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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to caninized murine antibodies to human PD-1 that have specific sequences and a high binding affinity for canine PD-1. The invention also relates to use of the antibodies of the present invention in the treatment of cancer in dogs.

BACKGROUND OF THE INVENTION

[0002] An immunoinhibitory receptor that is primarily expressed on activated T and B cells, Programmed Cell Death Receptor 1, also referred to as Programmed Death Receptor (PD-1), is a member of the immunoglobulin superfamily related to CD28 and CTLA-4. PD-1 and like family members are type I transmembrane glycoproteins containing an extracellular Ig Variable-type (V-type) domain that binds its ligands and a cytoplasmic tail that binds signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif).

[0003] PD-1 attenuates T-cell responses when bound to Programmed Cell Death Ligand 1, also referred to as Programmed Death Ligand 1 (PD-L1), and/or Programmed Cell Death Ligand 2, also referred to as Programmed Death Ligand 2 (PD-L2). The binding of either of these ligands to PD-1 negatively regulates antigen receptor signaling. Blocking the binding of PD-L1 to PD-1 enhances tumor-specific $CD8^+$ T-cell immunity, while aiding the clearance of tumor cells by the immune system. The three-dimensional structure of murine PD-1, as well as the co-crystal structure of mouse PD-1 with human PD-L1 have been reported [Zhang et al., Immunity 20: 337-347 (2004); Lin et al., Proc. Natl. Acad. Sci. USA 105: 3011-3016 (2008)]. PD-L1 and PD-L2 are type I transmembrane ligands that contain both IgV- and IgC-like domains in the extracellular region along with short cytoplasmic regions with no known signaling motifs. Both PD-L1 and PD-L2 are either constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as various tumor types. PD-L1 is not only expressed on B, T, myeloid and dendritic cells (DCs), but also on peripheral cells, such as microvascular endothelial cells and non-lymphoid organs *e.g.*, heart or lung. In contrast, PD-L2 is only found on macrophages and DCs. The expression pattern of PD-1 ligands suggests that PD-1 plays a role in maintaining peripheral tolerance and may further serve to regulate self-reactive T- and B-cell responses in the periphery.

[0004] In any case, it is now abundantly clear that PD-1 plays a critical role in at least certain human cancers, presumably by mediating immune evasion. Accordingly, PD-L1 has been shown to be expressed on a number of mouse and human tumors and is inducible by IFN *gamma* in the majority of PD-L1 negative tumor cell lines [Iwai et al., Proc. Natl. Acad. Sci.

U.S.A. 99: 12293-12297 (2002); Strome et al., Cancer Res., 63: 6501-6505 (2003)]. Furthermore, the expression of PD-1 on tumor infiltrating lymphocytes and/or PD-L1 on tumor cells has been identified in a number of primary human tumor biopsies. Such tumor tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas, as well as tumors of the head and neck [Brown et al., J. Immunol. 170: 1257-1266 (2003); Dong et al., Nat. Med. 8: 793-800 (2002); Wintterle et al., Cancer Res. 63: 7462-7467 (2003); Strome et al., Cancer Res., 63: 6501-6505 (2003); Thompson et al., Cancer Res. 66: 3381-5 (2006); Thompson et al., Clin. Cancer Res. 13: 1757-1761 (2007); Nomi et al., Clin. Cancer Res. 13: 2151-2157. (2007)]. More strikingly, PD-ligand expression on tumor cells has been correlated to poor prognosis of human cancer patients across multiple tumor types [reviewed in Okazaki and Honjo, Int. Immunol. 19: 813-824 (2007)].

[0005] Moreover, Nomi et al. [Clin. Cancer Res. 13: 2151-2157 (2007)] demonstrated the therapeutic efficacy of blocking the binding of PD-L1 to PD-1 in a murine model of aggressive pancreatic cancer through administering either PD-1 or PD-L1 directed antibody. These antibodies effectively promoted tumor reactive $CD8^+$ T cell infiltration into the tumor resulting in the upregulation of anti-tumor effectors including IFN *gamma,* granzyme B, and perforin. Similarly, the use of antibodies to block the binding of PD- L1 and PD-1 significantly inhibited tumor growth in a model of mouse squamous cell carcinoma [Tsushima et al., Oral Oncol. 42: 268-274 (2006)].

[0006] In other studies, transfection of a murine mastocytoma line with PD-L1 led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-L1 monoclonal antibody was added [Iwai et al., Proc. Natl. Acad. Sci. U.S.A. 99: 12293-12297 (2002)]. *In vivo,* blocking the PD1/PD-L1 interaction was shown to increase the efficacy of adoptive T cell transfer therapy in a mouse tumor model [Strome et al., Cancer Res. 63: 6501-6505 (2003)]. Further evidence for the role of PD-1 in cancer treatment comes from experiments performed with PD-1 knockout mice in which PD-L1 expressing myeloma cells grew only in wild-type animals (resulting in tumor growth and associated animal death), but not in PD-1 deficient mice [Iwai Y. et al., Proc. Natl. Acad. Sci. U.S.A. 99: 12293-12297 (2002)]. More recently, antibodies against PD-1 (including humanized murine monoclonal antibodies against human PD-1) have shown at least initial success in cancer therapy in humans [*see e.g.,* US 8,354,509 B2, US 8,008,449 B2, and US 7,595,048 B2]. WO2008/083174 discloses antibodies that specifically bind to PD-1 and block the binding of PD-1 to PD-L-1, and the use thereof to treat cancer or infections. This document teaches that the subject to be treated can be a dog.

[0007] WO2008/156712 discloses the murine PD-1-08A antibody which was raised against human PD-1 and was canonized in the present application.

[0008] Anti-PD-1 antibodies may also be useful in chronic viral infection. Memory CD8 T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by

varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber et al. [Nature 439: 682-687 (2006)] showed that mice infected with a laboratory strain of LCMV developed chronic infection resulted in high levels of virus in the blood and other tissues. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. Barber *et al.* found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-L1.

[0009] 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

[0010] The present invention relates to caninized murine anti-human PD-1 antibodies that have a high binding affinity to canine PD-1, as well as having the ability to block the binding of canine PD-1 to canine PD-L1. The present invention also relates to use of such antibodies in the treatment of disease such as cancer and/or those due to infections. Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the animal body by therapy or for diagnosis.

[0011] The invention is defined by the appended claims .

[0012] Accordingly, the present invention provides an isolated caninized antibody or antigen binding fragment thereof as defined in the claims that specifically binds Programmed Death Receptor 1 (PD-1) comprising a canine IgG heavy chain and a canine *kappa* light chain. In particular embodimenents of this type, the canine *kappa* or *lambda* light chain that comprises three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and the canine IgG heavy chain comprises three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3) obtained from a mammalian PD-1 antibody. Particular embodiments of the caninized antibodies and fragments thereof of the present invention bind canine PD-1 and/or block the binding of canine PD-1 to canine Programmed Death Ligand 1 (PD-L1).

[0013] In certain embodiments, canine light chain is a kappa chain. In particular embodiments of this type, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20. In related embodiments the CDRL1 comprises a conservatively modified variant of SEQ ID NO: 20. In other embodiments, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22. In related embodiments the CDRL2 comprises a conservatively modified variant of SEQ ID NO: 22. In still other embodiments the CDRL3 comprises the amino acid sequence of SEQ ID

NO: 24. In related embodiments the CDRL3 comprises a conservatively modified variant of SEQ ID NO: 24. In yet other embodiments the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14. In related embodiments the CDRH1 comprises a conservatively modified variant of of SEQ ID NO: 14. In still other embodiments the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16. In related embodiments the CDRH2 comprises a conservatively modified variant of SEQ ID NO: 16. In yet other embodiments the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18. In related embodiments the CDRH3 comprises a conservatively modified variant of SEQ ID NO: 18.

[0014] In specific embodiments the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20 or a conservatively modified variant of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22 or a conservatively modified variant of SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24 or a conservatively modified variant of SEQ ID NO: 24.

[0015] In other specific embodiments the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14 or a conservatively modified variant of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16 or a conservatively modified variant of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18 or a conservatively modified variant of SEQ ID NO: 18.

[0016] In a more specific embodiment the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20 or a conservatively modified variant of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22 or a conservatively modified variant of SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24 or a conservatively modified variant of SEQ ID NO: 24, and the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14 or a conservatively modified variant of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16 or a conservatively modified variant of SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18 or a conservatively modified variant of SEQ ID NO: 18.

[0017] In an even more specific embodiment the CDRL1 comprises the amino acid sequence of SEQ ID NO: 20, the CDRL2 comprises the amino acid sequence comprising SEQ ID NO: 22, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 14, the CDRH2 comprises the amino acid sequence comprising SEQ ID NO: 16, and the CDRH3 comprises the amino acid sequence of SEQ ID NO: 18.

[0018] In other embodiments the IgG heavy chain comprises an amino acid sequence of SEQ ID NO: 28

[0019] In certain embodiments the *kappa* light chain comprises an amino acid sequence of SEQ ID NO: 32.

[0020] In particular embodiments the *kappa* light chain comprises an amino acid sequence of SEQ ID NO: 34.

[0021] In a more particular embodiment, an isolated caninized antibody comprises the amino acid sequence of SEQ ID NO: 28 and of SEQ ID NO: 34.

[0022] The present invention further provides isolated nucleic acids that encode any one of the light chains of the caninized antibody of the present invention. Similarly, the present invention further provides isolated nucleic acids that encode any one of the heavy chains of the caninized antibody of the present invention. The present invention further provides expression vectors that comprise one or more of the isolated nucleic acids of the present invention. The present invention further provides host cells that comprise one or more expression vectors of the present invention.

[0023] In particular embodiments, the antibody is a recombinant antibody or an antigen binding fragment thereof. In related embodiments, the variable heavy chain domain and variable light chain domain are connected by a flexible linker to form a single-chain antibody.

[0024] In particular embodiments, the antibody or antigen binding fragment is a Fab fragment.

[0025] In other embodiments, the antibody or antigen binding fragment is a Fab' fragment. In other embodiments, the antibody or antigen binding fragment is a $(Fab')_2$ fragment. In still other embodiments, the antibody or antigen binding fragment is a diabody. In particular embodiments, the antibody or antigen binding fragment is a domain antibody. In particular embodiments, the antibody or antigen binding fragment is a camelized single domain antibody.

[0026] In particular embodiments, the caninized murine anti-human PD-1 antibody or antigen binding fragment increases the immune response of the canine subject being treated.

[0027] The present invention further provides isolated nucleic acids that encode the caninized murine anti-human PD-1 antibodies or antigen binding fragments as disclosed herein. In related embodiments such antibodies or antigen binding fragments can be used for the preparation of a medicament to treat cancer in a canine subject. Alternatively, or in conjunction, the present invention provides for the use of any of the antibodies or antibody fragments of the present invention for diagnostic use. In yet additional embodiments, a kit is provided comprising any of the caninized antibodies or antigen binding fragments disclosed herein.

[0028] In yet additional embodiments, an expression vector is provided comprising an isolated nucleic acid encoding any of the caninized murine anti-human PD-1 antibodies or antigen binding fragments of the invention. The invention also relates to a host cell comprising any of the expression vectors described herein. In particular embodiments, these nucleic acids, expression vectors or polypeptides of the invention are useful in methods of making an antibody.

[0029] The present invention further includes pharmaceutical compositions comprising an antibody or antigen binding fragment thereof together with a pharmaceutically acceptable carrier or diluent. In addition, the present invention provides methods of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of such pharmaceutical compositions. In certain embodiments the method is used for the treatment of cancer. In other embodiments, the method is used in the treatment of an infection or infectious disease. In still other embodiments, a caninized antibody of the present invention or antigen binding fragment thereof is used as a vaccine adjuvant.

[0030] 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

[0031]

Figure 1 shows the reactivity of murine anti-human PD-1 monoclonal antibody 08A [mAb 08A; as first described in US 8,354,509 B2 in relation to human PD-1] against the His-tagged extracellular domain of canine PD-1.

Figure 2 shows the reactivity of murine anti-human PD-1 monoclonal antibody 08A (see above) against canine PD-1 proteins expressed on CHO cells using CELISA. Murine anti-human PD-1 monoclonal antibody 08A and its caninized variants were found to react with canine PD-1 in a dose dependent manner.

Figure 3 depicts the ligand blockade by murine and caninized monoclonal antibodies. Murine anti-human PD-1 monoclonal antibody 08A (see above) and its caninized variants blocked the binding of canine PD-L1 to PD-1 expressed on CHO cell surface.

Figure 4 provides the alignment of canine IgGB constant heavy chains (CHs) lacking ADCC function. The canine wild type IgB [cIgGB wt], Canine IgGB(+)A-hinge [cIgGB(+) A-hinge], Canine IgGB(+) D-hinge [cIgGB(+) D-hinge], and Canine IgGB (-)ADCC [cIgGB(-) ADCC] are depicted. The (+) A-hinge is the replacement with IgG-A hinge *plus* a lysine and asparagine amino acid replacement as shown; the (+) D-hinge is the replacement with IgG-D hinge *plus* a lysine and the asparagine amino acid replacement as shown. The (-)ADCC is the lysine and asparagine amino acid replacement.

DETAILED DESCRIPTION

Abbreviations

[0032] Throughout the detailed description and examples of the invention the following abbreviations will be used:

ADCC
: Antibody-dependent cellular cytotoxicity

CDC
: Complement-dependent cytotoxicity

CDR
: Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system

CHO
: Chinese hamster ovary

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.

HRP
: Horseradish peroxidase

IFN
: interferon

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)

MES
: 2-(N-morpholino)ethanesulfonic acid

MOA
: Mechanism of action

NHS
: Normal human serum

PCR
: Polymerase chain reaction

PK
: Pharmacokinetics

SEB
: Staphylococcus Enterotoxin B

TT
: Tetanus toxoid

V region
: The segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.

VH
: Immunoglobulin heavy chain variable region

VK
: Immunoglobulin *kappa* light chain variable region

DEFINITIONS

[0033] 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.

[0034] 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.

[0035] "Activation" as it applies to cells or to receptors refers to the activation or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. "Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compounds derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors.

[0036] "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.

[0037] "Administration" and "treatment," as it applies to an animal, *e.g.*, a canine experimental 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 means *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 an animal, more preferably a mammal (*e.g.*, canine, feline, or human) and most preferably a canine.

[0038] "Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies or antigen binding fragments of the present invention, internally or externally to a canine subject or patient having one or more disease symptoms, or being suspected of having a disease, 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 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 symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease 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 symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veteranarians 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 symptom(s) in every subject, it should alleviate the target disease 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.

[0039] "Treatment," as it applies to a human, veterinary (*e.g.*, canine) or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a human, veterinary (*e.g.*, canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies or antigen binding fragments of the present invention to a canine or other animal subject, a cell, tissue, physiological compartment, or physiological fluid.

[0040] Canine PD-1 has been found to comprise the amino acid sequence of SEQ ID NO: 2. In a specific embodiment canine PD-1 is encoded by a nucleic acid that comprises the nucleotide sequence of SEQ ID NO: 1. Canine PD-1 sequences may differ by having, for example, conserved variations in non-conserved regions, but the canine PD-1 will have substantially the same biological function as the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2. For example, a biological function of PD-1 is to attenuate T-cell responses when bound to PD-L1 and/or PD-L2. That is, PD-1 may be considered a negative regulator. Notably, the

cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif). In addition, a biological function of canine PD-1 may be having, for example, an epitope in the extracellular domain that is specifically bound by an antibody of the instant disclosure.

[0041] Canine PD-L1 has been found to comprise the amino acid sequence of SEQ ID NO: 8. In a specific embodiment canine PD-L1 is encoded by a nucleotide sequence comprising SEQ ID NO: 7. Canine PD-L1 sequences may differ by having, for example, conserved variations in non-conserved regions, but the canine PD-L1 will have substantially the same biological function as the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8. For example, one biological function of PD-L1 is to attenuate T-cell responses when bound to PD-1.

[0042] A particular canine PD-1 or PD-L1 amino acid sequence respectively, will generally be at least 90% identical to the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain cases, a canine PD-1 or PD-L1 respectively, may be at least 95%, or even at least 96%, 97%, 98% or 99% identical to the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain embodiments, a canine PD-1 or a PD-L1 amino acid sequence will display no more than 10 amino acid differences from the canine PD-1 comprising the amino acid sequence of SEQ ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. In certain embodiments, the canine PD-1 or the PD-L1 amino acid sequence respectively, may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the canine PD-1 comprising the amino acid sequence ID NO: 2, or the canine PD-L1 comprising the amino acid sequence of SEQ ID NO: 8, respectively. Percent identity can be determined as described herein below.

[0043] The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the mammalian body (e.g., canine body) of cancerous cells, cells or tissues infected with pathogens, or invading pathogens.

Caninized Anti-Human PD-1 Antibodies

[0044] The present invention provides isolated caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof as defined in the claims that bind canine PD-1 and uses of such antibodies or fragments.

[0045] As used herein, a caninized murine anti-human PD-1 antibody refers to a caninized antibody that specifically binds to mammalian PD-1. An antibody that specifically binds to mammalian PD-1, and in particular canine PD-1, is an antibody that exhibits preferential

binding to mammalian PD-1 as compared to other antigens, but this specificity does not require absolute binding specificity. A caninized murine anti-human PD-1 antibody is considered "specific" for canine PD-1 if its binding is determinative of the presence of canine PD-1 in a biological sample obtained from a canine, or if it is capable of altering the activity of canine PD-1 without unduly interfering with the activity of other canine proteins in a canine sample, *e.g.* without producing undesired results such as false positives in a diagnostic context or side effects in a therapeutic context. The degree of specificity necessary for a caninized murine anti-human PD-1 antibody may depend on the intended use of the antibody, and at any rate is defined by its suitability for use for an intended purpose. The antibody, or binding compound derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, preferably at least ten-times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with any other canine antigen.

[0046] As used herein, an antibody is said to bind specifically to a polypeptide comprising a given sequence (in this case canine PD-1) if it binds to polypeptides comprising the sequence of canine PD-1, but does not bind to other canine proteins lacking the amino acid sequence of canine PD-1. For example, an antibody that specifically binds to a polypeptide comprising canine PD-1 may bind to a FLAG®-tagged form of canine PD-1, but will not bind to other FLAG®-tagged canine proteins.

[0047] 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 PD-1) 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.

[0048] Typically, a caninized antibody or antigen binding fragment thereof of the invention retains at least 10% of its canine PD-1 binding activity (when compared to the corresponding 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 PD-1 binding affinity as the parental antibody. It is also intended that an 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.

[0049] "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.

[0050] The variable regions of each light/heavy chain pair form the antigen binding site of the antibody. 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.

[0051] 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 flanked 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)].

[0052] 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.* CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and 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), definining 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.

[0053] As used herein, the term "canine" includes all domestic dogs, Canis lupus familiaris or Canis familiaris, unless otherwise indicated.

[0054] 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 antibody) 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 canine antibody, and/or to modify the Fc function, as discussed below.

[0055] There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa.*

[0056] Besides binding and activating of canine immune cells, a canine or caninized antibody against PD-1 optimally has two attributes:

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

[0057] 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 displays undesirable ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D display no ADCC activity. (IgG-C has considerable ADCC activity). The present invention overcomes this difficulty by providing mutant canine IgG-B antibodies specific to PD-1; such antibodies lack effector functions such as ADCC and can be easily of purified using industry standard protein A chromatography.

[0058] As used herein, the term "caninized antibody" refers to an antibody that comprises the three heavy chain CDRs and the three light chain CDRS from a murine anti-human PD-1 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 canine PD-1 and/or its ability to block the binding of canine PD-1 to canine PD-L1.

[0059] "Homology" 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 monomer subunit, 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.

[0060] "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.

[0061] The phrase "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to use promoters, polyadenylation signals, and enhancers.

[0062] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. It should also be readily understood that when a nucleic acid sequence is provided herein, it may include a stop codon. However, as stop codons are interchangeable the inclusion of a specific stop codon in a sequence should not be viewed as a necessary portion of that sequence.

[0063] As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that not all progeny will have precisely identical DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0064] As used herein, "germline sequence" refers to a sequence of unrearranged immunoglobulin DNA sequences. Any suitable source of unrearranged immunoglobulin sequences may be used. Human germline sequences may be obtained, for example, from JOINSOLVER® germline databases on the website for the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the United States National Institutes of Health. Mouse germline sequences may be obtained, for example, as described in Giudicelli et al. [Nucleic Acids Res. 33:D256-D261 (2005)].

Properties of the Exemplary Caninized Murine Anti-human PD-1 Antibodies

[0065] The present invention provides isolated caninized murine anti-human PD-1 antibodies as defined in the claims and methods of use of the antibodies or antigen binding fragments thereof in the treatment of disease *e.g.,* the treatment of cancer in canines. Examples of caninized murine anti-human PD-1 antibodies that bind canine PD-1 include, but are not limited to: antibodies that comprise canine IgG-A, IgG-B, and IgG-D heavy chains and/or canine *kappa* light chains together with murine anti-human PD-1 CDRs. Accordingly, the present invention provides isolated caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof as defined in the claims that bind to canine PD-1 and block the binding of canine PD-1 to canine PD-L1.

[0066] In a further embodiment, the isolated antibody or antigen-binding fragment thereof that binds canine PD-1 comprises a canine antibody *kappa* light chain comprising a murine light chain CDR-1, CDR-2 and CDR-3 and a canine antibody heavy chain IgG comprising a murine heavy chain CDR-1, CDR-2 and CDR-3.

[0067] Also described herein are antibodies or antigen binding fragments thereof that specifically binds PD-1 and have canine antibody *kappa* light chains comprising CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID NOs: 20, 22, and/or 24 and canine antibody heavy chain IgG with CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID NOs: 14, 16, and/or 18, while still exhibiting the desired binding and functional properties. In another instance the antibody or antigen binding fragment comprises a canine frame comprising of a combination of IgG heavy chain sequence (comprising an amino acid sequence of SEQ ID NO: 26, 28, or 30 with and without signal sequence) with a *kappa* light chain (comprising an amino acid sequence of SEQ ID NO: 32, or 34 with and without signal sequence) having up to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more conservative or non-conservative amino acid substitutions, while still exhibiting the desired binding and functional properties. In a particular instance the number of conservative amino acid substitutions is between 0 to 5 for the IgG heavy chain and 0 to 5 for the *kappa* light chain.

[0068] "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 nonessential 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. In some instances of the antibody or antigen binding fragment may comprise polypeptide chains with the sequences disclosed herein, *e.g.,* SEQ ID NOs: 26, 28, 30, 32, and/or 34, or polypeptide chains comprising up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more conservative amino acid substitutions. Exemplary conservative substitutions are set forth in Table I.

TABLE I.

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

[0069] 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 speficity. 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 I.

Nucleic Acids

[0070] The present invention further comprises the nucleic acids encoding the immunoglobulin chains of caninized murine anti-human PD-1 antibodies and antigen binding fragments thereof defined in the claims

[0071] For example, the present invention includes the nucleic acids listed in Tables 2 and 3 and the Sequence Listing Table below that encode the claimed antibodies.

[0072] Herein described 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 antibodies 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.

[0073] Also described are 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.

[0074] 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. Sequence similarity includes identical residues and nonidentical, biochemically related amino acids. Biochemically related amino acids that share similar properties and may be interchangeable are discussed above.

[0075] 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).

[0076] This present invention also provides expression vectors comprising the isolated nucleic acids of the invention, wherein the nucleic acid is operably linked to control sequences that are recognized by a host cell when the host cell is transfected with the vector. Also provided are host cells comprising an expression vector of the present invention and methods for producing the antibody or antigen binding fragment thereof disclosed herein comprising culturing a host cell harboring an expression vector encoding the antibody or antigen binding fragment defined in the claims in culture medium, and isolating said antigen or antigen binding fragment thereof from the host cell or culture medium.

Epitope Binding and Binding Affinity

[0077] Herein described are also antibodies or antigen binding fragments thereof that bind to the same epitope on canine PD-1 as the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and/or of SEQ ID NO: 32, or the caninized murine anti-human PD-1 antibody comprising the amino acid sequence ID NO: 28 and/or ID NO: 34. The caninized murine anti-human PD-1 antibodies or antigen binding fragments thereof are capable of inhibiting the binding of canine PD-1 to canine PD-L1.

[0078] The caninized murine anti-human PD-1 antibody can be produced recombinantly as described below in the examples. 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.

[0079] 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.

[0080] In general, glycoproteins produced in a particular cell line or transgenic animal will have a glycosylation pattern that is characteristic for glycoproteins produced in the cell line or transgenic animal. Therefore, the particular glycosylation pattern of an antibody will depend on the particular cell line or transgenic animal used to produce the antibody. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein, comprise the instant invention, independent of the glycosylation pattern that the antibodies may have. Similarly, in particular embodiments, antibodies with a glycosylation pattern comprising only non-fucosylated *N*-glycans may be advantageous, because these antibodies have been shown to typically exhibit more potent efficacy than their fucosylated counterparts both *in vitro* and *in vivo* [*See* for example, Shinkawa et al., J. Biol. Chem. 278: 3466-3473 (2003); U.S. Patent Nos. 6,946,292 and 7,214,775].

[0081] The present invention further includes antibody fragments of the caninized murine anti-human PD-1 antibodies defined in the claims. The antibody fragments include $F(ab)_2$ fragments, which may be produced by enzymatic cleavage of an IgG by, for example, pepsin. Fab fragments may be produced by, for example, reduction of $F(ab)_2$ with dithiothreitol or mercaptoethylamine. A Fab fragment is a V_L-C_L chain appended to a V_H-C_{H1} chain by a disulfide bridge. A $F(ab)_2$ fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an $F(ab)_2$ molecule includes a portion of the F_c region between which disulfide bridges are located. An F_V fragment is a V_L or V_H region.

[0082] In one embodiment, 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 another embodiment, the antibody or antigen binding fragment comprises a light chain constant region, *e.g.,* a canine light chain constant region, such as a kappa light chain region. By way of example, and not limitation the canine heavy chain constant region can be from IgG-D and the canine light chain constant region can be from *kappa.*

Antibody Engineering

[0083] The caninized murine anti-human PD-1 antibodies of the present invention have been engineered to include modifications to framework residues within the variable domains of a parental (i.e., canine) monoclonal antibody, e.g. to improve the properties of the antibody.

Experimental and diagnostic uses

[0084] Caninized murine anti-human PD-1 antibodies or antigen-binding fragments thereof of the present invention may also be useful in diagnostic assays for canine PD-1 protein, *e.g.*,

detecting its expression in specific tumor cells, tissues, or serum. Such diagnostic methods may be useful in various disease diagnoses, particularly certain cancers in canines.

[0085] For example, such a method comprises the following steps:

1. (a) coat a substrate *(e.g.,* surface of a microtiter plate well, *e.g.,* a plastic plate) with caninized murine anti-human PD-1 antibody or an antigen-binding fragment thereof;
2. (b) apply a sample to be tested for the presence of canine PD-1 to the substrate;
3. (c) wash the plate, so that unbound material in the sample is removed;
4. (d) apply detectably labeled antibodies (*e.g.*, enzyme-linked antibodies) which are also specific to the PD-1 antigen;
5. (e) wash the substrate, so that the unbound, labeled antibodies are removed;
6. (f) if the labeled antibodies are enzyme linked, apply a chemical which is converted by the enzyme into a fluorescent signal; and
7. (g) detect the presence of the labeled antibody.

[0086] In a further embodiment, the labeled antibody is labeled with peroxidase which react with ABTS [*e.g.*, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] or 3,3',5,5'-Tetramethylbenzidine to produce a color change which is detectable. Alternatively, the labeled antibody is labeled with a detectable radioisotope (*e.g.*, ^{3}H) which can be detected by scintillation counter in the presence of a scintillant. Caninized murine anti-human PD-1 antibodies of the invention may be used in a Western blot or immuno protein blot procedure.

[0087] Such a procedure forms part of the present invention and includes for example:

1. (i) contacting a membrane or other solid substrate to be tested for the presence of bound canine PD-1 or a fragment thereof with a caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof of the present invention. Such a membrane may take the form of a nitrocellulose or vinyl-based [*e.g.*, polyvinylidene fluoride (PVDF)] membrane to which the proteins to be tested for the presence of canine PD-1 in a nondenaturing PAGE (polyacrylamide gel electrophoresis) gel or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gel have been transferred (*e.g.*, following electrophoretic separation in the gel). Before contact of membrane with the caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof, the membrane is optionally blocked, *e.g.*, with non-fat dry milk or the like so as to bind non-specific protein binding sites on the membrane.
2. (ii) washing the membrane one or more times to remove unbound caninized murine anti-human PD-1 antibody or an antigen-binding fragment thereof and other unbound substances; and
3. (iii) detecting the bound caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof.

[0088] Detection of the bound antibody or antigen-binding fragment may be by binding the antibody or antigen-binding fragment with a secondary antibody (an anti-immunoglobulin antibody) which is detectably labeled and, then, detecting the presence of the secondary antibody.

[0089] The caninized murine anti-human PD-1 antibodies and antigen-binding fragments thereof disclosed herein may also be used for immunohistochemistry. Such a method forms part of the present invention and comprises, *e.g.*, (1) contacting a cell to be tested for the presence of canine PD-1 with a caninized murine anti-human PD-1 antibody or antigen-binding fragment thereof of the present invention; and (2) detecting the antibody or fragment on or in the cell. If the antibody or antigen-binding fragment itself is detectably labeled, it can be detected directly. Alternatively, the antibody or antigen-binding fragment may be bound by a detectably labeled secondary antibody which is detected.

[0090] Certain caninized murine anti-human PD-1 antibodies and antigen-binding fragments thereof disclosed herein may also be used for *in vivo* tumor imaging. Such a method may include injection of a radio labeled caninized murine anti-human PD-1 antibodies or antigen-binding fragment thereof into the body of a canine to be tested for the presence of a tumor associated with canine PD-1 expression followed by nuclear imaging of the body of the patient to detect the presence of the labeled antibody or antigen-binding fragment *e.g.*, at loci comprising a high concentration of the antibody or antigen-binding fragment which are bound to the tumor.

[0091] Imaging techniques include SPECT imaging (single photon emission computed tomography) or PET imaging (positron emission tomography). Labels include *e.g.*, iodine-123 (^{123}I) and technetium-99m (^{99m}Tc), *e.g.,* in conjunction with SPECT imaging or ^{11}C, ^{13}N, ^{15}O or ^{18}F, *e.g.,* in conjunction with PET imaging or Indium-111 [*See e.g.,* Gordon et al., International Rev. Neurobiol. 67:385-440 (2005)].

Pharmaceutical Compositions and Administration

[0092] To prepare pharmaceutical or sterile compositions of the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof is 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)].

[0093] 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, anti-PD-1 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.

[0094] 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.

[0095] 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.

[0096] In particular embodiments, the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof can be administered by an invasive route such as by injection. In further embodiments of the invention, a caninized murine anti-human PD-1 antibody or antigen binding fragment thereof, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, intratumorally, 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.

[0097] 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.

[0098] 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. Patent No. 4,487,603, which discloses an implantable microinfusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which

discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent. No. 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.

[0099] Alternately, one may administer the caninized murine anti-human PD-1 antibody in a local rather than systemic manner, for example, via injection of the antibody directly into an arthritic joint or pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the caninized murine anti-human PD-1 antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, arthritic joint or pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

[0100] The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibody, the level of symptoms, the immunogenicity of the therapeutic antibody, and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibody to effect improvement in the target disease state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibody 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)].

[0101] Determination of the appropriate dose is made by the veteranarian, *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 symptoms of, *e.g.,* the inflammation or level of inflammatory cytokines produced.

[0102] Antibodies or antigen binding fragments thereof disclosed herein may be provided by continuous infusion, or by doses administered, *e.g.*, daily, 1-7 times per week, weekly, biweekly, 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 the caninized murine anti-human PD-1 antibody in the subject's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 µg/ml or more. In other embodiments, a caninized murine anti-human PD-1 antibody of the present invention is 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.

[0103] 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 with a disorder, disease or symptom, or with the potential to develop such a disorder, disease or symptom.

[0104] As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, 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 binding compound 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 an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. 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 disease severity.

Other Combination Therapies

[0105] As previously described, the caninized murine anti-human PD-1 antibody or antigen binding fragment thereof may be coadministered with one or other more therapeutic agents (such as a chemotherapeutic agent). The antibody may be linked to the agent (as an

immunocomplex) or can be administered separately from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be coadministered with other known therapies.

Kits

[0106] Further provided are kits comprising one or more components that include, but are not limited to, an antibody or antigen binding fragment, as discussed herein, which specifically binds PD-1 (*e.g.,* a caninized murine anti-human PD-1 antibody or antigen binding fragment thereof of the present invention) in association with one or more additional components including, but not limited to a pharmaceutically acceptable carrier and/or a chemotherapeutic agent, as discussed herein. The binding composition and/or the chemotherapeutic agent can be formulated as a pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

[0107] In one embodiment, the kit includes a binding composition of the invention (the caninized murine anti-human PD-1 antibody comprising the amino acid sequence of SEQ ID NO: 28 and ID NO: 32 or 34, or a pharmaceutical composition thereof in one container (*e.g.*, in a sterile glass or plastic vial) and a pharmaceutical composition thereof and/or a chemotherapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial).

[0108] In another embodiment, the kit comprises a combination of the invention, including a binding composition component (e.g., the caninized murine anti-human PD-1 antibody comprising the amino acid sequence ID NO: 28 and of SEQ ID NO: 32 or 34) along with a pharmaceutically acceptable carrier, optionally in combination with one or more therapeutic agent component formulated together, optionally, in a pharmaceutical composition, in a single, common container.

[0109] If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above. The kit can also include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids pet owners and veteranarians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

[0110] As a matter of convenience, an antibody or specific binding agent disclosed herein can be provided in a kit, *i.e.,* a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic or detection assay. Where the antibody is labeled

with an enzyme, the kit will include substrates and cofactors required by the enzyme (*e.g.,* a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

EXAMPLES

EXAMPLE 1

CANINE PD-1 AND PD-L1

Identification and Cloning of Canine PD-1:

[0111] A nucleic acid encoding a full length canine PD-1 (cPD-1) was identified through a search of the NCBI gene bank data bases (accession number XM_543338.4, SEQ ID NO: 1). The translated amino acid sequence SEQ ID NO: 2 (accession number XP-543338.3) corresponds to putative canine PD-1 protein which was further identified through searching the gene bank (NCBI) protein databases and aligning the identified amino acid sequence with murine, feline, and human PD-1 amino acid sequences. The DNA sequence corresponding to the full length canine PD-1 gene that was codon optimized for CHO cells was synthesized and cloned into a plasmid designated p96793. Comparison of DNA and protein sequences of predicted canine PD-1 with known PD-1 DNA and protein sequences led to the identification of the DNA sequences encoding the extra-cellular domain (ECD) of canine PD-1 (SEQ ID NO: 3) and the amino acid sequence of the ECD of canine PD-1 (SEQ ID NO: 4).

[0112] A DNA sequence encoding the ECD of canine PD-1 in addition to a GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2726. A nucleic acid sequence (SEQ ID NO: 5) corresponding to the canine PD-1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene was chemically synthesized and cloned into a plasmid designated LPD2727. Canine PD-1 ECD and the Fc part of human IgG1 Fc comprises the amino acid sequence of SEQ ID NO: 6.

Identification and Cloning of Canine PD-L1:

[0113] A nucleic acid encoding a full length canine PD-L1 was identified through a search of the NCBI gene bank data bases (accession number XM_541302.4; SEQ ID NO: 7). The translated amino acid sequence (accession number XP-541302.4; SEQ ID NO: 8) corresponding to the putative canine PD-L1 protein was identified by searching the gene bank (NCBI) protein databases and alignment of the identified sequence with known PD-L1 mouse and human sequences.

[0114] Comparison of DNA encoding canine PD-L1 with known PD-L1 sequences identified the DNA sequence corresponding to the ECD domain of canine PD-L1 (SEQ ID NO: 9; which was codon optimized for CHO cells). The predicted amino acid sequence of the ECD of canine PD-L1 is SEQ ID NO: 10. DNA encoding PD-L1 ECD plus GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2695.

[0115] A DNA sequence encoding the amino acid sequence of canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 Fc (SEQ ID NO: 11) was chemically synthesized and cloned into a plasmid designated LPD2697. Canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 comprises the amino acid sequence ID NO: 12. Table 1 contains a description of the expression plasmids mentioned above.

Table 1

PLASMIDS COMPRISING DNA ENCODING PD-1 or PD-L1	
PLASMID NAME	EXPRESSED GENE
P96793	Canine PD-1
LPD2726	Canine PD-1 ECD-8HIS
LPD2727	Canine PD-1 ECD-/Human IgG1 Fc
LPD2695	Canine PD-L1 ECD-8HIS
LPD2697	Canine PD-L1 ECD-/Human IgG1 Fc

Expression of PD-1 and PD-L1 proteins:

[0116] Expression plasmids encoding the PD-1ECD-HIS, PD-1ECD-Fc, PDL-1 ECD-HIS, and PD-L1ECD-Fc proteins were transfected into HEK 293 cells and the proteins were purified from the supernatant of transfected cells using Protein A for Fc fusion proteins or Nickel (Ni^{2+}) column chromatography for HIS-tagged proteins. Purified proteins were used for: ELISA or binding assays as detailed below. Expressed proteins were analyzed by SDS-PAGE gels.

EXAMPLE 2

IDENTIFICATION OF MURINE ANTI-HUMAN MONOCLONAL ANTIBODIES THAT BIND

CANINE PD-1

Confirmation of monoclonal antibodies reactivity against canine PD-1

[0117] One of the mouse monoclonal antibodies that previously had been raised against human PD-1 [hPD-1.08A, identified in US 8,354,509 B2, hereby incorporated by reference in its entirety] also was found to strongly react with canine PD-1. Purified hPD-1.08A was tested for reactivity with the HIS-tagged ECD domain of canine PD-1 by ELISA as follows: HIS-tagged canine PD-1 ECD protein is diluted to 10µg/mL in coating buffer (Carbonate/Bicarbonate pH 9.0) and dispensed at 100 µl/well in 96-well flat bottomed ELISA plates (NUNC). The plates are incubated at 4°C overnight. The plates are then washed three times with phosphate buffered saline containing 0.05% Tween-20 (PBST). Next, 200 µl of blocking buffer (5% skim milk in PBST) is added to each well and the plates are incubated at 37°C for 60 minutes.

[0118] The plates are then washed three times with PBST. Next, 100 µl of test monoclonal antibodies (mAbs) diluted in blocking buffer is added to the first wells of the appropriate columns. Test mAbs are then diluted two-fold to the appropriate plate position. Following incubation of the plates at 37°C for 60 minutes, the plates are washed three times with PBST. Next, 100 µl per well of a 1:2,000 dilution of a horseradish peroxidase conjugated goat anti-mouse IgG (KPL) is added to the plates, which are then incubated at 37°C for 60 minutes. Then the plates are washed three times with PBST, and 100 µl/well of 3,3',5,5' tetramethyl benzidine, (TMB) substrate (from KPL) is added to the plates. The color reaction is allowed to develop for 5-20 minutes at 37°C prior to measuring absorbance at 650nm.

CHO cells expressing canine PD-1 protein

[0119] The full length canine PD-1 gene was cloned into plasmid p96793. In this plasmid the expression of the canine PD-1 protein is driven by an hCMV promoter. CHO DXB11 cells (dhfr-) were maintained in MEM-alpha (Gibco) supplemented with 10% fetal bovine serum.

[0120] Transfection of CHO cells with plasmid p96793 was carried out in 75 cm^2 flasks containing approximately 6 $\times 10^6$ cells by liposome-mediated gene delivery using Lipofectamine (Invitrogen). After 48 hours, cells were passaged into MEM-*alpha* medium without nucleosides, supplemented with 10% FBS and 400µg/mL hygromycin B (selective medium). Limited-dilution cloning was performed on the pool of dhfr+, hygromycin resistant cells. Clones were assessed for expression of canine PD-1 by immunofluorescence assay. Briefly, cell monolayers were fixed in 96 well plates with 80% acetone. Fixed and dried cell monolayers were then incubated for 1 hour with a polyclonal goat anti-human PD-1 antibody (R&D Systems). Plates were washed with PBS, then incubated for 1 hour with a fluoresceinlabeled rabbit anti-goat IgG

antibody (KPL). Plates were washed with PBS. Clones exhibiting fluorescence were expanded and cell stocks were established.

Reactivity of mouse mabs against Canine PD-1 proteins expressed on CHO cells

[0121] The reactivity of mouse anti-human PD-1 mAbs with canine PD-1 on CHO cells was determined by a cell-based assay using CHO cells that express PD-1. Briefly, the CHO cells expressing canine PD-1 were cultured to 80-100% confluency in 50 µl media (DMEM/HAM's F12, 10% FBS; "CHO Media"). Next, 50 µl of media containing various concentrations of purified mAbs were added for 1 hour at 37°C. Following three washes with PBS-TWEEN, 100 µl of goat anti-mouse horse raddish peroxidase (HRP) diluted 1:1000 in culture media was added for one hour at 37°C. After three additional washes with PBS-TWEEN, bound mAbs were visualized with a perioxidase substrate (TMB). The absorbance increase due to perioxidase activity at 450 nm was measured in a microplate reader. Color development is stopped by adding 50 µL per well of 1 M phosphoric acid.

Ligand blockade by mouse and caninized anti-PD-1 mAbs

[0122] For mouse anti-human PD-1 mAbs which react with canine PD-1, a cell-based ELISA (CELISA) assay based on the CHO cell line expressing canine PD-1 was used. Ligand blockade was confirmed using this assay in conjunction with biotinylated cPD-L1/Fc protein. Briefly, seed cPD-1 CHO cells in 96-well plates at 4 $\times 10^4$ cells per well and incubate cells at 37°C for 18-24 hours till they are 95-100% confluent. Aspirate cell culture media, wash the plates 3 times with PBS + 0.05% Tween20 and 1x CHO media. Make 3-fold serial dilutions of anti-cPD1 mAbs in CHO media, starting at 30 µg/mL, and add 50 µL/well of each antibody dilution to the plate. Incubate at 37°C, 5% CO_2 with shaking for 30 min. Add 50 µL/well of cPD-L1-Fc -biotin (2 ug/ml in CHO media stock) and continue to incubate at 37°C, 5% CO_2 with shaking for 45 min. Wash the plates six times with PBS + 0.05% Tween 20. Add 100u1/well of 1:2000 Streptavidin-Horse Raddish Peroxidase (Streptavidin-HRP) in CHO media and incubate 30-60 min at 37°C/5% CO_2. Wash the plates five times with PBS + 0.05% Tween20. Add 100 µl/well of TMB color developing substrate. Stop color development by adding 50 µl/well of 1M phosphoric acid. Measure optical density (O.D.) at A450 - A620 using an ELISA plate reader.

Cloning and identification of DNA sequences corresponding to mouse Hpd-.08A mAb

[0123] The DNA sequence of mouse VH and VL chains and the DNA sequences encoding their CDRs are identified as described US 8,354,509 [*see,* Table IV of US 8,354,509; provided in Table 2 directly below].

Table 2

Mouse anti-Human PD-1 CDRs from hPD-1.08A of US 8,354,509				
CDR	Heavy Chain (SEQ ID NO:)		Light Chain (SEQ ID NO:)	
	N.A.	A.A.	N.A.	A.A.
CDR-1	13	14	19	20
CDR-2	15	16	21	22
CDR-3	17	18	23	24

EXAMPLE 3

CANINIZATION OF MOUSE ANTI-HUMAN PD-1 MONOCLONAL ANTIBODIES

[0124] In order to execute the process of caninization, the 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. There are four known IgG subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. There are two types of light chains in canine antibodies referred to as kappa and lambda. Table 3 lists both the amino and nucleic acid sequences of modified canine heavy (IgG-A, IgG-B, IgG-D) and light (*Kappa*) antibody chains of the present invention that comprise the murine anti-human PD-1 CDRs of Table 2.

Table 3

MODIFIED CANINE HEAVY AND LIGHT CHAIN SEQUENCES#			
Chain type	Subtype	Nucleic Acid SEQ ID NO:	Amino Acid SEQ ID NO:
H	IgG-A	25	26
H	IgG-B	27	28
H	IgG-D	29	30
L	*Kappa* (1011)	31	32
L	*Kappa* (1022)	33	34
# Sequences do not include the signal sequence.			

Construction of Caninized anti PD-1 Antibodies

[0125] Without being bound by any specific approach, the process of producing variants of caninized anti-PD-1 mAbs with various contents of canine and mouse sequences involved the

general following scheme:

1. i) Determine DNA sequence of VH and VL chains of mouse mabs
2. ii) Identify the H and L chain CDRs of mouse mabs
3. iii) Identify a suitable H and L chain of canine IgG
4. iv) Write down the DNA sequence of canine IgG H and L chains
5. v) Replace the DNA sequence encoding endogenous dog H and L chain CDRs with DNA sequences encoding the respective mouse CDRs. Also, optionally replace some canine frame residues with selected residues from the corresponding mouse frame regions.
6. vi) Synthesize the DNA from step (v) and clone it into a suitable expression plasmid
7. vii) Transfect plasmids into HEK 293 cells
8. viii) Purify expressed antibody from HEK 293 supernatant
9. ix) Test purified antibody for binding to canine PD-1

[0126] The above outlined steps resulted in a set of variant antibodies with various contents of canine and mouse sequences. The present invention identifies the caninized murine anti-human PD-1 antibodies comprising SEQ ID NO: 28 and ID NO: 32 or 34 as having particularly tight binding with canine PD-1.

[0127] Full length canine PD-1 DNA sequence: signal sequence **underlined and in bold**

Nucleotide sequence **SEQ ID NO: 1 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 35 includes the signal sequence.**

atggggagccggcggggccctggccgctcgtctgggccgtgctgcagctgggctggtggccaggatggctcctag
actcccctgacaggccctggagcccgctcaccttctccccggcgcagctcacggtgcaggagggagagaacgccac
gttcacctgcagcctggccgacatccccgacagcttcgtgctcaactggtaccgcctgagcccccgcaaccagacg
gacaagctggccgccttccaggaggaccgcatcgagccgggccgggacaggcgcttccgcgtcatgcggctgccca
acgggcgggacttccacatgagcatcgtcgctgcgcgcctcaacgacagcggcatctacctgtgcggggccatcta
cctgccccccaacacacagatcaacgagagtccccgcgcagagctctccgtgacggagagaaccctggagcccccc
acacagagccccagccccccacccagactcagcggccagttgcaggggctggtcatcggcgtcacgagcgtgctgg
tgggtgtcctgctactgctgctgctgacctgggtcctggccgctgtcttccccagggccacccgaggtgcctgtgt
gtgcgggagcgaggacgagcctctgaaggagggccccgatgcagcgcccgtcttcaccctggactacggggagctg
gacttccagtggcgagagaagacgccggagcccccggcgccctgtgccccggagcagaccgagtatgccaccatcg
tcttcccgggcaggccggcgtccccgggccgcagggcctcggccagcagcctgcagggagcccagcctccgagccc
cgaggacggacccggcctgtggcccctctga

[0128] Full length canine PD-1 Amino acid sequence: signal sequence **underlined and in bold**

Amino acid sequence **SEQ ID NO: 2 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 36 includes the signal sequence.**

MGSRRGPWPLVWAVLQLGWWPGWLLDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRNQT
DKLAAFQEDRIEPGRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLEPP
TQSPSPPPRLSGQLQGLVIGVTSVLVGVLLLLLLTWVLAAVFPRATRGACVCGSEDEPLKEGPDAAPVFTLDYGEL
DFQWREKTPEPPAPCAPEQTEYATIVFPGRPASPGRRASASSLQGAQPPSPEDGPGLWPL

[0129] Canine PD-1 extracellular domain_DNA sequence: **SEQ ID NO: 3** (Codon optimized for expression in CHO cells)

ctggattcccccgacagaccctggagccctctcaccttctcccctgcccagctgaccgtccaggaaggcgagaatg
ccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggtacagactgagccccaggaacca
gaccgacaagctggccgctttccaggaggacaggatcgaacccggcagggacaggaggtttagggtcatgaggctg
cccaacggcagggacttccacatgtccatcgtggccgccagactgaacgactccggcatctacctgtgcggcgcta
tctacctgccccccaacacccagatcaacgagagccccagggccgaactgagcgtgacagagagaaccctggaacc
tcccacccagagcccttcccctcctcctagactgagcggacagctgcagggcctggtg

[0130] Canine PD-1 extracellular domain: **SEQ ID NO:** 4:

LDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRNQTDKLAAFQEDRIEPGRDRRFRVMRL
PNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLEPPTQSPSPPPRLSGQLQGLV

[0131] Canine PD-1 extracellular domain - human IgG1 Fc DNA sequence: **SEQ ID NO: 5** (Codon optimized for expression in HEK-293 cells)

ctggattcccccgacagaccctggagccctctcaccttctcccctgcccagctgaccgtccaggaaggcgagaatg
ccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggtacagactgagccccaggaacca
gaccgacaagctggccgctttccaggaggacaggatcgaacccggcagggacaggaggtttagggtcatgaggctg
cccaacggcagggacttccacatgtccatcgtggccgccagactgaacgactccggcatctacctgtgcggcgcta
tctacctgccccccaacacccagatcaacgagagccccagggccgaactgagcgtgacagagagaaccctggaacc
tcccacccagagcccttcccctcctcctagactgagcggacagctgcagggcctggtgggtaccgacaaaactcac
acatgcccaccgtgcccagcacctgaactcctgggggaccgtcagtcttcctcttcccccaaaacccaaggaca
ccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtt
caactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtac
cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaaca
aagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct
gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgac
atcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg

gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt
gatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

[0132] Canine PD-1 extracellular domain - human IgG1 Fc fusion protein: signal sequence **underlined and in bold: SEQ ID NO: 6; SEQ ID NO: 53 includes the signal sequence.**

MNFLLSWVHWSLALLLYLHHAKWSQALDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRN
QTDKLAAFQEDRIEPGRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLE
PPTQSPSPPPRLSGQLQGLVGTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

[0133] Full length canine PD-L1 DNA sequence: signal sequence **underlined and in bold**

Nucleotide sequence **SEQ ID NO: 7 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 37 includes the signal sequence.**

atgagaatgtttagtgtctttacattcatggcctactgccatttgctaaaagcatttacgatcacagtttctaagg
acctgtatgtggtagagtatggtggcaatgtgacaatggaatgcaaattcccggtggaaaaacagttaaacttgtt
tgcactaatcgtctactgggaaatggaggataaaaaaattatacaatttgtgaatggaaaggaagacctgaaagtt
cagcacagcagctacagccagagggctcagctattgaaggaccagctcttcttggggaaggctgcgcttcagatca
cagatgtgagattgcaggatgcaggggtttactgctgcttgatcggctatggcggtgctgactacaagcggattac
tttgaaagttcatgccccgtaccgcaacatcagccaaagaatttctgtggatcctgtcacctctgaacatgaacta

atgtgtcaggctgagggttaccctgaggctgaagtcatctggacaagcagtgaccaccgagtcctgagtggcaaaa
ccaccatcactaattccaatagggaagagaagctttcaatgtgaccagcacgctgaacatcaatgcaacagctaa
tgagattttctactgcacttttcaaagatcaggtcctgaggaaaacaatactgccgagttggtcatcccagaacga
ctgcccgttccagcaagtgagaggactcatttcatgattctgggacctttcctgttgcttcttggtgtagtcctgg
cagtcactttctgtctaaaaaaacatgggagaatgatggatgtggaaaaatgttgcacccgagataggaactcaaa
gaaacgaaatgatatacaatttgaagagacataa

[0134] Full length canine PD-L1: signal sequence **underlined and in bold**

Amino acid sequence **SEQ ID NO: 8 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 38 includes the signal sequence**.

MRMFSVFTFMAYCHLLKAFTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKV
QHSSYSQRAQLLKDQLFLGKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKVHAPYRNISQRISVDPVTSEHEL
MCQAEGYPEAEVIWTSSDHRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRSGPEENNTAELVIPER
LPVPASERTHFMILGPFLLLLGVVLAVTFCLKKHGRMMDVEKCCTRDRNSKKRNDIQFEET

[0135] Canine PD-L1 extracellular domain DNA sequence: **SEQ ID NO: 9** (Codon optimized for expression in CHO cells

tttaccatcaccgtgtccaaggacctgtacgtggtcgagtacggcggcaatgtgaccatggagtgcaagttccccg
tggagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggacaagaagatcatccagttcgtgaa
cggcaaggaggacctgaaggtgcagcactccagctactcccagagagcccagctgctgaaggaccagctgttcctg
ggcaaggccgccctgcagatcaccgacgtgagactgcaggacgccggcgtgtattgctgcctgatcggctacggag
gcgccgactacaagaggatcaccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcc
cgtgaccagcgagcacgagctgatgtgccaagccgagggctatcccgaggccgaagtgatctggaccagcagcgac
cacagggtcctgagcggcaagaccaccatcaccaacagcaacagggaggagaagctgttcaacgtgaccagcaccc
tcaacatcaacgccaccgccaacgagatcttctactgcaccttccagaggagcggccccgaagagaacaacaccgc
cgagctggtgatccccgagagactgcctgtgcctgccagcgagaggacccac

[0136] Canine PD-L1 extracellular domain protein: **SEQ ID NO: 10**

FTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQRAQLLKDQLFL
GKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKVHAPYRNISQRISVDPVTSEHELMCQAEGYPEAEVIWTSSD
HRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRSGPEENNTAELVIPERLPVPASERTH

[0137] Canine PD-L1 extracellular domain - human IgG1 Fc DNA sequence: **SEQ ID NO: 11** (Codon optimized for expression in HEK-293 cells)

tttaccatcaccgtgtccaaggacctgtacgtggtcgagtacggcggcaatgtgaccatggagtgcaagttccccg
tggagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggacaagaagatcatccagttcgtgaa
cggcaaggaggacctgaaggtgcagcactccagctactcccagagagcccagctgctgaaggaccagctgttcctg
ggcaaggccgccctgcagatcaccgacgtgagactgcaggacgccggcgtgtattgctgcctgatcggctacggag
gcgccgactacaagaggatcaccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcc
cgtgaccagcgagcacgagctgatgtgccaagccgagggctatcccgaggccgaagtgatctggaccagcagcgac
cacagggtcctgagcggcaagaccaccatcaccaacagcaacagggaggagaagctgttcaacgtgaccagcaccc
tcaacatcaacgccaccgccaacgagatcttctactgcaccttccagaggagcggccccgaagagaacaacaccgc
cgagctggtgatccccgagagactgcctgtgcctgccagcgagaggacccacggtaccgacaaaactcacacatgc
ccaccgtgcccagcacctgaactcctgggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctca
tgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactg
gtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtg
gtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccc
atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgcc
gtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgca
tgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

[0138] Canine PD-L1 extracellular domain - human IgG1 Fc fusion protein: **SEQ ID NO: 12**

FTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQRAQLLKDQLFL
GKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKVHAPYRNISQRISVDPVTSEHELMCQAEGYPEAEVIWTSSD
HRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRSGPEENNTAELVIPERLPVPASERTHGTDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

08A VH : CDR H1 DNA: **SEQ ID NO: 13:**
agttattatc tgtac

08A VH : CDR H1 protein: **SEQ ID NO: 14:**
Ser Tyr Tyr Leu Tyr

08A VH : CDR H2 DNA: **SEQ ID NO: 15:**
ggggttaatc ctagtaatgg tggtactaac ttcagtgaga agttcaag

08A VH : CDR H2 protein: **SEQ ID NO: 16:**
Gly Val Asn Pro Ser Asn Gly Gly Thr Asn Phe Ser Glu Lys Phe Lys

08A VH : CDR H3 DNA: **SEQ ID NO: 17:**
agggattcta actacgacgg gggctttgac tac

08A VH : CDR H3 protein: **SEQ ID NO: 18:**
Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr

08A VL : CDR L1 DNA: **SEQ ID NO: 19:** agggccagca aaagtgtcag tacatctggc tttagttatt tgcac

08A VL : CDR L1 protein: **SEQ ID NO: 20:** Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His

08A VL : CDR L2 DNA: **SEQ ID NO: 21:** cttgcatcca acctagagtc t

08A VL : CDR L2 protein: **SEQ ID NO: 22:**
Leu Ala Ser Asn Leu Glu Ser

08A VL : CDR L3 DNA: **SEQ ID NO: 23:**
cagcacagtt gggagcttcc gctcacg

08A VL : CDR L3 protein: **SEQ ID NO: 24:**
Gin His Ser Trp Glu Leu Pro Leu Thr

Caninized Murine Anti-Human PD-1 Antibody 08A

[0139]

canVH-can**IgGB**-Fc (12G8 signal sequence **underlined and in bold**): HEAVY CHAIN

Nucleotide sequence **SEQ ID NO: 27 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 41 includes the signal sequence.**

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
CCGGCGGCGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTCACCAGCTA
CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCCTGAGCGTGGACAAGGCCAAGAACACCGCCTACATGCAGC
TGAACTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCGA
CTACTGGGGACAGGGAACCCTGCTGACCGTGTCCAGCGCTTCCACAACCGCGCCATCAGTCTTTCCGTTGGCCCCA
TCATGCGGGTCGACGAGCGGATCGACTGTGGCCCTGGCGTGCTTGGTGTCGGGATACTTTCCCGAACCCGTCACGG
TCAGCTGGAACTCCGGATCGCTTACGAGCGGTGTGCATACGTTCCCCTCGGTCTTGCAATCATCAGGGCTCTACTC
GCTGTCGAGCATGGTAACGGTGCCCTCATCGAGGTGGCCCTCCGAAACGTTCACATGTAACGTAGCACATCCAGCC
TCCAAAACCAAGGTGGATAAACCCGTGCCGAAAAGAGAGAATGGGCGGGTGCCTCGACCCCCTGATTGCCCCAAGT
GTCCGGCTCCGGAAATGCTCGGTGGACCCTCAGTGTTTATCTTCCCTCCGAAGCCCAAGGACACTCTGCTGATCGC
GCGCACTCCAGAAGTAACATGTGTAGTGGTGGACCTTGATCCCGAGGACCCCGAAGTCCAGATCTCCTGGTTTGTA
GATGGGAAACAGATGCAGACCGCAAAAACTCAACCCAGAGAGGAGCAGTTCAACGGAACATACCGAGTGGTATCCG
TCCTTCCGATTGGCCACCAGGACTGGTTGAAAGGGAAGCAGTTTACGTGTAAAGTCAACAATAAGGCGTTGCCTAG
CCCTATTGAGCGGACGATTTCGAAAGCTAGGGGACAGGCCCACCAGCCATCGGTCTATGTCCTTCCGCCTTCCCGC
GAGGAGCTCTCGAAGAATACAGTGAGCCTTACATGCCTCATTAAGGATTTCTTCCCGCCTGATATCGACGTAGAGT
GGCAATCAAACGGTCAACAGGAGCCGGAATCCAAGTATAGAACCACTCCGCCCCAGCTTGACGAGGACGGATCATA
CTTTTTGTATTCAAAACTGTCGGTGGATAAGAGCCGGTGGCAGAGAGGTGACACCTTCATCTGTGCGGTGATGCAC
GAAGCACTCCATAATCACTACACCCAAGAGAGCCTCTCGCATTCCCCCGGAAAGTGA

Amino acid sequence **SEQ ID NO: 28 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 42 includes the signal sequence.**

MAVLGLLFCLVTFPSCVLSEVQLVQSGGDLVKPGGSVRLSCVASGYTFTSYYLYWVRQAPGKGLQWIGGVNPSNGG
TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLLTVSSASTTAPSVFPLAP
SCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPA
SKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFV
DGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSR
EELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMH
EALHNHYTQESLSHSPGK

canVH-can**IgGA**-Fc (12G8 signal sequence **underlined and in bold**): HEAVY CHAIN

Nucleotide sequence **SEQ ID NO: 25 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 39 includes the signal sequence.**

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
CCGGCGGCGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTCACCAGCTA
CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCCTGAGCGTGGACAAGGCCAAGAACACCGCCTACATGCAGC
TGAACTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCGA
CTACTGGGGACAGGGAACCCTGCTGACCGTGTCCAGCGCTTCCACAACGGCTCCGTCGGTGTTTCCCCTGGCACCT
AGCTGCGGGTCGACCTCGGGTAGCACAGTGGCGCTGGCGTGTTTGGTGTCGGGATACTTTCCCGAGCCGGTAACGG
TGTCATGGAACTCAGGGTCACTTACATCAGGAGTCCATACTTTTCCGTCCGTGCTGCAGTCAAGCGGCTTGCATTC
ACTGTCCTCGATGGTGACGGTGCCTTCGTCGAGGTGGCCCAGCGAAACGTTCACTTGTAACGTAGTACACCCGGCC

TCCAACACGAAAGTCGATAAACCGGTATTCAATGAGTGCAGATGTACAGACACCCCTCCCTGTCCGGTACCCGAAC
CCCTTGGAGGGCCGAGCGTCCTCATCTTCCCTCCCAAGCCAAAAGACATCTTGCGCATTACGAGGACACCAGAAGT
CACGTGCGTAGTGCTTGATCTCGGTAGAGAAGATCCCGAGGTCCAGATCTCGTGGTTTGTGGATGGAAAGGAGGTC
CACACCGCAAAGACTCAGTCGCGCGAGCAGCAGTTCAATGGCACGTATCGGGTCGTGAGCGTGCTTCCTATCGAGC
ATCAGGACTGGCTCACCGGGAAGGAGTTCAAATGCCGGGTCAATCATATCGACCTCCCGTCACCAATCGAGCGGAC
CATCTCGAAGGCTAGAGGAAGGGCGCACAAACCTTCGGTCTATGTGCTTCCCCCATCGCCCAAAGAGCTTTCCTCG
TCGGATACGGTGTCCATTACATGCTTGATTAAGGACTTCTATCCTCCTGATATTGATGTGGAATGGCAATCAAACG
GACAGCAGGAGCCGGAACGCAAGCACCGAATGACCCCACCGCAATTGGACGAAGATGGTAGCTACTTTCTCTACTC
AAAGCTCTCAGTCGACAAATCCCGATGGCAGCAGGGAGATCCCTTCACTTGCGCCGTGATGCACGAGACACTCCAA
AATCATTACACGGACCTTTCGTTGAGCCACTCGCCCGGAAAG

Amino acid sequence **SEQ ID NO: 26 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 40 includes the signal sequence.**

MAVLGLLFCLVTFPSCVLSEVQLVQSGGDLVKPGGSVRLSCVASGYTFTSYYLYWVRQAPGKGLQWIGGVNPSNGG
TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLLTVSSASTTAPSVFPLAP
SCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLHSLSSMVTVPSSRWPSETFTCNVVHPA
SNTKVDKPVFNECRCTDTPPCPVPEPLGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEV
HTAKTQSREQQFNGTYRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKELSS
SDTVSITCLIKDFYPPDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHETLQ
NHYTDLSLSHSPGK

canVH-can**IgGD**-Fc (12G8 signal sequence **underlined and in bold**): HEAVY CHAIN

Nucleotide sequence **SEQ ID NO: 29 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 43 includes the signal sequence.**

ATGGCCGTGCTGGGGCTGCTCTTCTGCCTGGTGACATTCCCAAGCTGTGTGCTAAGCGAGGTGCAGCTGGTGCAGT
CCGGCGGCGATCTGGTGAAGCCTGGAGGCAGCGTGAGACTGAGCTGCGTGGCCAGCGGCTACACCTTCACCAGCTA
CTACCTGTACTGGGTGAGGCAGGCTCCTGGCAAAGGACTGCAGTGGATCGGCGGCGTGAATCCTAGCAACGGCGGC
ACCAACTTCAGCGAGAAGTTCAAGAGCAGGGCCACCCTGAGCGTGGACAAGGCCAAGAACACCGCCTACATGCAGC
TGAACTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCACCAGGAGGGACAGCAACTACGACGGCGGCTTCGA
CTACTGGGGACAGGGAACCCTGCTGACCGTGTCCAGCGCTTCAACCACAGCGCCGAGCGTGTTCCCTCTGGCGCCG
TCGTGCGGTTCCACCTCGGGATCAACAGTGGCCCTCGCCTGTCTCGTGAGCGGATACTTTCCGGAGCCTGTCACGG
TGTCGTGGAATAGCGGATCACTCACGTCGGGCGTGCATACTTTTCCATCCGTCTTGCAATCGAGCGGATTGTACTC
ACTCTCCTCAACCGTCACTGTCCCCTCGTCGCGCTGGCCCTCGGAGACTTTTACGTGCAATGTAGTCCATCCGGCG
AGCAACACGAAGGTCGACAAGCCCGTACCCAAGGAATCAACATGCAAGTGCATCTCGCCCTGTCCCGTCCCCGAAT
CCCTTGGTGGCCCCTCAGTGTTTATCTTCCCTCCGAAGCCTAAAGACATCTTGAGAATCACAAGAACACCGGAAAT
CACGTGTGTGGTCCTTGACTTGGGACGCGAGGACCCTGAGGTACAAATCTCGTGGTTTGTGGACGGGAAAGAGGTG
CACACAGCAAAGACACAACCACGCGAGCAGCAGTTTAACTCAACGTACAGGGTAGTATCCGTACTTCCCATTGAAC
ACCAGGATTGGCTCACCGGTAAAGAATTCAAATGCCGAGTGAATCACATCGGGCTTCCTAGCCCAATTGAGCGGAC
GATTTCCAAAGCTAGGGGTCAGGCCCACCAGCCGAGCGTATACGTGTTGCCGCCCTCCCCGAAGGAGCTGTCATCG
TCAGATACGGTAACGTTGACGTGTCTGATCAAAGATTTCTTTCCTCCCGAAATTGATGTGGAATGGCAAAGCAATG
GGCAGCCCGAGCCCGAGTCAAAGTACCATACTACTGCACCACAGCTGGACGAAGATGGATCGTATTTCCTCTACTC
GAAACTGTCCGTGGATAAGTCCCGGTGGCAGCAAGGGGACACCTTCACTTGCGCGGTCATGCACGAGGCACTTCAG
AACCACTATACGGACTTGAGCCTCTCGCATTCGCCAGGGAAG

Amino acid sequence **SEQ ID NO: 30 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 44 includes the signal sequence.**

MAVLGLLFCLVTFPSCVLSEVQLVQSGGDLVKPGGSVRLSCVASGYTFTSYYLYWVRQAPGKGLQWIGGVNPSNGG
TNFSEKFKSRATLSVDKAKNTAYMQLNSLRAEDTAVYYCTRRDSNYDGGFDYWGQGTLLTVSSASTTAPSVFPLAP
SCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSTVTVPSSRWPSETFTCNVVHPA
SNTKVDKPVPKESTCKCISPCPVPESLGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFVDGKEV
HTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKELSS
SDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQ
NHYTDLSLSHSPGK

canVL-can**Kappa** (1022)xHGF signal sequence **underlined and in bold**: LIGHT CHAIN

Nucleotide sequence **SEQ ID NO: 33 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 47 includes the signal sequence.**

ATGGATATGAGAGTACCTGCACAACTTCTGGGATTGCTGCTTCTTTGGCTGAGAGGGGCCCGCTGCGATATCGTCC
TGACCCAGACCCCTCCTAGCCTGTCCGTGAGCCCTGGAGAACCCGCCAGCATCAGCTGCAGGGCCTCCAAGAGCGT
GAGCACCAGCGGCTTCAGCTACCTGCACTGGTACAGGCAGAAGCCCGGACAGCCTCCTCAGCTGCTGATCTTCCTG
GCCAGCAACCTGGAGAGCGGCGTGCCTGACAGGTTTAGCGGAAGCGGCAGCGGCACCGACTTCACACTGAGGATCT
CCAGGGTGGAAGCCGACGACGCCGGAGTGTACTACTGCCAGCACAGCTGGGAACTGCCCCTGACCTTCGGCCAGGG
CACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCAT
ACGGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACG
GGGTAATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTC
AACCTTGACGATGTCAAGCACCGAGTATCTTAGCCATGAGCTGTATTCGTGCGAGATCACCCACAAGTCCCTCCCC
TCCACTCTTATCAAATCCTTTCAGCGGTCGGAATGTCAGCGGGTCGAT

Amino acid sequence **SEQ ID NO: 34 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 48 includes the signal sequence.**

MDMRVPAQLLGLLLLWLRGARCDIVLTQTPPSLSVSPGEPASISCRASKSVSTSGFSYLHWYRQKPGQPPQLLIFL
ASNLESGVPDRFSGSGSGTDFTLRISRVEADDAGVYYCQHSWELPLTFGQGTKVEIKRNDAQPAVYLFQPSPDQLH
TGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLP
STLIKSFQRSECQRVD

canVL-can**Kappa** (1011)(xHGF signal sequence **underlined and in bold**:LIGHT CHAIN

Nucleotide sequence **SEQ ID NO: 31 is without the signal sequence; and**

Nucleotide sequence **SEQ ID NO: 45 includes the signal sequence.**

ATGGATATGAGAGTACCTGCACAACTTCTGGGATTGCTGCTTCTTTGGCTGAGAGGGGCCCGCTGCGATATCGTCC
TGACCCAGACCCCTCTGAGCCTGTCCGTGAGCCCTGGAGAACCCGCCAGCATCAGCTGCAGGGCCTCCAAGAGCGT
GAGCACCAGCGGCTTCAGCTACCTGCACTGGTACAGGCAGAAGCCCGGACAGAGCCCTCAGCTGCTGATCTTCCTG
GCCAGCAACCTGGAGAGCGGCGTGCCTGACAGGTTTAGCGGAAGCGGCAGCGGCACCGACTTCACACTGAGGATCT
CCAGGGTGGAAGCCGACGACGCCGGAGTGTACTACTGCCAGCACAGCTGGGAACTGCCCCTGACCTTCGGCCAGGG
CACCAAGGTGGAGATCAAGAGGAACGACGCTCAGCCAGCCGTGTACCTCTTCCAGCCTTCGCCGGACCAGCTTCAT
ACGGGGTCAGCGTCGGTGGTGTGCCTGTTGAACTCGTTTTACCCCAAGGACATTAACGTGAAGTGGAAGGTAGACG
GGGTAATTCAAGACACTGGCATTCAAGAGTCCGTCACGGAACAAGACTCAAAAGACTCAACGTATTCACTGTCGTC
AACCTTGACGATGTCAAGCACCGAGTATCTTAGCCATGAGCTGTATTCGTGCGAGATCACCCACAAGTCCCTCCCC
TCCACTCTTATCAAATCCTTTCAGCGGTCGGAATGTCAGCGGGTCGAT

Amino acid sequence **SEQ ID NO: 32 is without the signal sequence; and**

Amino acid sequence **SEQ ID NO: 46 includes the signal sequence.**

MDMRVPAQLLGLLLLWLRGARCDIVLTQTPLSLSVSPGEPASISCRASKSVSTSGFSYLHWYRQKPGQSPQLLIFL
ASNLESGVPDRFSGSGSGTDFTLRISRVEADDAGVYYCQHSWELPLTFGQGTKVEIKRNDAQPAVYLFQPSPDQLH
TGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLP
STLIKSFQRSECQRVD

EXAMPLE 4

MUTANT CANINE IgG-B ANTIBODIES SPECIFIC TO PD-1

[0140] There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa.* However, besides binding and activating of canine immune cells, a canine or caninized antibody against PD-1 optimally has two attributes:

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

[0141] None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has a high level of ADCC activity. IgG-C also has considerable ADCC activity. On the other hand, IgG-A binds weakly to protein A, but displays undesirable ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D display no ADCC activity. The present invention overcomes this

difficulty by providing mutant canine IgG-B antibodies specific to PD-1; such antibodies lack effector functions such as ADCC and can be easily of purified using industry standard protein A chromatography. The exact modifications are shown in Figure 4.

[0142] The IgG-B variants with reduced effector functions described encompass a first IgG-B variant in which a lysine (D 277) and an asparagine (N 325) residue is each mutated to an alanine residue [cIgGB(-) ADCC], a second variant in which the hinge region of IgG-B is replaced by the hinge region of IgG-D [cIgGB(+) D-hinge], and a third variant in which the hinge region of IgG-B is replaced with the hinge region of IgG-A [cIgGB(+) A-hinge]. Additionally, the second and third variants also include replacement of the same lysine and asparagine residues of the first variant with an alanine residue. The numbering of the lysine and asparagine residues mutated in this invention is based on the numbering scheme described for canine IgG heavy chains in Tang et al., [Vet Immunol and Immunopathol, 80:259-270 (2001)].

Canine IgGB wt

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVD
LDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARG
QAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO:49

Canine IgGB(+)A-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**FNECRCTDTPPCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVVDLDPED
PEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQP

SVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRG
DTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO:50

Canine IgGB(+)D-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKESTCKCISPCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVVDLDPED
PEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQP
SVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRG
DTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO: 51

Canine IgGB(-)ADCC

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR
WPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVVD
LDPEDPEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARG
QAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQESLSHSPGK SEQ ID NO: 52

SEQUENCE LISTING TABLE 1

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
1	√		Canine PD-1 Full Length	18		√	hPD-1 CDR3 of V_H
2		√	Canine PD-1 Full Length	19	√		hPD-1 CDR1 of V_L
3	√		Canine PD-1 ECD	20		√	hPD-1 CDR1 of V_L

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
4		√	Canine PD-1 ECD	21	√		hPD-1 CDR2 of V_L
5	√		Canine PD-1 - Human IgG1	22		√	hPD-1 CDR2 of V_L
6		√	Canine PD-1 - Human IgG1	23	√		hPD-1 CDR3 of V_L
7	√		Canine PD-L1 Full Length	24		√	hPD-1 CDR3 of V_L
8		√	Canine PD-L1 Full Length	25	√		IgG-A Heavy
9	√		Canine PD-L1 ECD	26		√	IgG-A Heavy
10		√	Canine PD-L1 ECD	27	√		IgG-B Heavy
11	√		Canine PD-L1 - Human IgG1	28		√	IgG-B Heavy
12		√	Canine PD-L1 - Human IgG1	29	√		IgG-D Heavy
13	√		hPD-1 CDR1 of V_H	30		√	IgG-D Heavy
14		√	hPD-1 CDR1 of V_H	31	√		*Kappa* (Light) (1011)
15	√		hPD-1 CDR2 of V_H	32		√	*Kappa* (Light) (1011)
16		√	hPD-1 CDR2 of V_H	33	√		*Kappa* (Light) (1022)
17	√		hPD-1 CDR3 of V_H	34		√	*Kappa* (Light) (1022)

SEQUENCE LISTING TABLE 2 (with the LEADER SEQUENCES)

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
35	&		Canine PD-1 Full Length	42		&	IgG-B Heavy
36		&	Canine PD-1 Full Length	43	&		IgG-D Heavy
37	&		Canine PD-L1 Full Length	44		&	IgG-D Heavy
38		&	Canine PD-L1 Full Length	45	&		*Kappa* (Light) (1011)
39	&		IgG-A Heavy	46		&	*Kappa* (Light) (1011)
40		&	IgG-A Heavy	47	&		*Kappa* (Light) (1022)
41	&		IgG-B Heavy	48		&	*Kappa* (Light) (1022)
53		&	Canine PD-1 - Human IgG1				

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
			Full Length				

SEQUENCE LISTING TABLE 3

ID	N.A.	A.A.	Description	ID	N.A.	A.A.	Description
49	&		cIgGB wt	51	&		cIgGB(+)D-hinge
50	&		cIgGB(+)A-hinge	52	&		cIgGB(-)ADCC

[0143] All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (*e.g.* Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (*e.g.* Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

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CANINISEREDE, MURINE ANTI-CANIN-PD-1-ANTISTOFFER

PATENTKRAV

1. Isoleret, caniniseret antistof, der specifikt binder programmeret celledødsreceptor-1 (PD-1), hvor antistoffet omfatter en canin-IgG-tungkæde og en canin-*kappa*-letkæde;

hvor IgG-tungkæden omfatter aminosyresekvensen ifølge SEQ ID NO: 28; og

hvor *kappa*-letkæden omfatter aminosyresekvensen ifølge SEQ ID NO: 32 eller 34; og

hvor antistoffet binder canin-PD-1 og blokerer bindingen af canin-PD-1 til canin-programmeret celledødsligand 1 (PD-L1).

2. Farmaceutisk sammensætning, der omfatter det isolerede antistof ifølge krav 1, og en farmaceutisk acceptabel bærer eller fortynder, til anvendelse i behandling af en sygdom.

3. Farmaceutisk sammensætning til anvendelse ifølge krav 2, hvor anvendelsen er beregnet:

a. til behandling af cancer;

b. behandling af en infektion eller infektionssygdom eller

c. som et vaccineadjuvans.

DRAWINGS

Figure 1. Reactivity of mouse 08A mAb against His-tagged extracellular domain of canine PD-1.

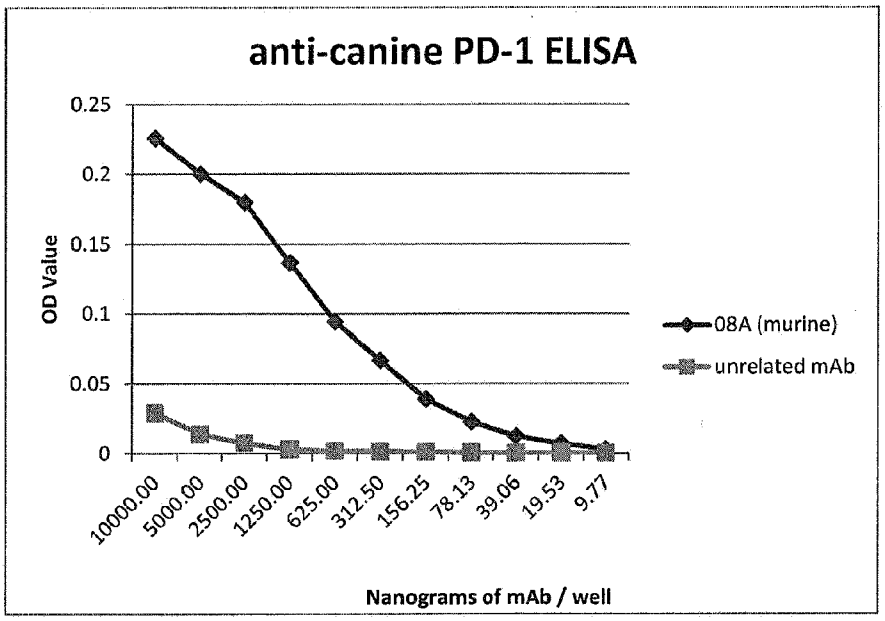


Figure 2: *Reactivity of mouse 08A mAbs against Canine PD-1 proteins expressed on CHO cells using CELISA. Murine 08A antibody and its caninized variants react with PD-1 in a dose dependent manner.*

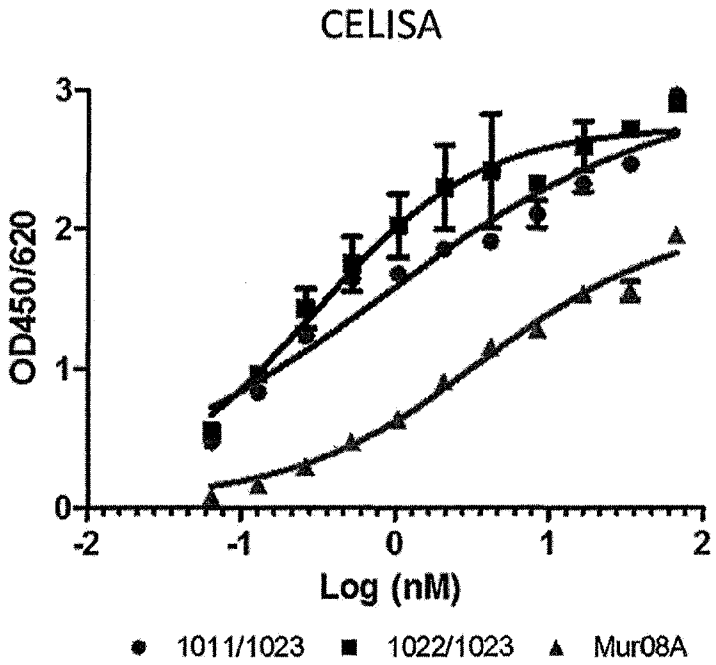


Figure 3. ligand blockade by mouse and caninized antibodies. Murine 08A and its caninized variants block binding of canine PD-1 to PD-1 expressed on CHO cell surface.

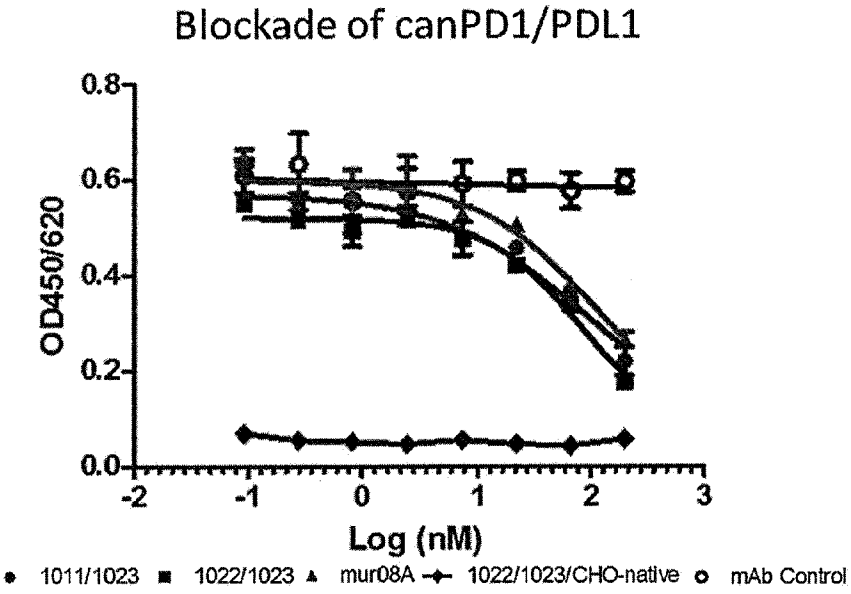
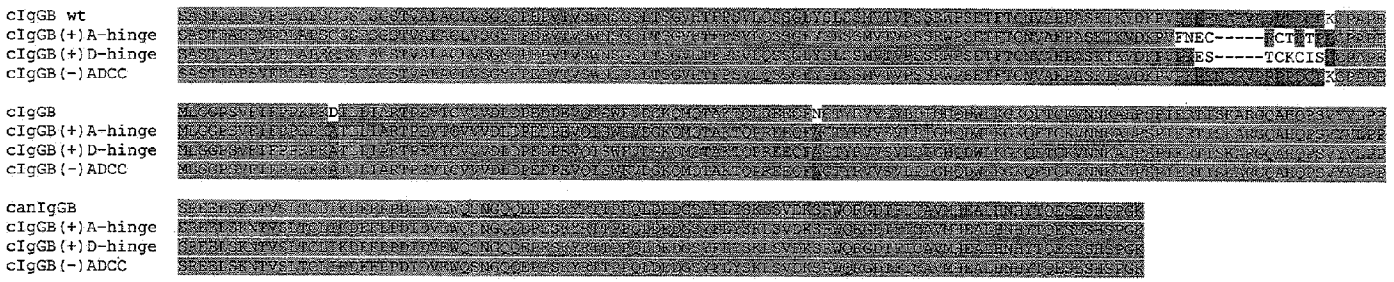


Figure 4. Alignment of canine IgGB CHs lacking ADCC function



cIgGB wt

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APEMLGGPSVFIFPPKPKDTLLIARTPEV
TCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGS
YFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cIgGB(+)A-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPV**FNECRCTDTPPCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVV
DLDPEDPEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYS
KLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cIgGB(+)D-hinge

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPV**PKESTCKCISPCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEVTCVVV
DLDPEDPEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYS
KLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cIgGB(-)ADCC

SASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPV**PKRENGRVPRPPDCPKCP**APEMLGGPSVFIFPPKPK**A**TLLIARTPEV
TCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQF**A**GTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGS
YFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

SEKVENSLISTE

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