", THE AAPS JOURNAL, SPRINGER INTERNATIONAL PUBLISHING, CHAM, vol. 21, no. 3, 13 March 2019 (2019-03-13) , pages 1-9, XP036777890, DOI: 10.1208/S12248-019-0304-3 [retrieved on 2019-03-13] the whole document abstract page 2-of-9, right column	41-49
X	GINA M. MICHELS ET AL: "A blinded, randomized, placebo-controlled, dose determination trial of lokivetmab (ZTS-00103289), a caninized, anti-canine IL-31 monoclonal antibody in client owned dogs with atopic dermatitis", VETERINARY DERMATOLOGY., vol. 27, no. 6, 1 December 2016 (2016-12-01), pages 478-e129, XP055397044, GB ISSN: 0959-4493, DOI: 10.1111/vde.12376 the whole document	88-92
X	DAVID P GEARING ET AL: "A fully caninised anti-NGF monoclonal antibody for pain relief in dogs", BMC VETERINARY RESEARCH, BIOMED CENTRAL, LONDON, GB, vol. 9, no. 1, 9 November 2013 (2013-11-09), page 226, XP021168653, ISSN: 1746-6148, DOI: 10.1186/1746-6148-9-226 the whole document figure 5	93-96

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/027836

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/027836

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 3494991 A1	12-06-2019	AU 2017305073 A1	20-12-2018
		BR 112019001902 A2	09-07-2019
		CA 3026050 A1	08-02-2018
		CN 109689099 A	26-04-2019
		EP 3494991 A1	12-06-2019
		JP 6527643 B2	05-06-2019
		JP 2019142968 A	29-08-2019
		JP WO2018025982 A1	14-02-2019
		KR 20190037048 A	05-04-2019
		KR 20200038562 A	13-04-2020
		RU 2019105510 A	07-09-2020
		SG 11201801024X A	30-05-2018
		TW 201818957 A	01-06-2018
		TW 202034941 A	01-10-2020
		US 2019169286 A1	06-06-2019
		WO 2018025982 A1	08-02-2018
WO 2018073185 A1	26-04-2018	CA 3040823 A1	26-04-2018
		CN 110114369 A	09-08-2019
		EP 3526246 A1	21-08-2019
		US 2020181258 A1	11-06-2020
		WO 2018073185 A1	26-04-2018
WO 2020142625 A2	09-07-2020	US 2020216536 A1	09-07-2020
		US 2020362035 A1	19-11-2020
		WO 2020142625 A2	09-07-2020
WO 2020082048 A1	23-04-2020	AU 2019360271 A1	29-04-2021
		CA 3114796 A1	23-04-2020
		EP 3866842 A1	25-08-2021
		WO 2020082048 A1	23-04-2020
WO 2020191289 A1	24-09-2020	NONE	