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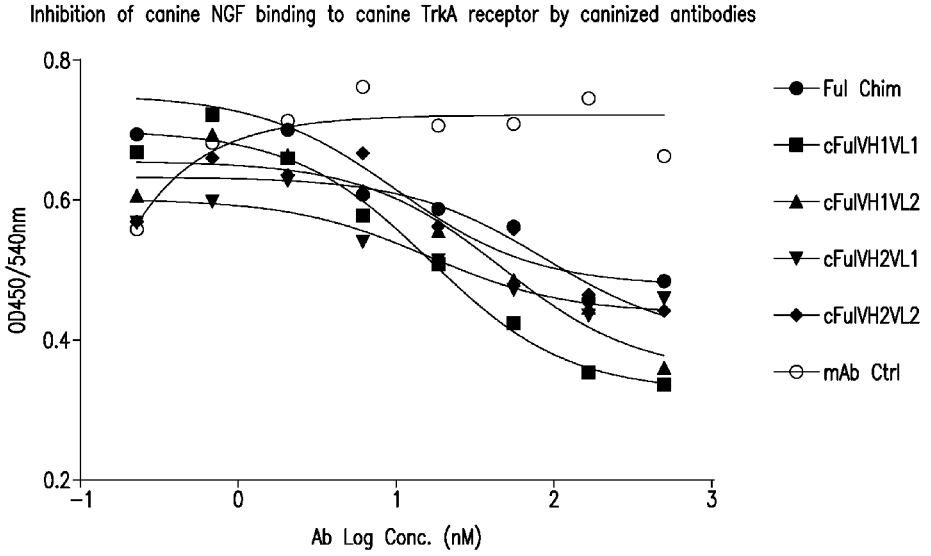

FIG.4

(57) Abstract: The present invention provides caninized anti-human NGF antibodies that have a high binding affinity for canine NGF. The invention also relates to use of these antibodies in the treatment of pain in canines and other companion animals.

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CANINIZED ANTIBODIES TO HUMAN NGF

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML file, created on December 6, 2022, is named 25370.xml. This sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) of provisional applications U.S. Serial No. 63/327,076, filed on April 4, 2022 and U.S. Serial No. 63/290,264 filed on December16, 2021. The subject matter of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to antibodies to proteins involved in pain. More particularly, the present invention further relates to caninized antibodies to human NGF that have a high binding affinity for canine NGF. The present invention also relates to use of the antibodies of the present invention in the treatment of pain in canines including in dogs with osteoarthritis.

BACKGROUND OF THE INVENTION

Nerve growth factor (NGF) is a well-characterized secreted protein that plays an important role in the development of the nervous system. In addition, NGF has also been shown to have biological effects on non-neuronal cells and tissues including cells of the immune system. NGF initially was isolated in the mouse submandibular gland as a complex composed of three non-covalently linked subunits. The *alpha* and *gamma* subunits of NGF belong to the kallikrein family of serine proteases, whereas the *beta* subunit of NGF complex exhibits the biological activities attributed to NGF. NGF (also referred to as *Beta* NGF) is produced as a prepropeptide with 18-amino acid residue signal peptide [Wiesmann and de Vos, CLMS:58, 748-759, (2001)]. Recombinant human *beta* -NGF is a homodimer of two 120 amino acid polypeptides. The C-terminal 120 amino acids of human NGF has approximately 98% homology to the predicted C-terminal end of NGF from other species, including canines and felines.

A number of studies indicate that NGF plays a key role in the transmission of pain. For example, in humans, NGF levels are elevated in the synovial fluids from patients with some arthritic conditions [Aloe, *et al.*, *Arch. Rheum.*, 35:351-355 (1992)]. Moreover, elevated levels of canine NGF expression have been demonstrated in synovial fluids of dogs with osteoarthritis [Isola, *et al.*, *Vet Comp. Orthop. Traumatol.*, 4:279 (2011)]. It also has been demonstrated that agents that inhibit the function of NGF such as neutralizing antibodies prevent hyperalgesia and allodynia in animal models of neuropathic pain [*see*, *e.g.*, Ramer *et al.*, *Eur. J. Neurosci.* 11:837-846 (1999) and Ro *et al.*, *Pain*, 79:265-274 (1999)]. The realization that NGF is involved in the transmission of pain in certain inflammatory and non-inflammatory conditions such as osteoarthritis and cancer led to interest in developing antibodies that can neutralize the biological activities of NGF. [Examples of anti-NGF antibodies known in the art include: WO01/78698, WO 01/64247, WO 02/096458, US 7,601,818 B2, and Gearing *et al.*, *BMC Veterinary Research*, 9:226, (2013)].

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

SUMMARY OF THE INVENTION

The present invention relates to caninized anti-human nerve growth factor (NGF) antibodies that have specific binding affinity for canine NGF, as well as having the ability to block the binding of canine NGF to the canine NGF receptor. The present invention includes the use of such antibodies in the treatment of hyperalgesia and allodynia in animal. The antibodies also can be used to treat pain in dogs with osteoarthritis.

Accordingly, the present invention provides novel caninized antibodies and antigen binding fragments thereof that are capable of binding and neutralizing canine NGF in which the caninized antibody or antigen binding fragment thereof comprises a heavy chain and a light chain. The heavy chain of the caninized antibody comprises a variable region (VH) and three constant regions, which includes the canine fragment crystallizable region (cFc or cFc region). The light chain also comprises a variable region (VL), but just one constant region. The respective variable regions of the heavy chain and light chain each comprise three hypervariable regions, *i.e.*, complementary determining regions (CDRs). Therefore, the light chain comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3) each comprising an amino acid sequence, whereas the heavy chain comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2

(CDRH2) and CDR heavy 3 (CDRH3) each comprising an amino acid sequence. The CDRH1 comprises the amino acid sequence of SEQ ID NO: 1, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 2, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 3, whereas CDRL1 comprises the amino acid sequence of SEQ ID NO: 4, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 5, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 6.

The caninized antibody also comprises a hinge region. The hinge region is preferably a canine hinge region. In certain embodiments, the hinge region comprises an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 45. In other embodiments, the hinge region comprises an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 46. In yet other embodiments, the hinge region comprises an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 47. In still other embodiments, the hinge region comprises an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 48. The present invention further provides antigen binding fragments of all of these antibodies.

The caninized antibodies of the present invention comprise a canine fragment crystallizable region (cFc region). In one embodiment, the canine cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence of SEQ ID NO: 49. In another embodiment, the canine cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence of SEQ ID NO: 50. In yet another embodiment, the canine cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence of SEQ ID NO: 52. In still another embodiment, the canine cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence of SEQ ID NO: 53. In yet another embodiment, the canine cFc region is a IgG-Bm that comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 51, in which both the aspartic acid residue (D) at position 31 of SEQ ID NO: 50 and the asparagine residue (N) at position 63 of SEQ ID NO: 50, are substituted by an alanine residue (A). The present invention further provides antigen binding fragments of all of these antibodies.

In certain embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 27. In other embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 28. In related embodiments, the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 29. In other related embodiments, the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 30. The present invention further provides antigen binding fragments of all of these antibodies.

In particular embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 27 and the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 29. In other embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 27 and the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 30. In yet other embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 28 and the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 29. In still other embodiments, the caninized antibody comprises a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 28 and the caninized antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 30. The present invention further provides antigen binding fragments of all of these antibodies.

In some embodiments, the caninized antibody comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 38. In other embodiments, the caninized antibody comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 39. In still other embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 36. In yet other embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 37. The present invention further provides antigen binding fragments of all of these antibodies.

In particular embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 36 and comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 38. In other embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 36 and comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 39. In still other

embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 37 and comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 38. In yet other embodiments, the caninized antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 37 and comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 39. The present invention further provides antigen binding fragments of all of these antibodies.

The present invention also provides nucleic acids, including isolated nucleic acids, that encode any of the caninized antibodies of the present invention and antigen binding fragments thereof. Therefore, the present invention provides nucleic acids (including isolated nucleic acids) that encode any one of the light chain variable regions of the caninized antibodies of the present invention. The present invention also provides nucleic acids that encode any one of the light chains of the caninized antibodies of the present invention. Similarly, the present invention further provides nucleic acids that encode any one of the heavy chain variable regions of the caninized antibodies of the present invention. In addition, the present invention further provides nucleic acids that encode any one of the heavy chains of the caninized antibodies of the present invention. The present invention further provides nucleic acids that encode any one of the antigen binding fragments of the antibodies of the present invention. In certain embodiments, the nucleic acid encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 38. In other embodiments, the nucleic acid encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 39. In related embodiments, the nucleic acid encodes a heavy chain that comprises the amino acid sequence of SEQ ID NO: 36. In other embodiments, the nucleic acid encodes a heavy chain that comprises the amino acid sequence of SEQ ID NO: 37. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain of a specific caninized antibody of the present invention and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain of said specific caninized antibody.

Accordingly, the present invention provides nucleic acids encoding the heavy chain variable regions of the caninized antibodies or antigen binding fragments thereof; the heavy chains of the caninized antibodies or antigen binding fragments thereof, the light chain variable regions of the caninized antibodies or antigen binding fragments thereof, and/or the light chains of the caninized antibodies or antigen binding fragments thereof. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the

antibodies of the present invention and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain of that (said) specific caninized antibody. The present invention also provides expression vectors that comprise such pairs of nucleic acids, or alternatively individual nucleic acids of the present invention. In addition, the present invention provides pairs of expression vectors, wherein one of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the caninized antibodies of the present invention, and the other of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the heavy chain of that (said) specific caninized antibody. Therefore, the present invention provides nucleic acids that encode the heavy chain variable region of a caninized antibody or an antigen binding fragment thereof of the present invention. The present invention further provides nucleic acids that encode the heavy chain of a caninized antibody or an antigen binding fragment thereof of the present invention. The present invention also provides nucleic acids that encode the light chain variable region of a caninized antibody or an antigen binding fragment thereof of the present invention. The present invention also provides nucleic acids that encode the light chain of a caninized antibody or an antigen binding fragment thereof of the present invention. In certain embodiments, the nucleic acid encoding the heavy chain variable region encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the light chain variable region encodes the light chain of that caninized antibody.

The present invention further provides as a pair, a nucleic acid encoding a set of the three heavy chain CDRs and a nucleic acid that encodes the corresponding set of the three light chain CDRs. In certain embodiments, the nucleic acid encoding the set of the three heavy chain CDRs encodes the heavy chain variable region of a caninized antibody and the corresponding nucleic acid encoding the set of the three light chain CDRs encodes the light chain variable region of that (said) caninized antibody. The present invention also provides a kit containing this pair of two nucleic acids. In certain embodiments, a nucleic acid encoding the set of the three heavy chain CDRs encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the set of the three light chain CDRs encodes the light chain of that caninized antibody.

In particular embodiments, a nucleic acid encodes a caninized antibody heavy chain that comprises a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3. In a related embodiments, a nucleic acid encodes a caninized antibody light chain that comprises a CDRL1 comprising the amino acid sequence of SEQ ID

NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6. The present invention further provides as a pair, a nucleic acid encoding a caninized antibody heavy chain that comprises a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3 and a nucleic acid encoding a caninized antibody light chain that comprises a CDRL1 comprising the amino acid sequence of SEQ ID NO: 4, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 6. The present invention also provides a kit containing this pair of two nucleic acids.

In specific embodiments, a nucleic acid of the present invention encodes a heavy chain variable region of a caninized antibody or antigen binding fragment thereof in which the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27. In a related embodiment, a nucleic acid encodes the light chain variable region of the caninized antibody or antigen binding fragment thereof in which the light chain variable region comprises the amino acid sequence of SEQ ID NO: 29. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 27 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain variable region that comprises the amino acid sequence of SEQ ID NO: 29. The present invention also provides a kit containing this pair of two nucleic acids. In certain embodiments, the nucleic acid encoding the heavy chain variable region encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the light chain variable region encodes the light chain of that caninized antibody.

In other specific embodiments, a nucleic acid of the present invention encodes a heavy chain variable region of a caninized antibody or antigen binding fragment thereof in which the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27. In a related embodiment, a nucleic acid encodes the light chain variable region of the caninized antibody or antigen binding fragment thereof in which the light chain variable region comprises the amino acid sequence of SEQ ID NO: 30. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 27 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain variable region that comprises the amino acid sequence of SEQ ID NO: 30. The present invention also

provides a kit containing this pair of two nucleic acids. In certain embodiments, the nucleic acid encoding the heavy chain variable region encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the light chain variable region encodes the light chain of that caninized antibody.

In other specific embodiments, a nucleic acid of the present invention encodes a heavy chain variable region of a caninized antibody or antigen binding fragment thereof in which the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28. In a related embodiment, a nucleic acid encodes the light chain variable region of the caninized antibody or antigen binding fragment thereof in which the light chain variable region comprises the amino acid sequence of SEQ ID NO: 29. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 28 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain variable region that comprises the amino acid sequence of SEQ ID NO: 29. The present invention also provides a kit containing this pair of two nucleic acids. In certain embodiments, the nucleic acid encoding the heavy chain variable region encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the light chain variable region encodes the light chain of that caninized antibody.

In still other specific embodiments, a nucleic acid of the present invention encodes a heavy chain variable region of a caninized antibody or antigen binding fragment thereof in which the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28. In a related embodiment, a nucleic acid encodes the light chain variable region of the caninized antibody or antigen binding fragment thereof in which the light chain variable region comprises the amino acid sequence of SEQ ID NO: 30. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 28 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain variable region that comprises the amino acid sequence of SEQ ID NO: 30. The present invention also provides a kit containing this pair of two nucleic acids. In certain embodiments, the nucleic acid encoding the heavy chain variable region encodes the heavy chain of a caninized antibody and the corresponding nucleic acid encoding the light chain variable region encodes the light chain of that caninized antibody.

In yet other specific embodiments, a nucleic acid of the present invention encodes a heavy chain of a caninized antibody or antigen binding fragment thereof in which the heavy chain comprises the amino acid sequence of SEQ ID NO: 36. In a related embodiment, a nucleic acid encodes the light chain of the caninized antibody or antigen binding fragment thereof in which the light chain comprises the amino acid sequence of SEQ ID NO: 38. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain that comprises the amino acid sequence of SEQ ID NO: 36 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 38. The present invention also provides a kit containing this pair of two nucleic acids.

In still other embodiments, a nucleic acid of the present invention encodes a heavy chain of a caninized antibody or antigen binding fragment thereof in which the heavy chain comprises the amino acid sequence of SEQ ID NO: 36. In a related embodiment, a nucleic acid encodes the light chain of the caninized antibody or antigen binding fragment thereof in which the light chain comprises the amino acid sequence of SEQ ID NO: 39. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain that comprises the amino acid sequence of SEQ ID NO: 36 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 39. The present invention also provides a kit containing this pair of two nucleic acids.

In specific embodiments, a nucleic acid of the present invention encodes a heavy chain of a caninized antibody or antigen binding fragment thereof in which the heavy chain comprises the amino acid sequence of SEQ ID NO: 37. In a related embodiment, a nucleic acid encodes the light chain of the caninized antibody or antigen binding fragment thereof in which the light chain comprises the amino acid sequence of SEQ ID NO: 38. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain that comprises the amino acid sequence of SEQ ID NO: 37 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 38. The present invention also provides a kit containing this pair of two nucleic acids.

In specific embodiments, a nucleic acid of the present invention encodes a heavy chain of a caninized antibody or antigen binding fragment thereof in which the heavy chain comprises the amino acid sequence of SEQ ID NO: 37. In a related embodiment, a nucleic acid encodes the

light chain of the caninized antibody or antigen binding fragment thereof in which the light chain comprises the amino acid sequence of SEQ ID NO: 39. The present invention further provides a pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain that comprises the amino acid sequence of SEQ ID NO: 37 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain that comprises the amino acid sequence of SEQ ID NO: 39. The present invention also provides a kit containing this pair of two nucleic acids.

The present invention further provides expression vectors that comprise and express one or more of the nucleic acids of the present invention. In particular embodiments, the expression vector comprises and expresses a nucleic acid encoding a heavy chain of a caninized antibody of the present invention and a nucleic acid encoding a light chain of that caninized antibody. The present invention also provides host cells that comprise one or more expression vectors of the present invention.

The present invention also provides pharmaceutical compositions comprising the caninized antibodies and/or antigen binding fragments of the antibodies and a pharmaceutically acceptable carrier or diluent. In addition, pharmaceutical compositions are provided that comprise a nucleic acid encoding a heavy chain of a caninized antibody of the present invention and a nucleic acid encoding a light chain of that caninized antibody and a pharmaceutically acceptable carrier or diluent. In other embodiments, the pharmaceutical compositions comprise a nucleic acid encoding both a heavy chain of a caninized antibody of the present invention and a light chain of that caninized antibody. In yet other embodiments, the pharmaceutical compositions comprise a pharmaceutically acceptable carrier or diluent and an expression vector that comprises one or more nucleic acids encoding a heavy chain of a caninized antibody of the present invention and a light chain of that caninized antibody and thereby, can express the caninized antibody and/or antigen binding fragments of the antibody of the present invention, *in vivo*.

The present invention further provides methods of treating a condition associated with pain in an animal subject. The method of treatment can comprise administering to an animal subject in need thereof, a therapeutically effective amount of a pharmaceutical composition of the present invention. In certain embodiments, the method is used for the treatment of osteoarthritis. In other embodiments, the method is used for the treatment of hyperalgesia. In still other embodiments, the method is used for the treatment of allodynia. In yet other embodiments, the method is used for the treatment of pain. In still other embodiments, the method is used for the

treatment of any combination of osteoarthritis, hyperalgesia, allodynia, and/or pain. The animal subject is preferably a canine.

The present invention also provides methods of producing a caninized antibody or antigen binding fragment thereof that binds canine NGF. In particular embodiments, the method includes culturing one or more host cells that comprise one or more expression vectors of the present invention that encode and express the light chain of a caninized antibody of the present invention and/or the heavy chain of that caninized antibody in a culture medium under conditions in which the nucleic acid or nucleic acids are expressed, thereby producing a polypeptide comprising the light chain of a caninized antibody of the present invention, and the heavy chain of that caninized antibody. The polypeptides are then recovered from the one or more host cells and/or culture medium. In certain embodiments, the polypeptides comprising the light chain of a caninized antibody of the present invention and the polypeptides comprising the heavy chain of that caninized antibody are combined with each under conditions that are conducive to form a caninized antibody.

The present invention further provides a pair of host cells, where in one of the pair of host cells comprises an expression vector that comprises one of a pair of nucleic acids that comprises a nucleotide sequence that encodes the heavy chain of a specific caninized antibody of present invention, whereas the other of the pair of host cells comprises an expression vector that comprises the other of the pair of nucleic acids that comprises the nucleotide sequence that encodes the light chain of said specific caninized antibody. The present invention further provides a method of producing a caninized antibody of the present invention that binds canine NGF comprising culturing each one of the pair of host cells in a culture medium either individually or in combination under conditions wherein the nucleic acids are expressed, thereby producing a polypeptide comprising the light chain of the caninized antibody, the heavy chain of the caninized antibody, or both and then recovering the light chain of the caninized antibody, the heavy chain of the caninized antibody, or both from the pair of host cells or culture medium.

These and other aspects of the present invention will be better appreciated by reference to the following Brief Description of the Drawings and the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the binding of human-canine chimeric Fulranumab (Ful Chimeric) and variants of caninized Fulranumab (cFul) antibodies to canine NGF.

Ful Chimeric (•), cFulVH1L1 (◙), cFulVH1L2 (▲), cFulVH2L1 (▼), cFulVH2L2 (♦), and mab control (o).

Figure 2 depicts the binding of human-canine chimeric Fasinumab (Fas Chimeric) and individual caninized Fasinumab (cFas) antibodies to canine NGF. Fas Chimeric (•), cFasVH2L2 (◙), cFasVH2L3 (▲), and mab control (o).

Figure 3 shows the binding of canine NGF to the canine TrkA receptor. The binding of canine NGF to canine NGF receptor (TrkA) was determined by ELISA. Canine NGF (•).

Figure 4 depicts the inhibition of canine NGF binding to canine TrkA receptor by the human-canine chimeric Fulranumab or by individual caninized antibodies. Ful Chimeric (•), cFulVH1L1 (◙), cFulVH1L2 (▲), cFulVH2L1 (▼), cFulVH2L2 (♦), and mab control (o).

Figure 5 shows the stimulation of TF-1 cell proliferation by canine NGF. [Canine NGF (•)].

Figure 6 shows the inhibition of TF-1 cell proliferation by human-canine chimeric Fulranumab (Ful Chimeric) or individual caninized anti-NGF antibodies. Ful Chimeric (•), cFulVH1L1 (◙), cFulVH1L2 (▲), cFulVH2L1 (▼), cFulVH2L2 (♦), and mab control (o).

DETAILED DESCRIPTION OF THE INVENTION

In response to need for better therapies for pain in canines, the present invention provides formulations and methodology that can achieve a significant effect to relieve the pain associated with and/or due to NGF. Accordingly, it was surprisingly found that whereas caninized antibodies comprising a set of CDRs from an antibody first raised against human NGF could both bind tightly to canine NGF and block the binding of canine NGF to the canine TrkA receptor, a caninized antibody comprising a set of CDRs from another antibody first raised against human NGF could not measurably bind to canine NGF. This was true even though both corresponding human-canine chimeric constructs could tightly bind to canine NGF.

ABBREVIATIONS

Throughout the detailed description and examples of the invention the following abbreviations will be used:

ADCC	Antibody-dependent cellular cytotoxicity
CDC	Complement-dependent cyotoxicity
CDR	Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system
EC50	concentration resulting in 50% efficacy or binding

ELISA	Enzyme-linked immunosorbant assay
FR	Antibody framework region: the immunoglobulin variable regions excluding the CDR regions.
IC50	concentration resulting in 50% inhibition
IgG	Immunoglobulin G
Kabat	An immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat [*Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]
mAb	Monoclonal antibody (also Mab or MAb)
V region	The segment of IgG chains which is variable in sequence between different antibodies.
VH	Immunoglobulin heavy chain variable region
VL	Immunoglobulin light chain variable region

DEFINITIONS

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

"Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, *e.g.*, adhesion, or activity in maintaining a structure of a cell, *e.g.,* cell membranes or cytoskeleton. "Activity" can also mean specific activity, *e.g.*, [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], concentration in a biological compartment, or the like. "Activity" may refer to modulation of components of the innate or the adaptive immune systems.

"Administration" and "treatment", as it applies to an animal, *e.g.*, a canine subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal *e.g.*, a canine subject, cell, tissue, organ, or

biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell.

"Administration" and "treatment" also mean *in vitro* and *ex vivo* treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably a non-human animal, more preferably a mammal (*e.g.*, canine or feline) and most preferably a canine.

"Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies of the present invention, internally or externally to *e.g.*, a canine subject or patient having one or more symptoms, or being suspected of having a condition, for which the agent has therapeutic activity. Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease/condition symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease/condition symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease/condition state, age, and weight of the patient (*e.g.*, canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease/condition symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veterinarians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (*e.g.*, a treatment method or article of manufacture) may not be effective in alleviating the target disease/condition symptom(s) in every subject, it should alleviate the target disease/condition symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a veterinary (*e.g.*, canine) or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a veterinary (*e.g.*, canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies of the present invention to *e.g.*, a canine or other animal subject (*e.g.*, feline), a cell, tissue, physiological compartment, or physiological fluid.

As used herein, the term "canine" includes all domestic dogs, Canis lupus familiaris or Canis familiaris, unless otherwise indicated.

As used herein, the term "feline" refers to any member of the *Felidae* family. Members of this family include wild, zoo, and domestic members, including domestic cats, pure-bred and/or mongrel companion cats, show cats, laboratory cats, cloned cats, and wild or feral cats.

As used herein the term “canine frame” refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in the majority of embodiments the amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse or human) in both chains. Optionally the heavy and/or light chains of the canine antibody may contain some foreign non-CDR residues, *e.g.*, so as to preserve the conformation of the foreign CDRs within the caninized antibody, and/or to modify the Fc region function, as exemplified below and/or disclosed in U.S. 10,106,607 B2, hereby incorporated by reference herein in its entirety.

The “Fragment crystallizable region” abbreviated as “Fc” or used interchangeably with “Fc region” corresponds to the CH3-CH2 portion of an antibody that interacts with cell surface receptors called Fc receptors. The canine fragment crystallizable region (cFc region) of each of the four canine IgGs were first described by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001); *see also*, Bergeron *et al.*, *Vet. Immunol. Immunopathol.* 157: 31-41 (2014) and U.S. 10,106,607 B2].

As used herein the canine Fc (cFc) “IgG-Bm” is canine IgG-B Fc comprising two (2) amino acid residue substitutions, D31A and N63A, as in the amino acid sequence of SEQ ID NO: 20 of IgG-B (*see* below) and preferably without the c-terminal lysine (‘K”) *i.e.*, SEQ ID NO: 51). Both the aspartic acid residue (D) at position 31 of SEQ ID NO: 50 and the asparagine residue (N) at position 63 of SEQ ID NO: 50, are substituted by an alanine residue (A) in IgG-Bm. These two amino acid residue substitutions serve to significantly diminish the antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of the naturally occurring canine IgG-B [*see*, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in their entirety]. Further amino acid substitutions to the IgG-Bm are also envisioned, which parallel those which can be made in IgG-B. The amino acid sequence of IgG-B, SEQ ID NO: 50 is:

```
1                                                    50
LGGPSVFIFP PKPKDTLLIA RTPEVTCVVV DLDPEDPEVQ ISWFVDGKQM
↳ CH2

51                                                   100
```

QTAKTQPREE QFNGTYRVVS VLPIGHQDWL KGKQFTCKVN NKALPSPIER

101 150

TISKARGQAH QPSVYVLPPS REELSKNTVS LTCLIKDFFP PDIDVEWQSN

CH3

151 200

GQQEPESKYR TTPPQLDEDG SYFLYSKLSV DKSRWQRGDT FICAVMHEAL

201 215

HNHYTQKSLS HSPGK

The amino acid sequence of IgG-Bm, SEQ ID NO: 51, is provided below.

LGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVS
LTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQESLSHSPG

The amino acid sequence of IgG-Bm, SEQ ID NO: 20, with the C-Terminal lysine (K):

LGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVS
LTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQESLSHSPGK

As used herein, a "substitution of an amino acid residue" with another amino acid residue in an amino acid sequence of an antibody for example, is equivalent to "replacing an amino acid residue" with another amino acid residue and denotes that a particular amino acid residue at a specific position in the amino acid sequence has been replaced by (or substituted for) by a different amino acid residue. Such substitutions can be particularly designed *i.e.*, purposefully replacing an alanine with a serine at a specific position in the amino acid sequence by *e.g.*, recombinant DNA technology. Alternatively, a particular amino acid residue or string of amino acid residues of an antibody can be replaced by one or more amino acid residues through more natural selection processes *e.g.*, based on the ability of the antibody produced by a cell to bind to a given region on that antigen, *e.g.*, one containing an epitope or a portion thereof, and/or for the antibody to comprise a particular CDR that retains the same canonical structure as the CDR it is replacing. Such substitutions/replacements can lead to "variant" CDRs and/or variant antibodies.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. An antibody can be a monomer, dimer, or larger multimer. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), caninized antibodies, fully canine antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as caninization of an antibody for use as a canine therapeutic antibody.

As used herein, an antibody of the present invention that "blocks" or is "blocking" or is "blocking the binding" of *e.g.*, a canine ligand to its binding partner (*e.g.*, its receptor), is an antibody that blocks (partially or fully) the binding of the canine ligand to its canine receptor and *vice versa*, as determined in standard binding assays (*e.g.*, BIACore®, ELISA, or flow cytometry).

Typically, an antibody or antigen binding fragment of the invention retains at least 10% of its canine antigen binding activity (when compared to the parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the canine antigen binding affinity as the parental antibody. It is also intended that an antibody or antigen binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

"Isolated antibody" refers to the purification status and in such context means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

As used herein, an antibody is said to bind specifically to a polypeptide comprising a given antigen sequence (in this case a portion of the amino acid sequence of canine NGF) if it binds to polypeptides comprising the portion of the amino acid sequence of canine NGF, but does not bind to other canine proteins lacking that portion of the sequence of canine NGF. For example, an antibody that specifically binds to a polypeptide comprising canine NGF, may bind

to a FLAG®-tagged form of canine NGF, but will not bind to other FLAG®-tagged canine proteins.

As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, *i.e.* antibody fragments that retain the ability to bind specifically to the antigen (*e.g.*, canine NGF) bound by the full-length antibody, *e.g.* fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, *e.g.*, sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

An antibody, or binding compound derived from the antigen-binding site of an antibody, binds to its canine antigen, or a variant or mutein thereof, "with specificity" when it has an affinity for that canine antigen or a variant or mutein thereof which is at least ten-times greater, more preferably at least 20-times greater, and even more preferably at least 100-times greater than its affinity for any other canine antigen tested. An antibody that binds canine NGF "with specificity" may still bind an NGF from another species (*e.g.*, feline NGF and/or human NGF).

As used herein, a "chimeric antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, where the first and second antibodies are from different species. [U.S. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 6851-6855 (1984)]. Typically the variable domains are obtained from an antibody from an experimental animal (the "parental antibody"), such as a rodent (or a rodent that comprises a human immune system) and the constant domain sequences are obtained from the animal subject antibodies, *e.g.*, canine so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a canine subject respectively, than the parental (*e.g.,* rodent) antibody.

As used herein, the term "caninized antibody" refers to forms of antibodies that contain sequences from both canine and non-canine (*e.g.*, mouse or human) antibodies. In general, the caninized antibody will comprise substantially all of at least one or more typically, two variable domains in which all or substantially all of the hypervariable loops correspond to those of a non-canine immunoglobulin (*e.g.*, comprising 6 CDRs as exemplified below), and all or substantially all of the framework (FR) regions (and typically all or substantially all of the remaining frame) are those of a canine immunoglobulin sequence. A caninized antibody can comprise both the three heavy chain CDRs and the three light chain CDRS from *e.g.*, a human anti-human NGF antibody together with a canine frame or a modified canine frame. A modified canine frame

comprises one or more amino acids changes as exemplified herein that further optimize the effectiveness of the caninized antibody, *e.g.*, to increase its binding to its canine antigen and/or its ability to block the binding of that canine antigen to the canine antigen's natural binding partner.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same. Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of *Sequences of Proteins of Immunological Interest*, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978); Kabat, *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987) or Chothia, *et al.*, *Nature* 342:878-883 (1989)].

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.*, LCDR1 or CDRL1, LCDR2 or CRDL2, and LCDR3 or CDRL3 in the light chain variable domain and HCDR1 or CDRH1, HCDR2 or CDRH2, and HCDR3 or CDRH3 in the heavy chain variable domain). [*See* Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), defining the CDR regions of an antibody by sequence; *see also* Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987) defining the CDR regions of an antibody by structure]. As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A or IgGA, IgG-B or IgGB, IgG-C or IgGC, and IgG-D or IgGD. The two known canine light chain subtypes are referred to as *lambda* and *kappa*. Each of the two heavy chains consists of one variable domain (VH) and three constant domains referred to as CH-1, CH-2, and CH-3.

The CH-1 domain is connected to the CH-2 domain *via* an amino acid sequence referred to as the "hinge" or alternatively as the "hinge region".

In specific embodiments of the invention, besides binding canine NGF, a canine or caninized antibody against its antigen of the present invention optimally has two attributes:

1. Lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
2. be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has high level of ADCC activity. On the other hand, IgG-A binds weakly to protein A, but also displays ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D displays no ADCC activity. (IgG-C has considerable ADCC activity). One way the present invention addresses these issues in certain embodiments is by providing modified canine IgG-B antibodies of the present invention specific to an antigen of the present invention that lack the effector functions such as ADCC and can be easily purified using industry standard protein A chromatography.

"Homology", as used herein, refers to sequence similarity between two polynucleotide sequences or between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same base or amino acid residue, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared ×100. For example, if 6 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology. Sequence identity refers to the degree to which the amino acids of two polypeptides are the same at equivalent positions when the two sequences are optimally aligned. As used herein one amino acid sequence is 100% "identical" to a second amino acid sequence when the amino acid residues of both sequences are identical.

Accordingly, an amino acid sequence is 50% "identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.,* a protein, or a portion of the polypeptide being compared. In particular

embodiments, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account. Sequence similarity includes identical residues and nonidentical, biochemically related amino acids, *e.g.*, biochemically related amino acids that share similar properties and may be interchangeable.

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity [*see, e.g.*, Watson *et al., Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.; 1987)]. In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table A directly below.

TABLE A

Exemplary Conservative Amino Acid Substitutions

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys; His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala; Gly

Original residue	Conservative substitution
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants," as used herein, refers to antibodies or fragments in which one or more amino acid residues have been changed without altering a desired property, such an antigen affinity and/or specificity. Such variants include, but are not limited to, replacement of an amino acid with one having similar properties, such as the conservative amino acid substitutions of Table A above.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The present invention provides isolated caninized antibodies of the present invention, methods of use of the antibodies in the treatment of a condition *e.g.*, the treatment of osteoarthritis in canines.

The nucleic acid and amino acid sequences of these four heavy chains were first identified by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001)]. The amino acid and nucleic sequences for these heavy chains are also available from the GenBank data bases. For example, the amino acid sequence of IgGA heavy chain has accession number AAL35301.1, IgGB has accession number AAL35302.1, IgGC has accession number AAL35303.1, and IgGD has accession number (AAL35304.1). Canine antibodies also contain two types of light chains, *kappa* and *lambda*. The DNA and amino acid sequence of these light chains can be obtained

from GenBank Databases. For example, the *kappa* light chain amino acid sequence has accession number ABY 57289.1 and the *lambda* light chain has accession number ABY 55569.1.

The known amino acid sequences of the four unmodified canine Fcs are:

cIgG-A [SEQ ID NO: 49]

LGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQFNGT
YRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKELSSSDTV
SITCLIKDFYPPDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCA
VMHETLQNHYTDLSLSHSPGK

cIgG-B [SEQ ID NO: 50]

LGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVS
LTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQESLSHSPGK

cIgG-C [SEQ ID NO: 52]

LGGPSVFIFPPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQVQTANTQPREEQSNGT
YRVVSVLPIGHQDWLSGKQFKCKVNNKALPSPIEEIISKTPGQAHQPNVYVLPPSRDEMSKNTVT
LTCLVKDFFPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQISLSHSPGK

cIgG-D [SEQ ID NO: 53]

LGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQFNST
YRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKELSSSDTV
TLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDTFTCA
VMHEALQNHYTDLSLSHSPGK

In the present invention, the amino acid sequence for each of the four canine IgG Fc regions is based on the identified boundary of CH1 and CH2 domains as determined by Tang *et al, supra.* Caninized mammalian (*e.g.*, mouse or human) anti-human NGF antibodies that bind canine NGF of the present invention include, but are not limited to: antibodies of the present invention that comprise canine IgG-A, IgG-B, IgG-C, and IgG-D heavy chains and/or canine *kappa* or *lambda* light chains together with the anti-human NGF CDRs. Accordingly, the present invention provides caninized mouse or human antibodies of the present invention, including isolated caninized mouse or human anti-human NGF antibodies, that bind to canine NGF and that preferably also block the binding of that canine NGF to canine TrkA.

Accordingly, the present invention further provides caninized NGF antibodies and methods of use of the caninized antibodies of the present invention in the treatment of pain *e.g.*, osteoarthritis in canines.

The present invention further provides full length caninized heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized mouse or human anti-NGF antibodies (including isolated caninized human anti-human NGF antibodies) of the present invention and methods of use of the antibodies of the present invention in the treatment of a condition *e.g.*, the treatment of pain in canines.

The present invention also provides antibodies of the present invention that comprise a canine fragment crystallizable region (cFc region) in which the cFc region has been genetically modified to augment, decrease, or eliminate one or more effector functions. In one aspect of the present invention, the genetically modified cFc region decreases or eliminates one or more effector functions. In another aspect of the invention the genetically modified cFc region augments one or more effector function. In certain embodiments, the genetically modified cFc region is a genetically modified canine IgGB Fc region. In another such embodiment, the genetically modified cFc region is a genetically modified canine IgGC Fc region. In a particular embodiment, the effector function is antibody-dependent cytotoxicity (ADCC) that is augmented, decreased, or eliminated. In another embodiment, the effector function is complement-dependent cytotoxicity (CDC) that is augmented, decreased, or eliminated. In yet another embodiment, the cFc region has been genetically modified to augment, decrease, or eliminate both the ADCC and the CDC.

In order to generate variants of canine IgG that lack effector functions, a number of mutant canine IgGB heavy chains were generated. These variants may include one or more of the following single or combined substitutions in the Fc portion of the heavy chain amino acid sequence: P4A, D31A, N63A, G64P, T65A, A93G, and P95A. Variant heavy chains (*i.e.*, containing such amino acid substitutions) are cloned into expression plasmids and are transfected into HEK 293 cells along with a plasmid containing the gene encoding a light chain. Intact antibodies are expressed and purified from HEK 293 cells and then can be evaluated for binding to $Fc_{\gamma}RI$ and C1q to assess their potential for mediation of immune effector functions. [S*ee*, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in its entirety.]

The present invention also provides modified canine IgG-Ds which in place of its natural IgG-D hinge region they comprise a hinge region from:

IgG-A:	FNECRCTDTPPCPVPEP	SEQ ID NO: 45
IgG-B:	PKRENGRVPRPPDCPKCPAPEM	SEQ ID NO: 46; or
IgG-C:	AKECECKCNCNNCPCPGCGL	SEQ ID NO: 47.

Alternatively, the IgG-D hinge region can be genetically modified by replacing a serine residue with a proline residue, *i.e.*, PKESTCKCI**P**PCPVPES, SEQ ID NO: 48 (with the proline residue (P) in bold substituting for the naturally occurring serine residue). Such modifications can lead to a canine IgG-D lacking fab arm exchange. The modified canine IgG-Ds can be constructed using standard methods of recombinant DNA technology [*e.g.*, Maniatis *et al.*, *Molecular Cloning, A Laboratory Manual* (1982)]. In order to construct these variants, the nucleic acids encoding the amino acid sequence of canine IgG-D can be modified so that it encodes the modified IgG-Ds. The modified nucleic acid sequences are then cloned into expression plasmids for protein expression.

The six complementary determining regions (CDRs) of a caninized mouse or human anti-NGF antibody, as described herein can comprises a canine antibody *kappa* (*k*) or *lambda* (*l*) light chain comprising a mouse light chain LCDR1, LCDR2, and LCDR3 and a canine antibody heavy chain comprising a mouse or human heavy chain HCDR1, HCDR2, and HCDR3.

Nucleic Acids

The present invention also comprises the nucleic acids encoding the antibodies of the present invention (*see e.g.*, Examples below).

Also included in the present invention are nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the amino acid sequences of the caninized antibodies, with the exception of the CDRs which do not change, provided herein when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. The present invention further provides nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences when the comparison is performed with a BLAST algorithm, wherein the parameters of the algorithm are selected to give the largest match

between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

As used herein, nucleotide and amino acid sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.*, using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

The following references relate to BLAST algorithms often used for sequence analysis: BLAST ALGORITHMS: Altschul, S.F., *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish, W., *et al.*, *Nature Genet.* 3:266-272 (1993); Madden, T.L., *et al.*, *Meth. Enzymol.* 266:131-141(1996); Altschul, S.F., *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang, J., *et al.*, *Genome Res.* 7:649-656 (1997); Wootton, J.C., *et al.*, *Comput. Chem.* 17:149-163 (1993); Hancock, J.M. *et al.*, *Comput. Appl. Biosci.* 10:67-70 (1994); ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, (1978); *Natl. Biomed. Res. Found.*, Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, vol. 5, suppl. 3." (1978), M.O. Dayhoff (ed.), pp. 353-358 (1978), *Natl. Biomed. Res. Found.*, Washington, DC; Altschul, S.F., *J. Mol. Biol.* 219:555-565 (1991); States, D.J., *et al.*, *Methods* 3:66-70(1991); Henikoff, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 89:10915-10919 (1992); Altschul, S.F., *et al.*, *J. Mol. Evol.* 36:290-300 (1993); ALIGNMENT STATISTICS: Karlin, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 87:2264-2268 (1990); Karlin, S., *et al.*, *Proc. Natl. Acad. Sci.* USA 90:5873-5877 (1993); Dembo, A., *et al.*, *Ann. Prob.* 22:2022-2039 (1994); and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in *Theoretical and Computational Methods in Genome Research* (S. Suhai, ed.), pp. 1-14, Plenum, New York (1997).

Antibody Protein Engineering

By way of example, and not limitation, as indicated above, the canine heavy chain constant region can be from IgG-A, IgG-B, IgG-C, IgG-D, and the corresponding cFc can be a modified cFc, such as the IgG-Bm of the IgG-B heavy constant region used herein [*see*,

U.S. 10,106,607 B2, hereby incorporated by reference in its entirety] and the canine light chain can comprise the constant region from *kappa* or *lambda*.

The antibodies can be engineered to include modifications to the canine framework and/or the canine frame residues within the variable domains of a parental (*e.g.*, human) monoclonal antibody, *e.g.,* to improve the properties of the antibody.

The construction of caninized anti-NGF monoclonal antibodies can be performed by determining a DNA sequence that encodes the heavy and light chains of canine IgG were determined. The DNA and protein sequence of the canine heavy and light chains are known in the art and can be obtained by searching of the NCBI gene and protein databases. As indicated above, for canine antibodies there are four known IgG subtypes: IgG-A, IgG-B, IgG-C, and IgG-D, and two types of light chains, *i.e.*, *kappa* and *lambda*.

A caninized human anti-NGF antibody can be produced recombinantly by methods that are known in the field. Mammalian cell lines available as hosts for expression of the antibodies or fragments disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion or fragment thereof, the light chain and/or antigen-binding fragment thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in

connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

Accordingly, in certain embodiments, the antibody or antigen binding fragment comprises a heavy chain constant region, *e.g.*, a canine constant region, such as IgG-A, IgG-B, IgG-C and IgG-D canine heavy chain constant region or a variant thereof. In certain embodiments, the antibody or antigen binding fragment comprises a light chain constant region, *e.g.*, a canine light chain constant region, such as *lambda* or *kappa* canine light chain region or variant thereof. By way of example, and not limitation, the canine heavy chain constant region can be from IgG-B and the canine light chain constant region can be from *kappa*.

Caninized mammalian (*e.g.*, mouse or human) anti-human NGF antibodies that bind canine NGF of the present invention include, but are not limited to: antibodies of the present invention that comprise canine IgG-A, IgG-B, IgG-C, and IgG-D heavy chains and/or canine *kappa* or *lambda* light chains together with the anti-human NGF CDRs. Accordingly, the present invention provides caninized mouse or human antibodies of the present invention, including isolated caninized mouse or human anti-human NGF antibodies, that bind to canine NGF and that preferably also block the binding of that canine NGF to canine TrkA.

The present invention further provides caninized NGF antibodies and methods of use of the caninized antibodies of the present invention in the treatment of pain *e.g.*, osteoarthritis in canines.

The present invention further provides full length caninized heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized mouse or human anti-NGF antibodies (including isolated caninized human anti-human NGF antibodies) of the present invention

Pharmaceutical Compositions and Administration

To prepare pharmaceutical or sterile compositions comprising the antibodies of the present invention, these antibodies can be admixed with a pharmaceutically acceptable carrier or excipient. [*See*, *e.g.*, *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984)].

Formulations of therapeutic and diagnostic agents may be prepared by mixing with acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions or suspensions [see, *e.g.*, Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New

York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY]. In one embodiment, the antibodies of the present invention are diluted to an appropriate concentration in a sodium acetate solution pH 5-6, and NaCl or sucrose is added for tonicity. Additional agents, such as polysorbate 20 or polysorbate 80, may be added to enhance stability.

Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with another agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD_{50}/ ED_{50}). In particular aspects, antibodies exhibiting high therapeutic indices are desirable. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in canines. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The mode of administration can vary. Suitable routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial. In particular embodiments, the antibodies of the present invention can be administered by an invasive route such as by injection. In further embodiments of the invention, the antibodies of the present invention, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, or by inhalation, aerosol delivery. Administration by non-invasive routes (*e.g.*, orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle, including, *e.g.*, a prefilled syringe or autoinjector. The pharmaceutical compositions disclosed herein may also be administered with a needleless hypodermic injection device; such as

the devices disclosed in U.S. Patent Nos.: 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

The pharmaceutical compositions disclosed herein may also be administered by infusion. Examples of well-known implants and modules form administering pharmaceutical compositions include: U.S. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

Alternatively, one may administer the antibodies of the present invention in a local rather than systemic manner, often in a depot or sustained release formulation.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibodies, the level of symptoms, the immunogenicity of the therapeutic antibodies and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibodies to effect improvement in the target disease/condition state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibodies and the severity of the condition being treated. Guidance in selecting appropriate doses of therapeutic antibodies is available [*see, e.g.*, Wawrzynczak *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK (1996); Kresina (ed.) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY (1991); Bach (ed.) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY (1993); Baert, *et al. New Engl. J. Med.* 348:601-608 (2003); Milgrom *et al. New Engl. J. Med.* 341:1966-1973 (1999); Slamon *et al. New Engl. J. Med.* 344:783-792 (2001); Beniaminovitz *et al. New Engl. J. Med.* 342:613-619 (2000); Ghosh *et al. New Engl. J. Med.* 348:24-32 (2003); Lipsky *et al. New Engl. J. Med.* 343:1594-1602 (2000)].

Determination of the appropriate dose is made by the veterinarian, *e.g.*, using parameters or factors known or suspected in the art to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of the symptoms.

Antibodies provided herein may be provided by continuous infusion, or by doses administered, *e.g.*, daily, 1-7 times per week, weekly, bi-weekly, monthly, bimonthly, quarterly, semiannually, annually etc. Doses may be provided, *e.g.*, intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A total weekly dose is generally at least 0.05 μg/kg body weight, more generally at least 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/ml, 10 mg/kg, 25 mg/kg, 50 mg/kg or more [*see, e.g.*, Yang, *et al. New Engl. J. Med.* 349:427-434 (2003); Herold, *et al. New Engl. J. Med.* 346:1692-1698 (2002); Liu, *et al. J. Neurol. Neurosurg. Psych.* 67:451-456 (1999); Portielji, *et al. Cancer Immunol. Immunother.* 52:133-144 (2003)]. Doses may also be provided to achieve a pre-determined target concentration of antibodies of the present invention in the canine's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 μg/ml or more. In other embodiments, antibodies of the present invention are administered subcutaneously or intravenously, on a weekly, biweekly, "every 4 weeks," monthly, bimonthly, or quarterly basis at 10, 20, 50, 80, 100, 200, 500, 1000 or 2500 mg/subject.

As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with a disorder and/or a reduction in the severity of the symptoms of such disorder. The terms further include ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject (*e.g.*, a canine) with a disorder, condition and/or symptom, or with the potential to develop such a disorder, disease or symptom.

As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of antibodies of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, *e.g.*, canine, is effective to cause a measurable improvement in one or more symptoms of a disease or condition or the progression of such disease or condition. A therapeutically effective dose further refers to that amount of the antibodies sufficient to result in at least partial amelioration of symptoms, *e.g.,* treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially, or simultaneously. An effective amount of a therapeutic will result in an improvement of a diagnostic measure or parameter by at least 10%; usually by at least 20%;

preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. An effective amount can also result in an improvement in a subjective measure in cases where subjective measures are used to assess severity of the condition, *e.g.*, pain.

EXAMPLES

EXAMPLE 1

PRIOR ART ANTIBODIES TO HUMAN NGF REACTIVE WITH CANINE NGF

In an effort to develop a treatment for pain (*e.g.*, for osteoarthritis) in companion animals such as dogs, cats, and horses, an investigation was undertaken to learn whether two known human or humanized antibodies to human NGF [*see e.g.*, US 7,601,818 B2 (fulranumab abbreviated as ful herein), US 7,988,967 B2 (fasinumab; abbreviated as fas herein)] might also bind to NGF from dogs, cats or horses. It was found that both human/humanized monoclonal antibodies that bind to human NGF also bind to canine NGF. The set of the six prior art CDRs for these two previously disclosed antibodies are provide in Tables 1A and 1B. below.

TABLE 1A[1]

AMINO ACID SEQUENCES OF THE PRIOR ART

Ful CDRS IN THE CANINIZED ANTIBODIES

CDR	Amino Acid Sequence	SEQ ID NO:
H-1	SYSMN	1
H-2	YISRSSHTIFYADSVKG	2
H-3	VYSSGWHVSDYFDY	3
L-1	RASQGISSALA	4
L-2	DASSLES	5
L-3	QQFNSYPLT	6

TABLE 1B[2]

AMINO ACID SEQUENCES OF THE PRIOR ART

Fas CDRS IN THE CANINIZED ANTIBODIES

CDR	Amino Acid Sequence	SEQ ID NO:
H-1	ELSIH	7

[1] The amino acid sequences in Table 1A were previously obtained and disclosed in US 7,601,818 B2.

[2] The amino acid sequences in Table 1B were previously obtained and disclosed in US 7,988,967 B2.

H-2	GFDPEDGETIYAQKFQG	8
H-3	IGVVTNFDN	9
L-1	RASQAIRNDLG	10
L-2	AAFNLQS	11
L-3	QQYNRYPWT	12

EXAMPLE 2

CANINE NGF AND CANINE NGF TRKA RECEPTOR

The amino acid sequence of the canine NGF protein is available at the national center for biotechnology information (NCBI) under accession number NP_001181879.1 [SEQ ID NO: 13]. Canine NGF-HIS-Avi protein was produced as a fusion protein of canine NGF with a C-terminal addition of 6 histidine residues and an Avi tag sequence to facilitate purification and site-specific biotinylation of the NGF protein having the amino acid sequence of SEQ ID NO: 14. The predicted amino acid sequence of the high affinity canine nerve growth factor receptor (TrkA) is available at the national center for biotechnology information (NCBI) under accession number XP_038527745. The amino acid sequence of TrkA is SEQ ID NO: 15. A cNGF-hFc Fusion protein has an amino acid sequence of SEQ ID NO: 16. For the canine NGF receptor TrkA ECD-canine Fc fusion protein, the predicted amino acid sequence of TrkA ECD was produced as a fusion protein with a C-terminal addition of the cFc from canine IgG-B. The sequence of this fusion protein is shown in SEQ ID NO: 17.

TABLE 2

CANINE NGF, CANINE NGF RECEPTOR TrkA, And RELATED FUSION PROTEINS

PROTEIN	SEQ ID NO:	AMINO ACID SEQUENCE
Canine NGF	13	EPHPESHVPAGHAIPHAHWTKLQHSLDTALRRARSAPAGAIAARV TGQTRNITVDPKLFKKRRLRSPRVLFSTHPPPVAADAQDLDLEAG STASVNRTHRSKRSSSHPVFHRGEFSVCDSVSVWVGDKTTATDIK GKEVMVLGEVNINNSVFKQYFFETKCRDPTPVDSGCRGIDSKHWN SYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRRA
Canine NGF-HIS-Avi	14	EPHPESHVPAGHAIPHAHWTKLQHSLDTALRRARSAPAGAIAARV TGQTRNITVDPKLFKKRRLRSPRVLFSTHPPPVAADAQDLDLEAG

		STASVNRTHRSKRSSSHPVFHRGEFSVCDSVSVWVGDKTTATDIK GKEVMVLGEVNINNSVFKQYFFETKCRDPTPVDSGCRGIDSKHWN SYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRRAHH HHHHGLNDIFEAQKIEWHE
Canine NGF receptor TrkA	15	MLRGGRLGQRGGHGRAAGPGSLLAWLVLASAGAAPCPDVCCPHGP SGLRCTRAGALQSLHRLPGVENLTELYIDNQEHLQHLDAVHLKGL GMLRDLTIVKSGLRSVAPDAFHFTPRLRRLNLSFNALESLSWKTV QGLPLQELVLSGNPLHCSCALHWLLRWEEEGLGGVRGQRLQCPGQ GPLALLSNASCGVPVLKVQMPNASVEVGDDVLLQCQVEGQGLERA GWILPEVEELATVTQSGDLPSLGLTLANVTSDLNRKNVTCWAEND VGRAEVSVQVNVSFPASVQLHEAVELHHWCIPFSVDGQPAPSLRW LFNGSVLNETSFIFTEFLEPVANETVRHGCLRLNQPTHVNNGNYT LLAANPSGRAAAFVMAAFMDNPFEFNPEDPIPVSFSPVDTNSTSG DPVEKKDETPFGVSVAVGLAVFACLFLSTLFLALNKCGRRNKFGG NRAVVLAPEDGLAMSLHFMTLGGSSLSPTEGKGSGLQGHIIENPQ YFSDACVHHIKRQDIVLKWELGEGAFGKVFLAECHNLLPEQDKML VAVKALKEVSESARQDFQREAQLLTMLQHQHIVRFFGVCTEGRPL LMVFEYMRHGDLNRFLRSHGPDAKLLAGGEDVAPGPLGLGQLLAV ASQVAAGMVYLAGLHFVHRDLATRNCLVGQGLVVKIGDFGMSRDI YSTDYYRVGGRTMLPIRWMPPESILYRKFTTESDVWSFGVVLWEI FTYGKQPWYQLSNTEAIECITQGRELERPRACPPEVYAIMRGCWQ REPQQRHSIKDVHARLQALAQAPPVYLDVLG
cNGF-hFc Fusion protein	16	EPHPESHVPAGHAIPHAHWTKLQHSLDTALRRARSAPAGAIAARV TGQTRNITVDPKLFKKRRLRSPRVLFSTHPPPVAADAQDLLDLEAG STASVNRTHRSKRSSSHPVFHRGEFSVCDSVSVWVGDKTTATDIK GKEVMVLGEVNINNSVFKQYFFETKCRDPTPVDSGCRGIDSKHWN SYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAGRRAEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV

		LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK
cTrkA-ECD-IgG-B Fc fusion protein	17	AAPCPDVCCPHGPSGLRCTRAGALQSLHRLPGVENLTELYIDNQE HLQHLDAVHLKGLGMLRDLTIVKSGLRSVAPDAFHFTPRLRRLNL SFNALESLSWKTVQGLPLQELVLSGNPLHCSCALHWLLRWEEEGL GGVRGQRLQCPGQGPLALLSNASCGVPVLKVQMPNASVEVGDDVL LQCQVEGRGLERAGWILPEVEELATVTQSGDLPSLGLTLANVTSD LNRKNVTCWAENDVGRAEVSVQVNVSFPASVQLHEAVELHHWCIP FSVDGQPAPSLRWLFNGSVLNETSFIFTEFLEPVANETVRHGCLR LNQPTHVNNGNYTLLAANPSGRAAAFVMAAFMDNPFEFNPEDPIP VSFSPVDTNSTSGDPVEKKDETPFGVSVAVGVPKRENGRVPRPPD CPKCPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPED PEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLK GKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSK NTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGS YFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

EXAMPLE 3

GENERATION OF HUMAN-CANINE CHIMERIC NGF ANTIBODIES

Chimeric human-canine antibodies were constructed using the VH and VL sequences previously disclosed [*see,* Table 3 below] and then tested against canine NGF. Briefly, the VH and VL of each of a selected group of antibodies were genetically combined (fused) with the canine IgG-B heavy chain constant regions (CH1 - CH3) and light chain (kappa) constant region, respectively [*see* Table 4 for greater detail]. The human/humanized VH and VL regions of human-canine (H-C) chimeras listed in Table 4 were transiently expressed in HEK293 cells and then purified using a Protein A column. The binding activities of the individual chimeric antibodies were tested on ELISA plates coated with canine NGF, as described in Example 4 below.

TABLE 3

VH AND VL SEQUENCES OF PRIOR ART ANTIBODIES TO HUMAN NGF

VH AMINO ACID SEQUENCE FOR HUMAN/HUMANIZED ANTIBODIES	VL AMINO ACID SEQUENCE FOR HUMAN/HUMANIZED ANTIBODIES

hFul-VH (SEQ ID NO: 18)	hFul-VL (SEQ ID NO: 19)
EVQLVESGGGLVQPGGSLRLSCAASGFTLRS YSMNWVRQAPGKGLEWVSYISRSSHTIFYAD SVKGRFTISRDNAKNSLYLQMDSLRDEDTAM YYCARVYSSGWHVSDYFDYWGQGILVTVSS	AIQLTQSPSSLSASVGDRVTITCRASQGIS SALAWYQQKPGKAPKLLIYDASSLESGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ FNSYPLTFGGGTKVEIK
hFas-VH (SEQ ID NO: 21)	hFas-VL (SEQ ID NO: 22)
QVQLVQSGAEVKKPGASVKVSCKVSGFTLTE LSIHWVRQAPGKGLEWMGGFDPEDGETIYAQ KFQGRVTMTEDTSTDTAYMELTSLRSEDTAV YYCSTIGVVTNFDNWGQGTLVTVSS	DIQMTQSPSSLSASAGDRVTITCRASQAIR NDLGWYQQKPGKAPKRLIYAAFNLQSGVPS RFSGSGSGTEFTLTISSLQPEDLASYYCQQ YNRYPWTFGQGTKVEIK

CDRs are underlined.

TABLE 4

CHIMERIC HUMAN-CANINE ANTI-NGF

huFulVH-cIgGB （SEQ ID NO: 23）
EVQLVESGGGLVQPGGSLRLSCAASGFTLRSYSMNWVRQAPGKGLEWVSYISRSSHTIFYADS VKGRFTISRDNAKNSLYLQMDSLRDEDTAMYYCARVYSSGWHVSDYFDYWGQGILVTVSSAST TAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLS SMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIF PPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVL PIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLI KDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHE ALHNHYTQESLSHSPGK
huFulVL-cCk (SEQ ID NO: 24)
AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFS GSGSGTDFTLTISSLQPEDFATYYCQQFNSYPLTFGGGTKVEIKRNDAQPAVYLFQPSPDQLH

TGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHE
LYSCEITHKSLPSTLIKSFQRSECQRVD

hFAS-VH-cIgGB (SEQ ID NO: 25)

QVQLVQSGAEVKKPGASVKVSCKVSGFTLTELSIHWVRQAPGKGLEWMGGFDPEDGETIYAQK
FQGRVTMTEDTSTDTAYMELTSLRSEDTAVYYCSTIGVVTNFDNWGQGTLVTVSSASTTAPSV
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV
PSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPK
DTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQ
DWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFP
PDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNH
YTQESLSHSPGK

hFas-VL-cCk (SEQ ID NO: 26)

DIQMTQSPSSLSASAGDRVTITCRASQAIRNDLGWYQQKPGKAPKRLIYAAFNLQSGVPSRFS
GSGSGTEFTLTISSLQPEDLASYYCQQYNRYPWTFGQGTKVEIKRNDAQPAVYLFQPSPDQLH
TGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHE
LYSCEITHKSLPSTLIKSFQRSECQRVD

EXAMPLE 4

GENERATION OF CANINIZED NGF ANTIBODIES

Caninized antibodies were constructed using the two sets of 6 CDRs provided in Tables 1A – 1B. The binding activity of the chimeric and caninized antibodies to canine NGF was compared by ELISA (*see*, Example 5 below). As depicted in Figures 1 and 2, both chimeric antibodies show a strong affinity for canine NGF. In direct contrast, a control caninized monoclonal antibody (with the set of 6 CDRs obtained from a murine antibody raised against a non-related canine antigen) did not bind at all.

Accordingly, Figure 1 depicts a plot of the binding of human-canine chimeric Fulranumab (Ful Chim), and the caninized variants which contain the CDRs from Fulranumab, and an isotype control mAb (mAb ctrl) as determined by ELISA. The chimeric Fulranumab bound to canine NGF had an EC50 of 22 pM, whereas the caninized variants of Fulrnaumb

bound to canine NGF had a range of EC50 from 32 – 49 pM. These results demonstrate that these caninized antibodies have a strong binding affinity to canine NGF and thereby, make them suitable for development as drugs for treatment of pain in dogs.

Figure 2 depicts a plot of the binding of human-canine chimeric Fasinumab (Fas Chim), and the caninized variants containing CDRs from Fasinumab and isotype control mAb (mAb ctrl) as determined by ELISA. Surprisingly however, whereas the chimeric Fasinumab bound to canine NGF with an EC50 of 122 nM, the binding affinity for canine NGF of the corresponding caninized variants containing the CDRs from Fasinumab was too low to measure. This makes the caninized Fasinumab antibodies unsuitable for development for the treatment of pain in dogs. This demonstrates that is unpredictable whether a caninized antibody encoding a set of CDRs from a given antibody to human NGF would also bind to the canine NGF, even when the corresponding human-canine chimeria does bind well.

TABLE 5

VH AND VL AMINO ACID SEQUENCES OF
CANINIZED ANTIBODIES TO HUMAN AND CANINE NGF.

cFul_VH1 (SEQ ID NO: 27) EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYSMNWIRQAPGKGLQWVSYISRSSHTIFYADS VKGRFTISRDNAKNTLYLQMNSLRDEDTAVYYCARVYSSGWHVSDYFDYWGQGTLVTVSS cFul_VH2 (SEQ ID NO: 28) EVQLVESGGDLVKPGGSLRLSCVASGFTLRSYSMNWIRQAPGKGLQWVSYISRSSHTIFYADS VKGRFTISRDNAKNTLYLQMDSLRDEDTAVYYCARVYSSGWHVSDYFDYWGQGILVTVSS cFul-VL1 (SEQ ID NO: 29) EIVMTQSPASLSLSQEEKVTITCRASQGISSALAWYQQKPGQAPKLLIYDASSLESGVPSRFS GSGSGTDFSFTISSLEPEDVAVYYCQQFNSYPLTFGQGTKVEIK cFul-VL2 (SEQ ID NO: 30) EIQLTQSPASLSLSQEEKVTITCRASQGISSALAWYQQKPGQAPKLLIYDASSLESGVPSRFS GSGSGTDFSLTISSLEPEDFAVYYCQQFNSYPLTFGGGTKVEIK
cFas-VH1 (SEQ ID NO: 31)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFIELSIHWVRQAPGAGLDWMGGFDPEDGETIYAQK
FQGRVTLTADTSTSTAYMELSSLRAGDIAVYYCARIGVVTNFDNWGQGTLVTVSS

cFas-VH2 (SEQ ID NO: 32)

EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSIHWVRQAPGKGLDWMGGFDPEDGETIYAQK
FQGRVTLTEDTSTDTAYMELSSLRAGDIAVYYCSTIGVVTNFDNWGQGTLVTVSS

cFas-VL1 (SEQ ID NO: 33)

EIVMTQSPASLSLSQEEKVTITCRASQAIRNDLGWYQQKPGQAPKLLIYAAFNLQSGVPSRFS
GSGSGTDFSFTISSLEPEDVAVYYCQQYNRYPWTFGQGTKLEIK

cFas-VL2 (SEQ ID NO: 34)

DIVMTQTPLSLSVSPGETASISCRASQAIRNDLGWFRQKPGQSPQRLIYAAFNLQSGVPDRFS
GSGSGTDFTLRISRVEADDTGVYYCQQYNRYPWTFGQGTKLEIK

cFas-VL3 (SEQ ID NO: 35)

DIVMTQTPLSLSVSPGETASISCRASQAIRNDLGWFRQKPGKSPKRLIYAAFNLQSGVPDRFS
GSGSGTDFTLTISSVEADDTGVYYCQQYNRYPWTFGQGTKLEIK

CDRs are underlined

HEAVY AND LIGHT CHAINS OF CANINIZED ANTIBODIES

cFulVH1-cIgGB (SEQ ID NO: 36)

EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYSMNWIRQAPGKGLQWVSYISRSSHTIFYADSVK
GRFTISRDNAKNTLYLQMNSLRDEDTAVYYCARVYSSGWHVSDYFDYWGQGTLVTVSSASTTAPS
VFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTL
LIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKG
KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEW
QSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHS
PGK

cFulVH2-cIgGB (SEQ ID NO: 37)

EVQLVESGGDLVKPGGSLRLSCVASGFTLRSYSMNWIRQAPGKGLQWVSYISRSSHTIFYADSVK
GRFTISRDNAKNTLYLQMDSLRDEDTAVYYCARVYSSGWHVSDYFDYWGQGILVTVSSASTTAPS
VFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTL
LIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKG
KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEW
QSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHS
PGK

cFulVL1-cCk （SEQ ID NO: 38）

EIVMTQSPASLSLSQEEKVTITCRASQGISSALAWYQQKPGQAPKLLIYDASSLESGVPSRFSGS
GSGTDFSFTISSLEPEDVAVYYCQQFNSYPLTFGQGTKVEIKRNDAQPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFQRSECQRVD

cFulVL2-cCk (SEQ ID NO: 39)

EIQLTQSPASLSLSQEEKVTITCRASQGISSALAWYQQKPGQAPKLLIYDASSLESGVPSRFSGS
GSGTDFSLTISSLEPEDFAVYYCQQFNSYPLTFGGGTKVEIKRNDAQPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFQRSECQRVD

cFAS-VH1-cIgGB (SEQ ID NO: 40)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFIELSIHWVRQAPGAGLDWMGGFDPEDGETIYAQKFQ
GRVTLTADTSTSTAYMELSSLRAGDIAVYYCARIGVVTNFDNWGQGTLVTVSSASTTAPSVFPLA
PSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWP
SETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTLLIART
PEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTC
KVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQ
QEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cFAS-VH2-cIgGB (SEQ ID NO: 41)

EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSIHWVRQAPGKGLDWMGGFDPEDGETIYAQKFQ
GRVTLTEDTSTDTAYMELSSLRAGDIAVYYCSTIGVVTNFDNWGQGTLVTVSSASTTAPSVFPLA
PSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWP
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PEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTC
KVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQ
QEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cFAS-VL1-cCk (SEQ ID NO: 42)

EIVMTQSPASLSLSQEEKVTITCRASQAIRNDLGWYQQKPGQAPKLLIYAAFNLQSGVPSRFSGS
GSGTDFSFTISSLEPEDVAVYYCQQYNRYPWTFGQGTKLEIKRNDAQPAVYLFQPSPDQLHTGSA
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THKSLPSTLIKSFQRSECQRVD

cFAS-VL2-cCk (SEQ ID NO: 43)

DIVMTQTPLSLSVSPGETASISCRASQAIRNDLGWFRQKPGQSPQRLIYAAFNLQSGVPDRFSGS
GSGTDFTLRISRVEADDTGVYYCQQYNRYPWTFGQGTKLEIKRNDAQPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFQRSECQRVD

cFAS-VL3-cCk (SEQ ID NO: 44)

DIVMTQTPLSLSVSPGETASISCRASQAIRNDLGWFRQKPGKSPKRLIYAAFNLQSGVPDRFSGS
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THKSLPSTLIKSFQRSECQRVD

EXAMPLE 5

BINDING OF CHIMERIC AND CANINIZED ANTI-HUMAN NGF ANTIBODIES TO CANINE NGF

The binding of chimeric and caninized antibodies to canine NGF was determined by ELISA as follows:

1. Coat 100 ng/well canine NGF in an immunoplate and incubate the plate at 4°C overnight.
2. Wash the plate 3 times by PBS with 0.05% Tween 20 (PBST).
3. Block the plate by 0.5% BSA in PBS for 45 – 60 min at room temperature.
4. Wash the plate 3 times by PBST.
5. Make 3-fold dilution the antibodies in each column or row of dilution plate.
6. Transfer the diluted antibodies into each column or row of the immunoplate, and incubate the plate for 45 – 60 min at room temperature.
7. Wash the plate 3 times by PBST.
8. Add 1:2000 diluted horseradish peroxidase labeled anti – dog IgG Fc into each well of the plate and incubate the plate for 45 – 60 min at room temperature.
9. Wash the plate 3 times by PBST.

10. Add TMB Substrate into each well of the plate and incubate the plate for 10 to 15 min at room temperature for color development.

11. Add 100 μL of 1.5 M phosphoric acid into each well to stop the reaction.

12. Read the plate at 450 nm with 540 nm reference wavelength.

Figure 3 shows the binding of canine NGF to the canine TrkA receptor. The binding of canine NGF to canine NGF receptor (TrkA) was determined by ELISA in order to develop an assay to measure the ability of caninized anti-canine NGF antibodies to block the binding of canine NGF to its TrkA receptor. As shown in Figure 3, canine NGF binds to its canine TrkA receptor in a dose dependent manner and with an EC50 of 54 nM.

EXAMPLE 6

BLOCKING ACTIVITY OF THE CHIMERIC AND CANINIZED ANTIBODIES

The chimeric and caninized anti-NGF antibodies were tested for blocking the binding of canine NGF to the canine NGF receptor (TrkA) as follows:

1. Coat 100 ng/well canine TrkA-IgGBFc fusion protein in an immuno-plate and incubate the plate at 4°C overnight.

2. Wash the plate 3 times by PBS with 0.05% Tween 20 (PBST).

3. Block the plate by 0.5% BSA in PBS for 45 – 60 min at room temperature.

4. Wash the plate 3 times by PBST.

5. Make a 3-fold dilution of the antibodies in each column or row of dilution plate, and then add 100 ng/well biotinylated canine NGF and mix with the antibodies.

6. Transfer the diluted antibodies and canine NGF mixture into each column or row of the immunoplate, and incubate the plate for 45 – 60 min at room temperature.

7. Wash the plate 3 times by PBST.

8. Add 1:2000 diluted horseradish peroxidase conjugated streptavidin into each well of the plate and incubate the plate for 45 – 60 min at room temperature.

9. Wash the plate 3 times by PBST.

10. Add TMB Substrate into each well of the plate and incubate the plate for 10 to 15 min at room temperature for color development.

11. Add 100 μL of 1.5 M phosphoric acid into each well to stop the reaction.

12. Read the plate at 450 nm with 540 nm reference wavelength.

Figure 4 depicts the inhibition of canine NGF binding to canine TrkA receptor by caninized antibodies and the corresponding human-canine chimeric Fulranumab. The ability of human-canine chimeric Fulranumab (Ful Chim), and the caninized variants containing CDRs from Fulranumab to block the binding of canine NGF to its TrkA receptor was determined by ELISA. As shown, the chimeric Fulranumab and the caninized variants containing CDRs from Fulrnaumb both specifically and in a dose dependent manner inhibited the binding of canine NGF to its TrkA receptor with a range of IC50 from 10 – 87 nM. In direct contrast, the isotype control mAb (mAb ctrl) did not. These results indicate that the caninized antibodies are suitable for development for the treatment of pain in dogs.

EXAMPLE 7

INHIBITION OF CANINE NGF BIOACTIVITY IN TF-1 CELLS

TF-1 cell-based assay

TF-1 is a human erythroleukemic cell line that express human TrkA and proliferateS in response to NGF from various species. The effect of canine NGF on proliferation of TF-1 cells and the ability of chimeric and caninized anti-NGF antibodies to block proliferation of TF-1 cells were assessed as follows:

Materials

TF1 cell line (CRL-2003)

Growth medium: RPMI-1640 (ThermoFisher CAT#11875-085), 10% FBS and 2ng/mL rhGM-CSF (R&D, 7954-GM)

Assay Medium: RPMI-1640 (ThermoFisher CAT#11875-085) with 10% FBS

CELLTITER-GLO® One Solution Assay (Promega cat# G8461)

TF-1 cell culture:

1. Cells are incubated in T75 flask in a cell culture incubator at 37°C with 5% CO_2 and > 80% relative humidity. Cells are Passaged every 3-4 days when seeded as 4- 8 x 10^4 cells/mL in growth medium.
2. Passage the cells one day before cell proliferation assay conducted.

TF-1 cell proliferation mediated by canine beta-NGF assay:

1. Add 50 µL of assay medium to each well of 96-well plate.

2. Prepare 900 nM recombinant canine beta-NGF (cNGF) in assay medium. Add 25 µL of the cNGF to the first wells. In duplicate, 3-fold dilute across the plate and discard final 25 µL volume.

3. Harvest the TF-1 cells and wash 3X in assay medium. Resuspend the cells in assay medium to a concentration of 0.5 – 1 x 10^6 cells/mL.

4. Add 50 µL of the TF-1 cells to each well of the assay plate.

5. Incubate the plate for 48 hours (± 8 hours) in a cell culture incubator at 37°C with 5% CO2 and > 80% relative humidity.

6. Add 100 µL/well of CELLTITER-GLO ONE SOLUTION ASSAY into the plate. Mix contents for 2 minutes on an orbital shaker and incubate at room temperature for 15 minutes (± 5 minutes).

7. Measure luminescence intensity by plate reader.

Inhibition of cNGF mediated TF-1 cell proliferation by anti-cNGF antibodies:

1. Add 50 µL of assay medium to each well of 96-well plate.

2. Prepare 1800 nM antibody in assay medium. Add 25 µL of the antibody to the first wells. In duplicate, 3-fold dilute across the plate and discard final 25 µL volume. mAb iso-control, wells with assay medium and cell only are included

3. Prepare 60 nM cNGF in assay medium. Mix equal volume of the cNGF to the diluted antibody.

4. Harvest the TF-1 cells and wash 3X in assay medium. Resuspend the cells in assay medium to a concentration of 0.5 – 1 x 10^6 cells/mL.

5. Add 50 µL of the TF-1 cells to each well of a new 96-well plate. Transfer 50 µL of the mixed cNGF/antibody to each well of the cell plate.

6. Incubate the plate for 48 hours (± 8 hours) in a cell culture incubator at 37°C with 5% CO2 and > 80% relative.

7. Add 100 µL/well of CELLTITER-GLO ONE SOLUTION ASSAY into the plate. Mix contents for 2 minutes on an orbital shaker and incubate at room temperature for 15 minutes (± 5 minutes).

8. Measure luminescence intensity by plate reader.

Figure 5 shows the stimulation of TF-1 cell proliferation by canine NGF. The ability of canine NGF to stimulate proliferation of TF-1 cells was determined by a bioassay in order to develop an assay to measure the ability of caninized anti-canine NGF antibodies to block

downstream signaling and inhibit cell proliferation induced by the binding of canine NGF to the TrkA receptor on TF-1 cells. As shown, canine NGF binds in a dose dependent manner with EC50 of 28 nM to endogenous TrkA receptor expressed by to TF-1 cells and stimulates TF-1 cell proliferation. This result shows that the TF-1 cell-based assay can be used to test blocking activity of anti-canine NGF antibodies.

Figure 6 shows the inhibition of TF-1 cell proliferation by caninized anti-NGF antibodies. The ability of human-canine chimeric Fulranumab (Ful Chim), the caninized variants containing CDRS from Fulranumab identified in Figure 6, and isotype control mAb (mAb ctrl) to block TF-1 cell proliferation was determined in a bioassay with TF-1 cells. As shown, the chimeric and the caninized variants containing CDRs from Fulrnaumb specifically and in a dose dependent manner inhibited TF-1 cell proliferations with a range of IC50 from 0.26 – 0.4 nM. These results demonstrate that the caninized antibodies are suitable for development for treatment of pain in dogs.

ST.25 SEQUENCE LISTING FROM PRIORITY FILING

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Intervet International BV
Morsey, Mohamad
Zhang, Yuanzheng

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Ala Leu Lys Glu Val Ser Glu Ser Ala Arg Gln Asp Phe Gln Arg Glu
545 550 555 560

Ala Gln Leu Leu Thr Met Leu Gln His Gln His Ile Val Arg Phe Phe
565 570 575

Gly Val Cys Thr Glu Gly Arg Pro Leu Leu Met Val Phe Glu Tyr Met
580 585 590

Arg His Gly Asp Leu Asn Arg Phe Leu Arg Ser His Gly Pro Asp Ala
595 600 605

Lys Leu Leu Ala Gly Gly Glu Asp Val Ala Pro Gly Pro Leu Gly Leu
610 615 620

Gly Gln Leu Leu Ala Val Ala Ser Gln Val Ala Ala Gly Met Val Tyr
625 630 635 640

Leu Ala Gly Leu His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys
645 650 655

Leu Val Gly Gln Gly Leu Val Val Lys Ile Gly Asp Phe Gly Met Ser
660 665 670

Arg Asp Ile Tyr Ser Thr Asp Tyr Tyr Arg Val Gly Gly Arg Thr Met
675 680 685

Leu Pro Ile Arg Trp Met Pro Pro Glu Ser Ile Leu Tyr Arg Lys Phe
690 695 700

Thr Thr Glu Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp Glu Ile
705 710 715 720

Phe Thr Tyr Gly Lys Gln Pro Trp Tyr Gln Leu Ser Asn Thr Glu Ala
725 730 735

Ile Glu Cys Ile Thr Gln Gly Arg Glu Leu Glu Arg Pro Arg Ala Cys
740 745 750

Pro Pro Glu Val Tyr Ala Ile Met Arg Gly Cys Trp Gln Arg Glu Pro
755 760 765

Gln Gln Arg His Ser Ile Lys Asp Val His Ala Arg Leu Gln Ala Leu
770 775 780

Ala Gln Ala Pro Pro Val Tyr Leu Asp Val Leu Gly
785 790 795

<210> 16
<211> 455
<212> PRT
<213> Artificial Sequence

<220>
<223> canine-human fusion protein

<400> 16

Glu Pro His Pro Glu Ser His Val Pro Ala Gly His Ala Ile Pro His
1 5 10 15

Ala His Trp Thr Lys Leu Gln His Ser Leu Asp Thr Ala Leu Arg Arg
20 25 30

Ala Arg Ser Ala Pro Ala Gly Ala Ile Ala Ala Arg Val Thr Gly Gln
35 40 45

Thr Arg Asn Ile Thr Val Asp Pro Lys Leu Phe Lys Lys Arg Arg Leu
50 55 60

Arg Ser Pro Arg Val Leu Phe Ser Thr His Pro Pro Pro Val Ala Ala
65 70 75 80

Asp Ala Gln Asp Leu Asp Leu Glu Ala Gly Ser Thr Ala Ser Val Asn
85 90 95

Arg Thr His Arg Ser Lys Arg Ser Ser Ser His Pro Val Phe His Arg
100 105 110

Gly Glu Phe Ser Val Cys Asp Ser Val Ser Val Trp Val Gly Asp Lys
115 120 125

Thr Thr Ala Thr Asp Ile Lys Gly Lys Glu Val Met Val Leu Gly Glu
130 135 140

Val Asn Ile Asn Asn Ser Val Phe Lys Gln Tyr Phe Phe Glu Thr Lys
145 150 155 160

Cys Arg Asp Pro Thr Pro Val Asp Ser Gly Cys Arg Gly Ile Asp Ser
165 170 175

Lys His Trp Asn Ser Tyr Cys Thr Thr Thr His Thr Phe Val Lys Ala
180 185 190

Leu Thr Met Asp Gly Lys Gln Ala Ala Trp Arg Phe Ile Arg Ile Asp
195 200 205

Thr Ala Cys Val Cys Val Leu Ser Arg Lys Ala Gly Arg Arg Ala Glu
210 215 220

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

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

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 17
<211> 629
<212> PRT
<213> Canis familiaris

<400> 17

Ala Ala Pro Cys Pro Asp Val Cys Cys Pro His Gly Pro Ser Gly Leu
1 5 10 15

Arg Cys Thr Arg Ala Gly Ala Leu Gln Ser Leu His Arg Leu Pro Gly
20 25 30

Val Glu Asn Leu Thr Glu Leu Tyr Ile Asp Asn Gln Glu His Leu Gln
35 40 45

His Leu Asp Ala Val His Leu Lys Gly Leu Gly Met Leu Arg Asp Leu
50 55 60

Thr Ile Val Lys Ser Gly Leu Arg Ser Val Ala Pro Asp Ala Phe His
65 70 75 80

Phe Thr Pro Arg Leu Arg Arg Leu Asn Leu Ser Phe Asn Ala Leu Glu
85 90 95

Ser Leu Ser Trp Lys Thr Val Gln Gly Leu Pro Leu Gln Glu Leu Val
100 105 110

Leu Ser Gly Asn Pro Leu His Cys Ser Cys Ala Leu His Trp Leu Leu
115 120 125

Arg Trp Glu Glu Glu Gly Leu Gly Gly Val Arg Gly Gln Arg Leu Gln
130 135 140

Cys Pro Gly Gln Gly Pro Leu Ala Leu Leu Ser Asn Ala Ser Cys Gly
145 150 155 160

Val Pro Val Leu Lys Val Gln Met Pro Asn Ala Ser Val Glu Val Gly
165 170 175

Asp Asp Val Leu Leu Gln Cys Gln Val Glu Gly Arg Gly Leu Glu Arg
180 185 190

Ala Gly Trp Ile Leu Pro Glu Val Glu Glu Leu Ala Thr Val Thr Gln
195 200 205

Ser Gly Asp Leu Pro Ser Leu Gly Leu Thr Leu Ala Asn Val Thr Ser
210 215 220

Asp Leu Asn Arg Lys Asn Val Thr Cys Trp Ala Glu Asn Asp Val Gly
225 230 235 240

Arg Ala Glu Val Ser Val Gln Val Asn Val Ser Phe Pro Ala Ser Val
245 250 255

Gln Leu His Glu Ala Val Glu Leu His His Trp Cys Ile Pro Phe Ser

260 265 270

Val Asp Gly Gln Pro Ala Pro Ser Leu Arg Trp Leu Phe Asn Gly Ser
275 280 285

Val Leu Asn Glu Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro Val
290 295 300

Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg Leu Asn Gln Pro Thr
305 310 315 320

His Val Asn Asn Gly Asn Tyr Thr Leu Leu Ala Ala Asn Pro Ser Gly
325 330 335

Arg Ala Ala Ala Phe Val Met Ala Ala Phe Met Asp Asn Pro Phe Glu
340 345 350

Phe Asn Pro Glu Asp Pro Ile Pro Val Ser Phe Ser Pro Val Asp Thr
355 360 365

Asn Ser Thr Ser Gly Asp Pro Val Glu Lys Lys Asp Glu Thr Pro Phe
370 375 380

Gly Val Ser Val Ala Val Gly Val Pro Lys Arg Glu Asn Gly Arg Val
385 390 395 400

Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly
405 410 415

Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu Leu
420 425 430

Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu Asp Pro
435 440 445

Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met
450 455 460

Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly Thr Tyr
465 470 475 480

Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly
485 490 495

Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile
500 505 510

Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser Val
515 520 525

Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser
530 535 540

Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu
545 550 555 560

Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr
565 570 575

Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu
580 585 590

Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala
595 600 605

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser
610 615 620

His Ser Pro Gly Lys
625

<210> 18
<211> 123
<212> PRT
<213> Homo sapiens

<400> 18

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

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

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

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Asp Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser
115 120

<210> 19
<211> 107
<212> PRT
<213> Homo sapiens

<400> 19

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 20
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> modified canine

<400> 20

Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr
1 5 10 15

Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu
20 25 30

Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
35 40 45

Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
65 70 75 80

Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser

85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
100 105 110

Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr
115 120 125

Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp
130 135 140

Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
145 150 155 160

Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
165 170 175

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
180 185 190

Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser
195 200 205

Leu Ser His Ser Pro Gly Lys
210 215

<210> 21
<211> 118
<212> PRT
<213> Homo sapiens

<400> 21

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

Ser Val Lys Val Ser Cys Lys Val Ser Gly Phe Thr Leu Thr Glu Leu
20 25 30

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

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ser Thr Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 22
<211> 107
<212> PRT
<213> Homo sapiens

<400> 22

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

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

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Leu Ala Ser Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 23
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Human canine fusion protein

<400> 23

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

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

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

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Asp Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
130 135 140

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

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

Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
180 185 190

Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
195 200 205

Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
210 215 220

Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
225 230 235 240

Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
245 250 255

Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
275 280 285

Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
290 295 300

Phe Asn Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
305 310 315 320

Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala

325 330 335

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

His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
355 360 365

Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
370 375 380

Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
385 390 395 400

Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
405 410 415

Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
420 425 430

Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
435 440 445

Gln Glu Ser Leu Ser His Ser Pro Gly Lys
450 455

<210> 24
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Human canine fusion protein

<400> 24

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala

20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 25
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<223> Human canine fusion protein

<400> 25

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

Ser Val Lys Val Ser Cys Lys Val Ser Gly Phe Thr Leu Thr Glu Leu
20 25 30

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

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ser Thr Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala
130 135 140

Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser
180 185 190

Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

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

Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly
225 230 235 240

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

Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu Asp Pro
260 265 270

Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met
275 280 285

Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly
305 310 315 320

Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile
325 330 335

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

Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser

355 360 365

Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu
370 375 380

Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr
385 390 395 400

Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu
405 410 415

Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser
435 440 445

His Ser Pro Gly Lys
450

<210> 26
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Human canine fusion protein

<400> 26

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

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

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Leu Ala Ser Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 27
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 27

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

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val
35 40 45

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 28
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 28

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

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Leu Arg Ser Tyr

20 25 30

Ser Met Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val
35 40 45

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser
115 120

<210> 29
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 29

Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly

50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

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

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 30
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 30

Glu Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

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

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

100 105

<210> 31
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 31

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

Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Ile Glu Leu
20 25 30

Ser Ile His Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 32
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 32

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

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
20 25 30

Ser Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Leu Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
85 90 95

Ser Thr Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 33
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 33

Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

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

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 34
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 34

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

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

Leu Gly Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

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

Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 35
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> caninized human

<400> 35

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

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

Leu Gly Trp Phe Arg Gln Lys Pro Gly Lys Ser Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Glu Ala
65 70 75 80

Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 36
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 36

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

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val
35 40 45

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
130 135 140

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

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

Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
180 185 190

Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
195 200 205

Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
210 215 220

Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
225 230 235 240

Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
245 250 255

Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
275 280 285

Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
290 295 300

Phe Asn Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
305 310 315 320

Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
325 330 335

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

His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser

355 360 365

Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
370 375 380

Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
385 390 395 400

Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
405 410 415

Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
420 425 430

Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
435 440 445

Gln Glu Ser Leu Ser His Ser Pro Gly Lys
450 455

<210> 37
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 37

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

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Leu Arg Ser Tyr
20 25 30

Ser Met Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val
35 40 45

Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
130 135 140

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

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

Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
180 185 190

Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
195 200 205

Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
210 215 220

Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
225 230 235 240

Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
245 250 255

Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
275 280 285

Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
290 295 300

Phe Asn Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
305 310 315 320

Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
325 330 335

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

His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
355 360 365

Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
370 375 380

Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
385 390 395 400

Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
405 410 415

Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
420 425 430

Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
435 440 445

Gln Glu Ser Leu Ser His Ser Pro Gly Lys

450 455

<210> 38
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 38

Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

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

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln

145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 39
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 39

Glu Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu

85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 40
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 40

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

Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Ile Glu Leu

20 25 30

Ser Ile His Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala
130 135 140

Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser
180 185 190

Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

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

Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly
225 230 235 240

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

Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu Asp Pro
260 265 270

Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met
275 280 285

Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly
305 310 315 320

Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile
325 330 335

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

Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser
355 360 365

Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu
370 375 380

Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr
385 390 395 400

Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu
405 410 415

Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala

420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser
435 440 445

His Ser Pro Gly Lys
450

<210> 41
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 41

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

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
20 25 30

Ser Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Leu Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
85 90 95

Ser Thr Ile Gly Val Val Thr Asn Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe Pro

115 120 125

Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu Ala
130 135 140

Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser Ser
180 185 190

Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

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

Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu Gly
225 230 235 240

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

Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu Asp Pro
260 265 270

Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln Met
275 280 285

Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys Gly
305 310 315 320

Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro Ile
325 330 335

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

Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val Ser
355 360 365

Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val Glu
370 375 380

Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr Thr
385 390 395 400

Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu
405 410 415

Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys Ala
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu Ser
435 440 445

His Ser Pro Gly Lys
450

<210> 42
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 42

Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
1 5 10 15

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

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp

210 215

<210> 43
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 43

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

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

Leu Gly Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

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

Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln

145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 44
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Caninized human

<400> 44

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

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

Leu Gly Trp Phe Arg Gln Lys Pro Gly Lys Ser Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Phe Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Glu Ala
65 70 75 80

Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Tyr Asn Arg Tyr Pro Trp

85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
195 200 205

Gln Arg Ser Glu Cys Gln Arg Val Asp
210 215

<210> 45
<211> 17
<212> PRT
<213> Canis familiaris

<400> 45

Phe Asn Glu Cys Arg Cys Thr Asp Thr Pro Pro Cys Pro Val Pro Glu
1 5 10 15

Pro

<210> 46
<211> 22
<212> PRT
<213> Canis familiaris

<400> 46

Pro Lys Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys
1 5 10 15

Cys Pro Ala Pro Glu Met
20

<210> 47
<211> 20
<212> PRT
<213> Canis familiaris

<400> 47

Ala Lys Glu Cys Glu Cys Lys Cys Asn Cys Asn Asn Cys Pro Cys Pro
1 5 10 15

Gly Cys Gly Leu
20

<210> 48
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> modified canine

<400> 48

Pro Lys Glu Ser Thr Cys Lys Cys Ile Pro Pro Cys Pro Val Pro Glu
1 5 10 15

Ser

<210> 49
<211> 216

<212> PRT
<213> Canis familiaris

<400> 49

Leu Gly Gly Pro Ser Val Leu Ile Phe Pro Pro Lys Pro Lys Asp Ile
1 5 10 15

Leu Arg Ile Thr Arg Thr Pro Glu Val Thr Cys Val Val Leu Asp Leu
20 25 30

Gly Arg Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
35 40 45

Glu Val His Thr Ala Lys Thr Gln Ser Arg Glu Gln Gln Phe Asn Gly
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu
65 70 75 80

Thr Gly Lys Glu Phe Lys Cys Arg Val Asn His Ile Asp Leu Pro Ser
85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Arg Ala His Lys Pro
100 105 110

Ser Val Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp
115 120 125

Thr Val Ser Ile Thr Cys Leu Ile Lys Asp Phe Tyr Pro Pro Asp Ile
130 135 140

Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Arg Lys His
145 150 155 160

Arg Met Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr
165 170 175

Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Pro Phe

180 185 190

Thr Cys Ala Val Met His Glu Thr Leu Gln Asn His Tyr Thr Asp Leu
195 200 205

Ser Leu Ser His Ser Pro Gly Lys
210 215

<210> 50
<211> 215
<212> PRT
<213> Canis familiaris

<400> 50

Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr
1 5 10 15

Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Leu
20 25 30

Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
35 40 45

Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
65 70 75 80

Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
100 105 110

Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr
115 120 125

Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp
130 135 140

Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
145 150 155 160

Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
165 170 175

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
180 185 190

Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser
195 200 205

Leu Ser His Ser Pro Gly Lys
210 215

<210> 51
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> modified canine

<400> 51

Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr
1 5 10 15

Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu
20 25 30

Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
35 40 45

Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
65 70 75 80

Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
100 105 110

Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr
115 120 125

Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp
130 135 140

Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
145 150 155 160

Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
165 170 175

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
180 185 190

Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser
195 200 205

Leu Ser His Ser Pro Gly
210

<210> 52
<211> 215
<212> PRT
<213> Canis familiaris

<400> 52

Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Ile
1 5 10 15

Leu Val Thr Ala Arg Thr Pro Thr Val Thr Cys Val Val Val Asp Leu
20 25 30

Asp Pro Glu Asn Pro Glu Val Gln Ile Ser Trp Phe Val Asp Ser Lys
35 40 45

Gln Val Gln Thr Ala Asn Thr Gln Pro Arg Glu Glu Gln Ser Asn Gly
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
65 70 75 80

Ser Gly Lys Gln Phe Lys Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
85 90 95

Pro Ile Glu Glu Ile Ile Ser Lys Thr Pro Gly Gln Ala His Gln Pro
100 105 110

Asn Val Tyr Val Leu Pro Pro Ser Arg Asp Glu Met Ser Lys Asn Thr
115 120 125

Val Thr Leu Thr Cys Leu Val Lys Asp Phe Phe Pro Pro Glu Ile Asp
130 135 140

Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
145 150 155 160

Met Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
165 170 175

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
180 185 190

Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Ile Ser
195 200 205

Leu Ser His Ser Pro Gly Lys

210 215

<210> 53
<211> 216
<212> PRT
<213> Canis familiaris

<400> 53

Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Ile
1 5 10 15

Leu Arg Ile Thr Arg Thr Pro Glu Ile Thr Cys Val Val Leu Asp Leu
20 25 30

Gly Arg Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
35 40 45

Glu Val His Thr Ala Lys Thr Gln Pro Arg Glu Gln Gln Phe Asn Ser
50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu
65 70 75 80

Thr Gly Lys Glu Phe Lys Cys Arg Val Asn His Ile Gly Leu Pro Ser
85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
100 105 110

Ser Val Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp
115 120 125

Thr Val Thr Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Glu Ile
130 135 140

Asp Val Glu Trp Gln Ser Asn Gly Gln Pro Glu Pro Glu Ser Lys Tyr
145 150 155 160

His Thr Thr Ala Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr
165 170 175

Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Thr Phe
180 185 190

Thr Cys Ala Val Met His Glu Ala Leu Gln Asn His Tyr Thr Asp Leu
195 200 205

Ser Leu Ser His Ser Pro Gly Lys
210 215

We Claim:

1. A caninized antibody or antigen binding fragment thereof that binds canine Nerve Growth Factor (NGF) comprising a heavy chain and a light chain; wherein the light chain comprises three light chain complementary determining regions (CDRs) each comprising an amino acid sequence: CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and wherein the heavy chain comprises three heavy chain CDRs each comprising an amino acid sequence: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3):

(a) wherein CDRH1 comprises the amino acid sequence of SEQ ID NO: 1;

(b) wherein CDRH2 comprises the amino acid sequence of SEQ ID NO: 2;

(c) wherein CDRH3 comprises the amino acid sequence of SEQ ID NO: 3;

(d) wherein CDRL1 comprises the amino acid sequence of SEQ ID NO: 4;

(e) wherein CDRL2 comprises the amino acid sequence of SEQ ID NO: 5; and

(f) wherein CDRL3 comprises the amino acid sequence of SEQ ID NO: 6.

2. The caninized antibody or antigen binding fragment thereof of Claim 1, that comprises a hinge region that has an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48.

3. The caninized antibody or antigen binding fragment thereof of Claim 1 or 2, that comprises a canine fragment crystallizable region (cFc region); wherein the cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 52, and SEQ ID NO: 53.

4. The caninized antibody or antigen binding fragment thereof of Claim 1 or 2, that comprises a canine fragment crystallizable region (cFc region); wherein the cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence SEQ ID NO: 20 or SEQ ID NO: 51, wherein both the aspartic acid residue (D) at position 31 of SEQ ID NO: 50 and the asparagine residue (N) at position 63 of SEQ ID NO: 50, are substituted by an alanine residue (A).

5. The caninized antibody of any one of Claims 1-4, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27 and SEQ ID NO: 28.

6. The caninized antibody of any one of Claims 1-5, wherein the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 29 and SEQ ID NO: 30.

7. The caninized antibody of Claim 6, wherein

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 29; or

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 30; or

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 29; or

the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 30.

8. The caninized antibody of any one of Claims 1-7, wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 38 and SEQ ID NO: 39.

9. The caninized antibody of any one of Claims 1-8, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 36 and SEQ ID NO: 37.

10. The caninized antibody of Claim 9, wherein

the heavy chain comprises the amino acid sequence of SEQ ID NO: 37 and the light chain comprises the amino acid sequence of SEQ ID NO: 39; or

the heavy chain comprises the amino acid sequence of SEQ ID NO: 37 and the light chain comprises the amino acid sequence of SEQ ID NO: 38; or

the heavy chain comprises the amino acid sequence of SEQ ID NO: 36 and the light chain comprises the amino acid sequence of SEQ ID NO: 39; or

the heavy chain comprises the amino acid sequence of SEQ ID NO: 36 and the light chain comprises the amino acid sequence of SEQ ID NO: 38.

11. The caninized antibody of Claim 10, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 37 and the light chain comprises the amino acid sequence of SEQ ID NO: 39.

12. A nucleic acid that encodes the light chain of any one of Claims 1-11.

13. A nucleic acid that encodes the heavy chain of any one of Claims 1-11.

14. A pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain of a specific caninized antibody of any one of the antibodies of Claims 1-11 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain of said specific caninized antibody.

15. An expression vector comprising the pair of nucleic acids of Claim 14, or the nucleic acid of Claim 12, or Claim 13.

16. A pair of expression vectors, one comprising the one of the pair of nucleic acids of Claim 14, that comprises a nucleotide sequence that encodes the heavy chain of the specific caninized antibody and the other comprising the other of the pair of nucleic acids that comprises the nucleotide sequence that encodes the light chain of the specific caninized antibody.

17. A host cell comprising the expression vector of Claim 15 or the pair of expression vectors of Claim 16.

18. A pair of host cells, wherein one of the pair of host cells comprises an expression vector that comprises the one of the pair of nucleic acids of Claim 14, that comprises a nucleotide sequence that encodes the heavy chain of the specific caninized antibody, whereas the other of the pair of host cells comprises an expression vector that comprises the other of the pair of nucleic acids that comprises the nucleotide sequence that encodes the light chain of the specific caninized antibody.

19. A pharmaceutical composition comprising the caninized antibody of any one of Claims 1-11, or the expression vector of Claim 15, or the pair of expression vectors of Claim 16, or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

20. A method of aiding in a treatment of a condition associated with pain in a canine comprising administering to the canine a therapeutically effective amount of the pharmaceutical composition of Claim 19.

21. The method of claim 20, wherein said method is used for the treatment of osteoarthritis, hyperalgesia, allodynia, pain, or any combination thereof.

22. A method of producing a caninized antibody that binds canine NGF comprising:

a. culturing each one of the pair of host cells of Claim 18 in a culture medium either individually or in combination under conditions wherein the nucleic acids are expressed, thereby producing a polypeptide comprising the light chain of the caninized antibody, the heavy chain of the caninized antibody, or both; and

b. recovering the light chain of the caninized antibody, the heavy chain of the caninized antibody, or both from the pair of host cells or culture medium.

23. A pharmaceutical composition for the use of treatment of a condition associated with pain in a canine, comprising the caninized antibody of any one of Claims 1-11 or the expression vector of Claim 15 or the pair of expression vectors of Claim 16, or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

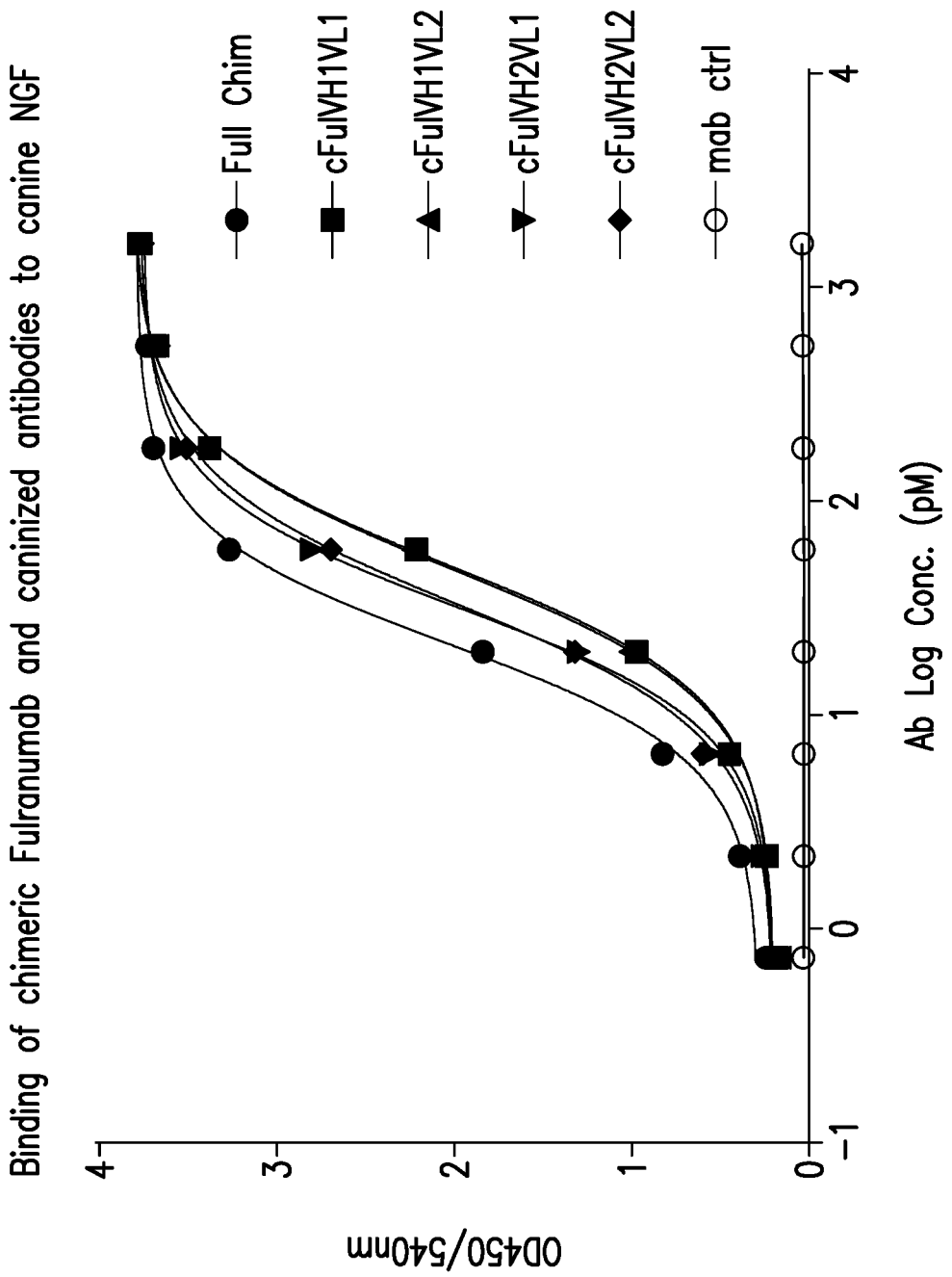
Binding of chimeric Fulranumab and caninized antibodies to canine NGF
OD450/540nm
Ab Log Conc. (pM)
Full Chim
cFulVH1VL1
cFulVH1VL2
cFulVH2VL1
cFulVH2VL2
mab ctrl
0
1
2
3
4
−1
0
1
2
3
4

FIG. 1

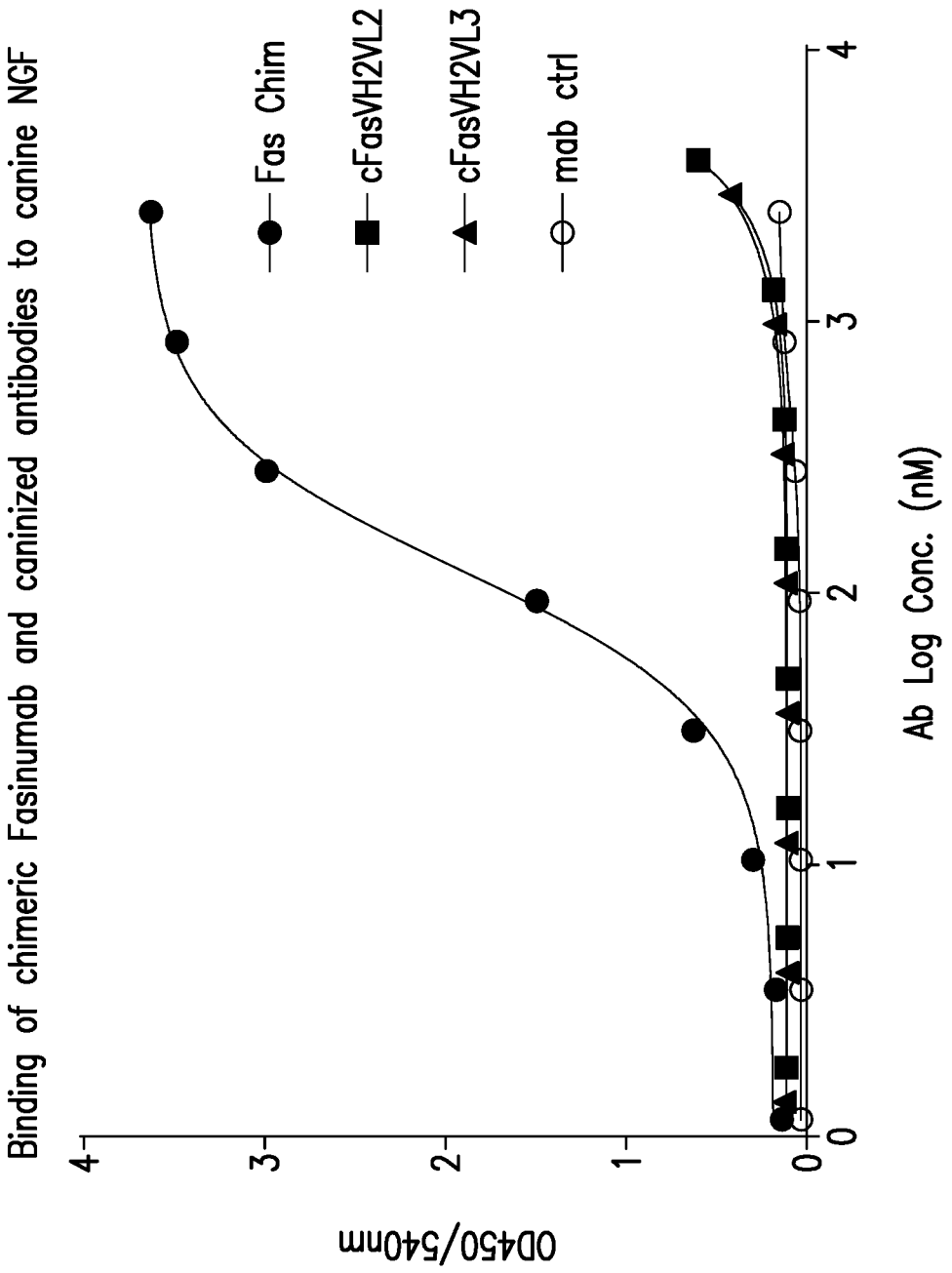
Binding of chimeric Fasinumab and caninized antibodies to canine NGF
Fas Chim
cFasVH2VL2
cFasVH2VL3
mab ctrl
OD450/540nm
Ab Log Conc. (nM)
0
1
2
3
4

FIG.2

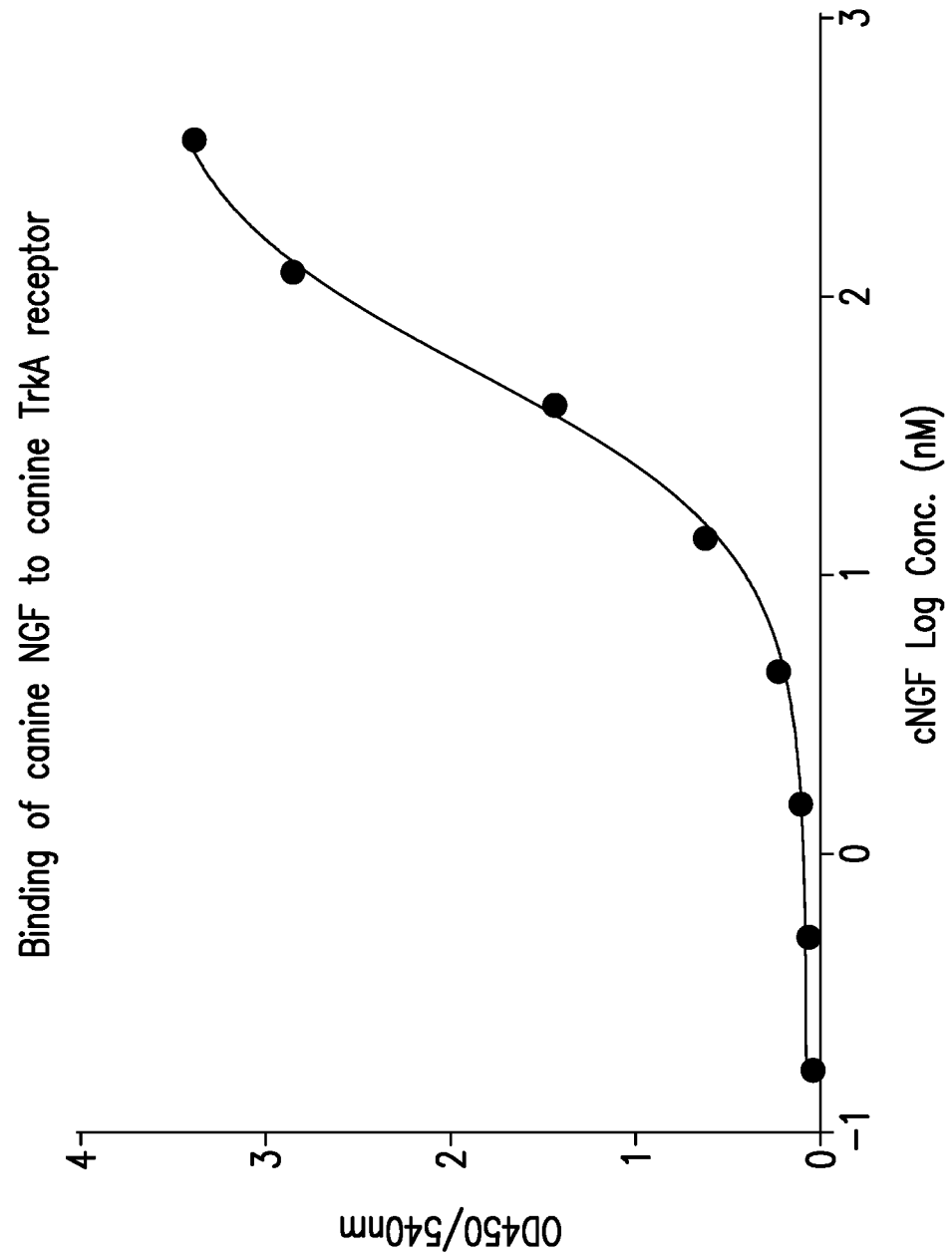
Binding of canine NGF to canine TrkA receptor
OD450/540nm
0
1
2
3
4
−1
0
1
2
3
cNGF Log Conc. (nM)

FIG.3

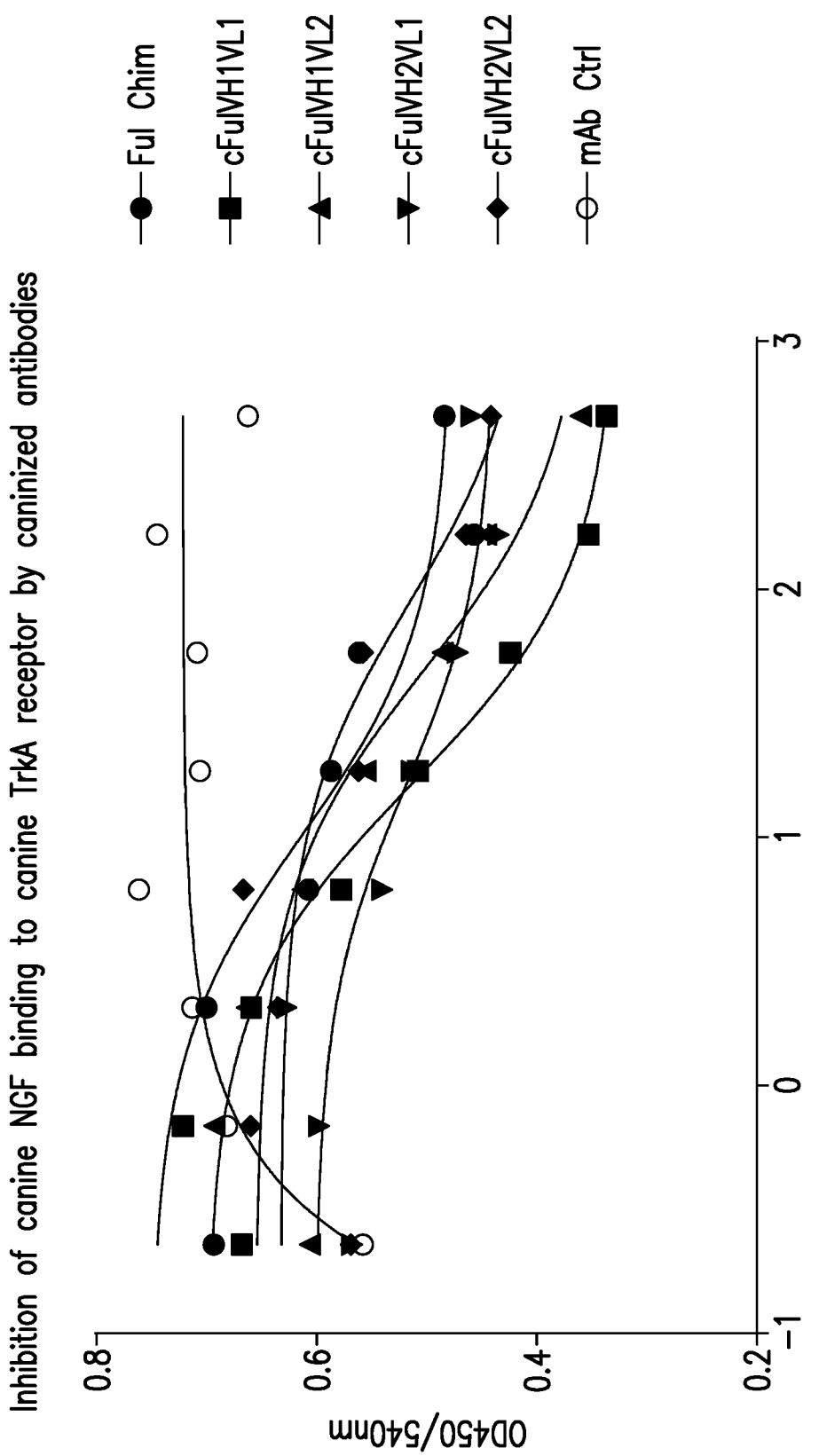
Inhibition of canine NGF binding to canine TrkA receptor by caninized antibodies
FuI Chim
cFuIVH1VL1
cFuIVH1VL2
cFuIVH2VL1
cFuIVH2VL2
mAb Ctrl
OD450/540nm
0.8
0.6
0.4
0.2
−1
0
1
2
3
Ab Log Conc. (nM)

FIG.4

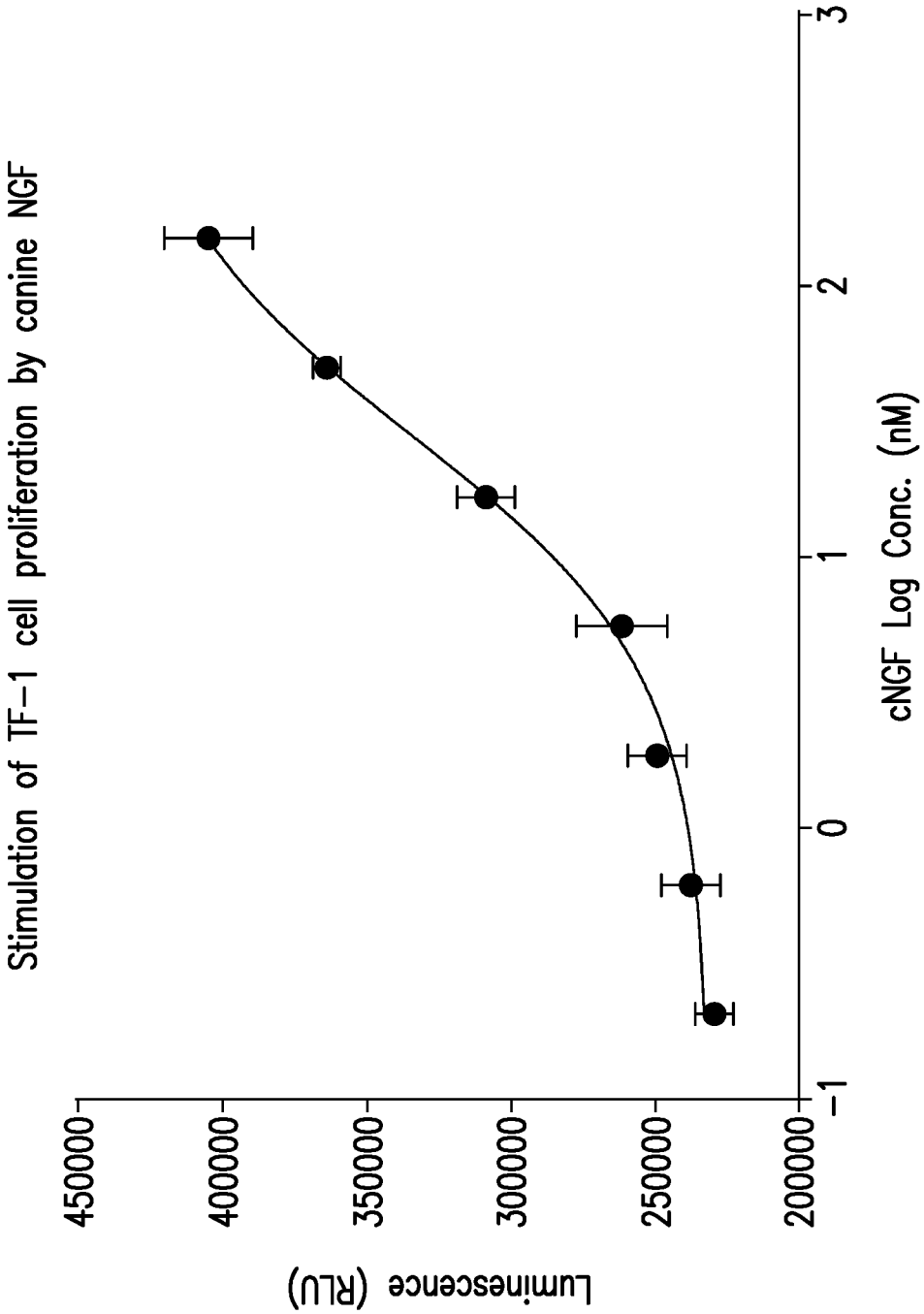
Stimulation of TF-1 cell proliferation by canine NGF
Luminescence (RLU)
450000
400000
350000
300000
250000
200000
-1
0
1
2
3
cNGF Log Conc. (nM)

FIG.5

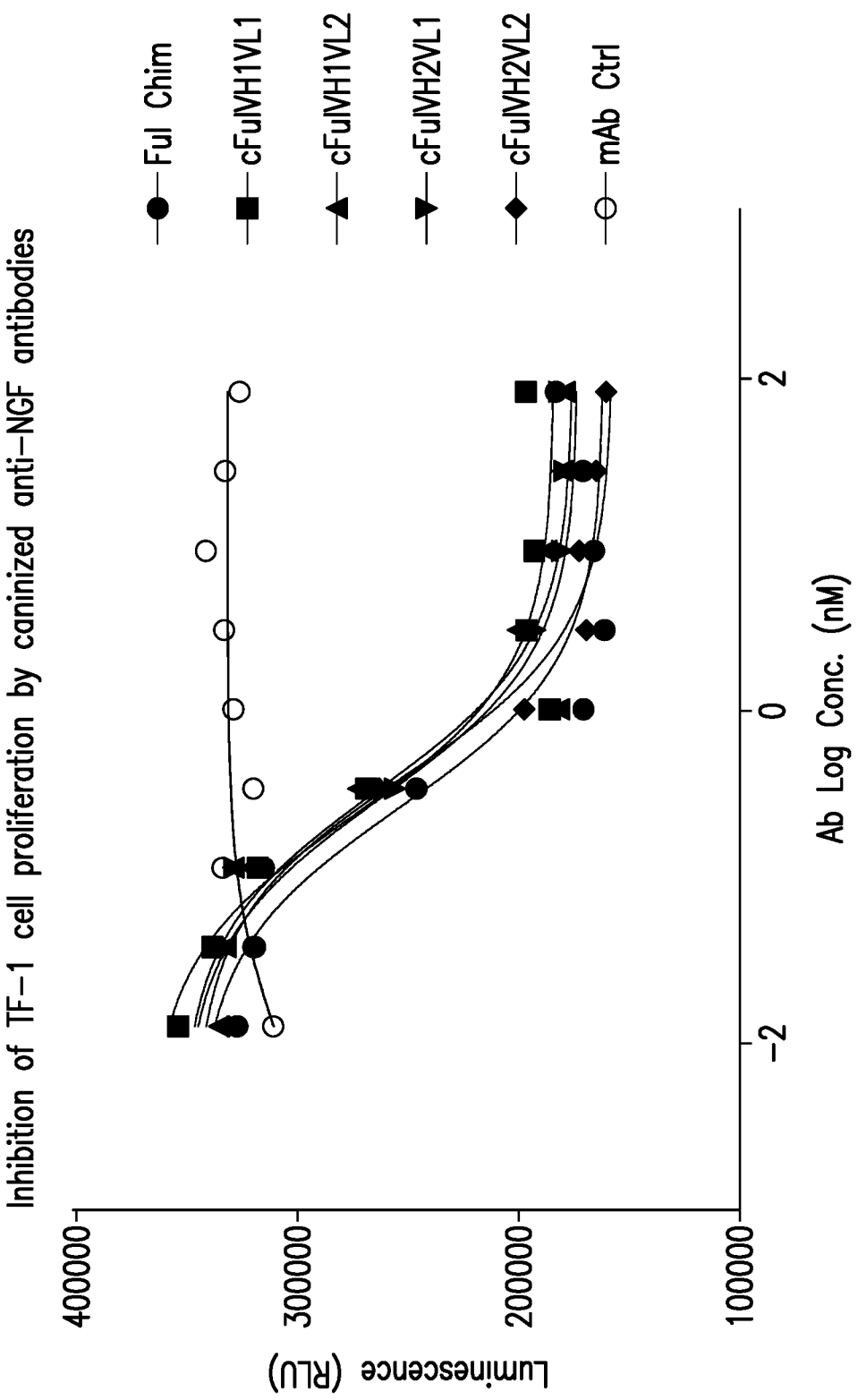
Inhibition of TF-1 cell proliferation by caninized anti-NGF antibodies
Ful Chim
cFulVH1VL1
cFulVH1VL2
cFulVH2VL1
cFulVH2VL2
mAb Ctrl
Luminescence (RLU)
400000
300000
200000
100000
-2
0
2
Ab Log Conc. (nM)

FIG.6