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(54) **Title:** MONOCLONAL ANTIBODIES DIRECTED TO CD20

(57) **Abstract:** The invention provides antibody to canine or feline or equine antigens. Specifically, antibodies directed to canine CD20 which have been caninized or felinized are provided. Also provided are methods for preparing high affinity antibodies to canine and feline CD20 as well as methods for treating B cell disorders in companion animals.

MONOCLONAL ANTIBODIES DIRECTED TO CD20

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 USC §119 of U.S. Provisional Application Serial No. 61/310,440 filed March 4, 2010, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention generally relates to monoclonal antibodies, including portions or variants thereof, directed to CD20 for the treatment of diseases, e.g., in mammals and particularly in companion animals, such as dogs, cats and horses. More particularly, the invention provides antibody constructs, and antibodies encoded by the constructs, which react with CD20 and are useful for detection of targets, diagnosis of disease and treatment of companion animals. Further disclosed herein are methods for the treatment of B cell disorders in companion animals. These methods are based upon the administration of an anti-CD20 antibody or antibodies targeting the CD20 of a target animal for the modulation of B-lymphocytes.

BACKGROUND OF THE INVENTION

[0003] The use of immunoglobulins as therapeutic treatment for a variety of diseases and disorders is rapidly increasing because they have shown to be safe and efficacious therapeutic agents. Approved therapeutic monoclonal antibodies for human use include Trastuzumab (antigen: 180 kD, HER2/neu), Cetuximab (antigens: 150 kD and 170 kD, EGF receptor), Alemtuzumab (antigen: 21-28 kD, CD52), and Rituximab (antigen: 35 kD, CD20). Additional therapeutic proteins are in various phases of clinical development for use in humans for a variety of diseases with the majority targeting various forms of cancer and inflammatory-related diseases.

[0004] Whereas antibodies have been studied and developed in several mammalian species such as humans and mice, they have been significantly less studied in companion animals such as canine, feline, and equine mammals. Treatments to address veterinary immune and inflammatory conditions have been borrowed from drugs developed for humans, often with imperfect results and generally consist of drugs classified as small molecules including non-steroidal anti-inflammatory agents, analgesic agents, steroidal agents, immunosuppressive agents or anti-metabolites, and chemotherapeutic agents. The arsenal of veterinary medicine is thus limited when it comes to addressing immune conditions and cancer. Additional drawback of these treatments is that they generally only address symptoms and they are associated with serious side effects as large doses have to be administered repeatedly for a long period of time with cumulative effects that often tend to be worse than the disease itself. There is thus a need for improved and more specific treatments and biologic agents for use in animals, such as companion animals. Heterochimeric antibodies and antibodies having enhanced effector regions for use in treating companion animals are generally

described in the Applicant's own international publications: US 2010/0061988A1 and US 2010/110838A2, the contents of each are incorporated herein by reference. There is still a need for highly specific antibodies which are not immunogenic in companion animals and which are effective to treat diseases characterized by over-proliferation of CD20-positive cells in companion animals.

SUMMARY OF THE INVENTION

[0005] The invention provides therapeutic antibodies useful for veterinary application, particularly antibodies directed to canine or feline or equine CD20, for example canine CD20, together with methods of making such antibodies using optimized immunogenic constructs and methods treatment using such antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[0006] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. It must be noted that, as used herein and in the appended claims, the singular forms include plural referents; the use of "or" means "and/or" unless stated otherwise. Thus, for example, reference to "a subject polypeptide" includes a plurality of such polypeptides and reference to "the agent" includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth. Moreover, it must be understood that the invention is not limited to the particular embodiments described, as such may, of course, vary. Further, the terminology used to describe particular embodiments is not intended to be limiting, since the scope of the present invention will be limited only by its claims.

[0007] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose.

[0008] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Suitable methods and materials are described below, however methods and materials similar or equivalent to those described herein can be used in the practice of the present invention. Thus, the materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

[0009] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, tissue culture and transfection (e.g., electroporation, lipofection, etc.). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished

in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Mayer and Walker, *Immunochemical Methods In Cell And Molecular Biology*, Academic Press, London (1987); Borrebaeck, *Antibody Engineering*, 2nd ed., Oxford Univ. Press (1995); Roitt et al., *Immunology* 6th ed., Mosby (2001); All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0010] The present invention provides methods for engineering heterochimeric antibodies and/or fragments thereof suitable for administration to a subject for treatment of a disease. The terms "**patient**," "**subject**," and "**individual**," are used interchangeably herein, to refer to mammals, including, but not limited to, humans, murines, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian farm and agricultural animals, mammalian sport animals, and mammalian pets. In certain embodiments of the invention, the subject is a companion animal, such as a dog, cat or horse.

[0011] Heterochimeric antibodies engineered thereof are the result of the fusion of portion of the variable domain nucleotide sequences to constant region nucleotide sequences and the co-expression of these sequences to produce heterochimeric recombinant antibodies. Furthermore, the invention relates to the use of such heterochimeric antibodies and/or fragments thereof as immunotherapeutic agents for the treatment of disease in animals and as diagnostic agents.

[0012] Heterochimeric antibodies offer several advantages, such as (i) reduced immunogenicity response upon repeated administration; (ii) increased potency mediated by an efficient recruitment of immune system responsible for effector functions in the targeted species; and (iii) increased half-life.

[0013] The present invention includes generation of antibodies and/or fragments thereof with the desired properties and their use in production. The antibodies from the present invention include a fragment of an antibody variable region derived from a species that is different than the species that contributes the constant region of the antibody. Thus, the antibodies and/or fragments thereof retain the specificities and high affinities with the desired effector functions of the target species.

[0014] The antibodies of the present invention in particular embodiments may recognize any therapeutic target suitable for antibody therapy, for example a tumor-related antigen, an allergy- or inflammation-related antigen, a cardiovascular disease-related antigen, an autoimmune disease-related antigen or a viral or bacterial infection-related antigen.

[0015] "**Native antibodies**" as used herein are usually glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the

heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (variable region) (V_H) followed by a number of constant domains (constant regions). Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain.

[0016] The "**light chains**" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda.

[0017] Depending on the amino acid sequence of the "**constant domain**" or "**constant region**" of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" or "isotypes", e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains corresponding to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

[0018] The term "**variable domain**" refers to certain portions of the immunoglobulin molecule that differ in sequence among antibodies and are required for antigen binding, thereby imparting specificity to each antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called "**hypervariable regions**" both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the "**framework region**" (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4). The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity and complement activation.

[0019] Papain digestion of antibodies produces two identical antigen-binding fragments, called "**Fab**" fragments, each with a single antigen-binding site, and a residual "**Fc**" fragment, whose name reflects its ability to readily crystallize. Pepsin treatment yields a binding cross-linking antigen.

[0020] "**Fv**" as used herein, refers to the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy chain and one light chain variable domain.

[0021] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other configurations of antibody fragments will also be well-known to the skilled artisan.

[0022] The term "**antibody**" is used herein in the broadest sense and specifically includes monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments exhibiting the desired biological or functional activity. The desired biological or functional activity will include at least binding to a cognate antigen and may further include complement activation and/or other effector functions. By "**full length antibody**" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions.

[0023] "**Antibody fragments**" or "**antigen-binding moiety**" comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments that bind 2 or more different antigens.

[0024] The term "**immunoconjugates**" refers to antibodies or fragment thereof conjugated to another molecule, particularly a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

[0025] As used herein the term "**valency**" refers to the number of potential target binding sites in a polypeptide. Each target binding site specifically binds one target molecule or specific site on a target molecule. When a polypeptide comprises more than one target binding site, each target binding site may specifically bind the same or different molecules (e.g., may bind to different molecules, e.g., different antigens, or different epitopes on the same molecule).

[0026] The term "**specificity**" refers to the ability to specifically bind (e.g., immunoreact with) a given target. A polypeptide may be monospecific and contain one or more binding sites which specifically bind a target or a polypeptide may be multispecific (e.g., bispecific or trispecific) and contain two or more binding sites which specifically bind the same or different targets.

[0027] An antibody of this invention which "**binds**" or which "**recognizes**" an antigen or epitope of interest is one that binds the antigen or epitope with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the antigen. With regard to the binding of

an antibody, in whole or part, to a target molecule, the term “**specific binding**” or “**specifically binds to**” or is “**specific to**” or is “**specifically immunoreactive to**” or “**specifically recognizes**” a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. It includes reference to the preferential association of an antibody, in whole or part, with a cell or tissue bearing the CD20 target molecule and not to cells or tissues lacking that target molecule. Specific binding typically results in greater than two-fold, preferably greater than five-fold, more preferably greater than ten-fold and most preferably greater than one hundred-fold increase in amount of bound ligand to the isolated polypeptide or cell or tissue bearing CD20 as compared to a cell or tissue lacking CD20 or to a non-specific polypeptide. It is further contemplated that specific binding may be ten-fold, twenty-fold, thirty-fold, forty-fold, fifty-fold, sixty-fold, seventy-fold, eighty-fold, or ninety-fold increase in amount of bound ligand to the isolated polypeptide or cell or tissue bearing CD20 as compared to a cell or tissue lacking CD20 or to a non-specific polypeptide. A variety of immunoassay formats are appropriate for selecting antibodies specifically immunoreactive with a particular protein. For example, ELISA immunoassays, FACS assays, Western Blots are routinely used to select monoclonal antibodies specifically immunoreactive with a protein.

[0028] An antibody binds “**the same epitope**” as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0029] The term “**monoclonal antibody**” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., Nature 256:495 (1975), or may be made by recombinant DNA methods. The monoclonal antibodies may also be isolated e.g. from phage antibody libraries.

[0030] Monoclonal antibodies are most frequently generated in mice by administration of an “**antigen**” and subsequent isolation of B-cells that make antibodies. The B-cells are then immortalized by fusion to another, stable cell type of the same species as the B cell to create a “**hybridoma**”. An individual B-cell makes one specific antibody (i.e. is clonally monospecific), which is defined by its primary amino acid sequence and its underlying gene sequence. As used herein, the terms “**heterohybridoma**” and “**heteromyeloma**” refer to lymphocyte cell lines immortalized by fusion of lymphocytes and myelomas from two different species.

[0031] Monoclonal antibodies can be initially generated, for example, by immunizing animals with an antigen or with cells that express the antigen. The generation of a hybridoma starts with the immunization of mice or companion animals such as dogs. Immunization can be performed with several types of cells in the presence or absence of adjuvants. Cells can also be used to identify the hybridoma cell lines with the desired properties by ELISA, Biacore, FACS or other methodologies available to those in the art.

[0032] Cells suitable for use in the methods of monoclonal antibody preparation according to the present invention include: (1) Peripheral Blood Mononuclear Cells (PBMC) or fractions of PBMC enriched in certain type of cells collected from healthy or diseased companion animals such as dogs, cats, or horses. Lymphocytes are pre-incubated in some instances with factors including factors including growth factors such as EPO, SCF, TNF α , TGF β , GMCSF, TPO, IL-1, IL-2, IL-3, IL-4, GCSF to increase the expression of the antigen prior to immunization. (2) Lymphoma cell lines or tumor cell lines established from healthy or diseased subjects optionally pre-incubated with factors listed above to increase the expression of the antigen prior to immunization. (3) Cell lines derived from tissues of healthy or diseased subjects pre-incubated in some instances with factors listed above to increase the expression of the antigen prior to immunization. (4) Cultured cells engineered to express an antigen coding region or fragment thereof, such as baculovirus-infected cells, bacterial cells, yeast cells, mammalian cells, plant cells, fungal cells and the like. The antigen in the form of DNA, RNA, protein, or peptide, can be included in any one of the fractions of the cell. (5) Magnetic Proteoliposome Particles (MPLs), which are prepared from cells expressing the antigen, such that the native conformation of the transmembrane receptor is maintained, have been described previously (see *e.g.*, Mirzabekov et al. *Nat. Biotechnol.* 18:649-654 (2000); Babcock et al. *J. Biol. Chem.* 276:38433-38440 (2001); PCT Publication WO 01/49265; U.S. Patent Application No. 20010034432).

[0033] In certain embodiments of the invention, the generation of monoclonal antibodies can be achieved using immunogens derived from DNA, peptides, or proteins. Hybridomas are generated by immunizing an animal, which can be for example, a mouse or a companion animal, or any animal that will give a suitable antibody response. In one aspect, immunization is performed by introducing into the animal an antigen-encoding nucleic acid, or a protein antigen, such as canine CD20 or an immunogenic fragment thereof, or a nucleic acid encoding CD20 or an immunogenic fragment thereof. The skilled artisan will appreciate that certain epitopes will be more immunogenic in an animal when removed from their native environment. Thus, a peptide corresponding to an epitope of an antigen conjugated to a carrier such as keyhole limpet hemocyanin, may elicit a stronger antibody response than either the peptide alone or the epitope when part of the native protein on which it is found. Such variations and other immunization schemes are known to the skilled artisan are included in the immunization methods of the invention.

[0034] The immunogen can be a plasmid carrying a nucleic acid sequence encoding an antigen or a fragment thereof. In other embodiments of the invention, monoclonal antibodies of the invention can be obtained by screening a library of antibody molecules or fragments thereof derived from immunization of animals. Monoclonal antibodies of the invention can also be obtained from libraries of antibodies or antibody-encoding nucleic acids.

[0035] As used herein the term "**antigen**" is understood to be any substance capable of stimulating antibody production. Also, the term "**immunogen**" is understood to include any substance used to induce an immune response.

[0036] The monoclonal antibodies herein may in some embodiments include "**chimeric**" antibodies in which a portion of the heavy and/or light chain is identical to or homologous with corresponding sequences from antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical to or homologous with corresponding sequences in antibodies from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, exhibiting the desired biological activity (See e.g., U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

[0037] "**Single-chain Fv**" or "**sFv**" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0038] The term "**diabodies**" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain ($V_H - V_L$). By using a linker that is short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

[0039] In certain aspects the present invention provides methods for adapting antibodies to the species of an intended therapeutic target. Generally, these methods include "**mammalization**" which is defined as a method for transferring donor antigen-binding information to a less immunogenic mammal antibody acceptor to generate useful therapeutic treatments. More specifically, the invention provides methods for felinezation, equinization and caninization of antibodies.

[0040] "**Caninization**" is defined as a method for transferring non-canine antigen-binding information from a donor antibody to a less immunogenic canine antibody acceptor to generate treatments useful as therapeutics in dogs.

[0041] "**Felination**" is defined as a method for transferring non-feline antigen-binding information from a donor antibody to a less immunogenic feline antibody acceptor to generate treatments useful as therapeutics in cats.

[0042] "**Equinization**" is defined as a method for transferring non-equine antigen-binding information from a donor antibody to a less immunogenic equine antibody acceptor to generate treatments useful as therapeutics in horses.

[0043] Caninized forms of non-canine antibodies provided herein are chimeric antibodies that contain minimal sequence derived from non-canine antibodies. For the most part, caninized antibodies are canine antibody sequences ("**acceptor**" or "**recipient**" antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-canine species ("**donor**" antibody) such as mouse, rat, rabbit, cat, dogs, goat, chicken, bovine, horse, llama, camel, dromedaries, sharks, non-human primates, human, humanized, recombinant sequence, or an engineered sequence having the desired properties. In some instances, framework region (FR) residues of the canine antibody are replaced by corresponding non-canine FR residues. Furthermore, caninized antibodies may include residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. The caninized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc) of a canine antibody.

[0044] As used herein, "**identity**" refers to the sequence matching between two polypeptides, molecules or between two nucleic acids. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit (for instance, if a position in each of the two DNA molecules is occupied by adenine, or a position in each of two polypeptides is occupied by a lysine), then the respective molecules are identical at that position. The "**percentage identity**" between two sequences is a function of the number of matching positions shared by the two sequences divided by the number of positions compared.times.100. Such alignment can be provided using, for instance, the program Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information NCBI.

[0045] In one embodiment, the recombinant polypeptides, or fragments, derivatives, or modifications thereof, are specifically administered into a patient. In another embodiment, the recombinant polypeptide of the invention, or fragments, derivatives, or modifications thereof, are introduced into cells and/or a tissue while under in vitro or ex vivo conditions, prior to the transplantation of the cells and/or a tissue into a mammalian organism for the purpose of treating, preventing, reducing or otherwise lowering disease conditions or symptoms associated or mediated by the disease.

[0046] The terms "**fragment**" and "**region**" refer to portions of a polypeptide or nucleic acid molecule that contains at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the reference nucleic acid molecule or polypeptide.

[0047] The terms "**polynucleotide**," "**nucleic acid**," and "**nucleic acid molecule**," are used interchangeably herein to refer to polymeric forms of nucleotides of any length. The polynucleotides can contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Polynucleotides can have any three-dimensional structure, and can perform any function, known or unknown. The term polynucleotide includes single-stranded, double-stranded, and triple helical molecules, and encompasses nucleic acids containing nucleotide analogs or modified backbone residues or linkages, which can be synthetic, naturally occurring, or non-naturally occurring, and which have similar binding properties as the reference nucleic acid.

[0048] "**Oligonucleotide**" refers generally to polynucleotides that are between 5 and about 100 nucleotides of single- or double-stranded DNA. Oligonucleotides may also refer to polynucleotides that are about 10, 20, 30, 40, 50, 60, 70, 80, or 90 nucleotides of single- or double-stranded DNA. For the purposes of this disclosure, the lower limit of the size of an oligonucleotide is two, and there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as "oligomers" or "oligos" and can be prepared by any method known in the art including isolation from naturally-occurring polynucleotides, enzymatic synthesis and chemical synthesis.

[0049] The terms "**polypeptide**," "**peptide**" and "**protein**" are used interchangeably herein to refer to a polymer of amino acid residues of any length. Polypeptides can have any three-dimensional structure, and can perform any function, known or unknown. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0050] The term "**amino acid**" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ carboxyglutamate, and O-phosphoserine. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0051] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0052] The terms "**conservatively modified variants**" or "**conservative variants**" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or substantially identical amino acid sequences; or for nucleic acids that do not encode an amino acid sequence, to nucleic acids that are substantially identical. As used herein, "substantially identical" means that two amino acid or polynucleotide sequences differ at no more than 10% of the amino acid or nucleotide positions, typically at no more than 5%, often at more than 2%, and most frequently at no more than 1% of the of the amino acid or nucleotide positions.

[0053] Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the alternate alanine codons without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one type of conservatively modified variants. Nucleic acid sequences encoding polypeptides described herein also encompass every possible silent variation of the nucleic acid. The skilled artisan will recognize that each amino acid codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be varied at one or more positions to code for the same amino acid. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence with respect to the expression product.

[0054] "**Complementarity**" as applied to nucleic acids, refers to the ability of the nucleic acid to form hydrogen bond(s) with another polynucleotide sequence by either traditional Watson-Crick or other non-traditional types of base pairing. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., enzymatic nucleic acid cleavage, RNA interference, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art. "**Percent complementarity**" refers to the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with another nucleic acid molecule. "**Perfectly complementary**" or "**100% complementarity**" means that all the contiguous nucleotides of a nucleic acid molecule will hydrogen bond with the same number of contiguous residues in a second nucleic acid molecule. "Substantial complementarity" and "substantially complementary" as used herein indicate that two nucleic acids are at least 90% complementary, typically at least 95% complementary, often at least 98% complementary, and most frequently at least 99% complementary over a region of more than about 15 nucleotides and more often more than about 19 nucleotides.

[0055] "**Homology**" is an indication that two nucleotide sequences represent the same gene or a gene product thereof, and typically means that the nucleotide sequence of two or more nucleic acid molecules are partially, substantially or completely identical. When from the same organism, homologous polynucleotides are representative of the same gene having the same chromosomal location, even though there may be individual differences between the polynucleotide sequences (such as polymorphic variants, alleles and the like). In certain embodiments, a homolog can be found in a non-native position in the genome, e.g. as the result of translocation.

[0056] The term "**heterologous**" refers to any two or more nucleic acid or polypeptide sequences that are not normally found in the same relationship to each other in nature. For instance, a heterologous nucleic acid is typically recombinantly produced, having two or more sequences, e.g., from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous polypeptide will often refer to two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0057] The term "**homolog**" refers to a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 55%, 57%, 60%, 65%, 68%, 70%, more preferably 80% or 85%, and most preferably 90%, 95%, 98%, or 99% identical at the amino acid level or nucleic acid to a reference sequence.

[0058] "**Similar**" sequences are those which, when aligned, share identical and similar amino acid residues, where similar residues are conservative substitutions for corresponding amino acid residues in an aligned reference sequence. In this regard, conservative residues in a sequence is a residue that is physically or functionally similar to the corresponding reference residue, e.g., that has a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the like. The "percentage similarity" between two sequences is a function of the number of positions that contain matching residues or conservative residues shared by the two sequences divided by the number of positions compared.times.100.

[0059] "**Amino acid consensus sequence**" as used herein refers to a hypothetical amino acid sequence that can be generated using a matrix of at least two, and preferably more, aligned amino acid sequences, and allowing for gaps in the alignment, such that it is possible to determine the most frequent amino acid residue at each position. The consensus sequence is that sequence which comprises the amino acids which are most frequently represented at each position. In the event that two or more amino acids are equally represented at a single position, the consensus sequence includes both or all of those amino acids. In some cases, amino acid consensus sequences correspond to a

sequence or sub-sequence found in nature. In other cases, amino acid consensus sequences are not found in nature, but represent only theoretical sequences.

[0060] The amino acid sequence of a protein can be analyzed at various levels. For example, conservation or variability can be exhibited at the single residue level, multiple residue level, multiple residues with gaps etc. Residues can exhibit conservation of the identical residue or can be conserved at the class level. The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). Other classes are known to one of skill in the art and may be defined using structural determinations or other data to assess substitutability.

[0061] Regarding amino acid sequences, one of skill in the art will recognize that individual substitutions, deletions or insertions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, inserts or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables detailing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude functionally equivalent polymorphic variants, homologs, and alleles of the invention.

[0062] As used herein, when one amino acid sequence (e.g., a first VH or VL sequence) is aligned with one or more additional amino acid sequences (e.g., one or more VH or VL sequences in a database), an amino acid position in one sequence (e.g., the first VH or VL sequence) can be compared to a "corresponding position" in the one or more additional amino acid sequences. As used herein, the "**corresponding position**" represents the equivalent position in the sequence(s) being compared when the sequences are optimally aligned, i.e., when the sequences are aligned to achieve the highest percent identity or percent similarity.

[0063] As used herein, the term "antibody database" refers to a collection of two or more antibody amino acid sequences (a "**plurality**" or "**multiplicity**" of sequences), and typically refers to a collection of tens, hundreds or even thousands of antibody amino acid sequences. An antibody database can store amino acid sequences of, for example, a collection of antibody VH regions, antibody VL regions or both, or can store a collection of framework sequences. In one embodiment, the antibody database is a database comprising or consisting of germline antibody sequences. In another embodiment, the antibody database is a database comprising or consisting of mature antibody sequences (e.g., a Kabat database of mature antibody sequences). In another embodiment, the

antibody database comprises or consists of sequences selected for one or more properties. In another embodiment, the antibody database comprises or consists of consensus sequences. In another embodiment, the antibody database comprises or consists of similar sequences. In yet another embodiment, the antibody database comprises or consists of sequences from major antibody clans (Das et al., *Immunogenetics*, 60:47-55 (2008); Das et al., *Proc. Natl. Ac. Sci. USA*. 105:16647-16652 (2008)).

[0064] As used herein, the term "**property**" or "**characteristic**" is a property of a polypeptide which is desirable and/or advantageous to one of skill in the art, e.g., in order to improve the manufacturing properties or therapeutic efficacy of the polypeptide. In one embodiment, the functional property is improved stability. In another embodiment, the functional property is improved solubility. In yet another embodiment, the functional property is non-aggregation. In still another embodiment, the functional property is an improvement in expression. In certain embodiments, the functional property is an improvement in antigen binding affinity.

[0065] The expression "**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 utilize promoters, polyadenylation signals, and enhancers.

[0066] 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.

[0067] The term "codon optimization" or "codon optimized sequences" refers to nucleotide sequences that have been optimized without altering the amino acid sequence of the original translated polypeptide and includes replacing any codons having a low usage frequency in the host species, elimination of spurious polyadenylation sequences, elimination of exon/intron splicing signals, elimination of transposon-like repeats, and optimization of GC content.

[0068] As used herein, the expressions "**cell**," "**cell line**," and "**cell culture**" are used interchangeably and all such designations include progeny. Thus, the words "**transformants**",

“**transfectants**”, “**transformed cells**” and “**transfected cells**” include the primary subject cell and cultures derived from.

[0069] Immunogenic, as used herein, refers to antigens, (including native antigens, fragments, mutant, and derivatives thereof, as well as recombinant and synthetic antigens), that, when introduced into an animal, elicit an immune response, such as a humoral or antibody response.

[0070] As used herein, the term “**not immunogenic**” or “**non-immunogenic**” means that an antigen, such as an antibody, or other molecule, does not raise an antibody response of sufficient magnitude to reduce the effectiveness of continued administration of the antibody in the majority of treated patients for sufficient time to achieve therapeutic efficacy.

[0071] As used herein, the term “**therapeutic**” encompasses the full spectrum of treatments for a “**disease**” or “**disorder**” or “**condition**”. A “therapeutic” agent of the invention may act in a manner that is prophylactic or preventive, including those that incorporate procedures designed to target individuals that can be identified as being at risk (pharmacogenetics); or in a manner that is ameliorative or curative in nature; or may act to slow the rate or extent of the progression of a disease or disorder; or may act to minimize the time required, the occurrence or extent of any discomfort or pain, or physical limitations associated with recuperation from a disease, disorder or physical trauma; or may be used as an adjuvant to other therapies and treatments.

[0072] “**Treatment**,” as used herein, covers any administration or application of remedies for disease in an animal, including a human, and includes inhibiting the disease, i.e., arresting its development; relieving the disease, i.e., causing its regression; and eliminating the disease, i.e., causing the removal of diseased cells or restoration of a non-diseased state. Treatment refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

[0073] A “**pharmaceutical composition**” or “**pharmaceutically acceptable composition**” of antibodies, polypeptides, or polynucleotides herein refers to a composition that usually contains a pharmaceutically acceptable carrier or excipient that is conventional in the art and which is suitable for administration into a subject for therapeutic, diagnostic, or prophylactic purposes. For example, compositions for oral administration can form solutions, suspensions, tablets, pills, capsules, sustained release formulations, oral rinses, or powders.

[0074] The term “**combination therapy**” refers to a therapeutic regimen that involves the provision of at least two distinct therapies to achieve an indicated therapeutic effect. For example, a combination therapy may involve the administration of two or more chemically distinct active ingredients, for example, a chemotherapeutic agent and an antibody. Alternatively, a combination therapy may involve the administration of an antibody and/or one or more chemotherapeutic agents, alone or together with the delivery of another treatment, such as radiation therapy and/or surgery. In

the context of the administration of two or more chemically distinct active ingredients, it is understood that the active ingredients may be administered as part of the same composition or as different compositions. When administered as separate compositions, the compositions comprising the different active ingredients may be administered at the same or different times, by the same or different routes, using the same or different dosing regimens, all as the particular context requires and as determined by the attending veterinarian or attending caregiver.

[0075] The term “**monotherapy**” refers to a treatment regimen based on the delivery of one therapeutically effective compound, whether administered as a single dose or several doses over time.

[0076] “**Immune conditions**” are a generic name for a wide range of diseases including arthritis, psoriasis, inflammatory bowel disease, multiple sclerosis, myocardial infarction, stroke, hemolytic anemia, atopic dermatitis, skin disorders, and the like, in which the immune system or a part thereof, such as a cell of the immune system, is abnormal or causes a disease state. Immune conditions include primary defects in an immune cell, tissue or organ, as well as “**autoimmune conditions**,” in which the normal mechanisms for preventing immune recognition of self antigens is defective, resulting in a disease or disorder involving a non-immune cell, tissue or organ type. Cancer such as leukemias and lymphomas are primary immune disorders, while multiple sclerosis and lupus are believed to be of autoimmune origin.

[0077] A multitude of therapeutic agents have been developed over the past few decades for the treatment of various types of immune conditions for humans and these have also been used for the treatment of immune conditions in companion animals. The most commonly used types of anti-immune agents include: immunosuppressant agents (e.g., cyclosporine, thiopurine, prednisone), and analgesic and antipyretic (e.g., aspirin, ibuprofen, naproxen, celecoxib, nimesulide, licoferone, omega-3-fatty acids), each of which may be administered simultaneously, sequentially or in a common dosage regimen with antibodies of the invention.

[0078] “**Cancer**” as used herein, refers to any abnormal cell or tissue growth, e.g., a tumor, which can be malignant or non-malignant. Cancer is characterized by uncontrolled proliferation of cells that may or may not invade the surrounding tissue and, hence, may or may not metastasize to new body sites. Cancer encompasses carcinomas, which are cancers of epithelial cells (e.g. squamous cell carcinoma, adenocarcinoma, melanomas, and hepatomas). Cancer also encompasses sarcomas, which are tumors of mesenchymal origin, (e.g. osteogenic sarcomas, leukemias, and lymphomas). Cancers can involve one or more neoplastic cell type. Cancer a generic name for a wide range of cellular malignancies characterized by unregulated growth, lack of differentiation, and the ability to invade local tissues and metastasize. These neoplastic malignancies affect, with various degrees of prevalence, every tissue and organ in the body. A multitude of therapeutic agents have been developed over the past few decades for the treatment of various types of cancer for humans and have

been used off-label or reformulated for the treatment of cancer in companion animals. The most commonly used types of anti-cancer agents include: DNA-alkylating agents (e.g., cyclophosphamide, ifosfamide), anti-metabolites (e.g., methotrexate, a folate antagonist, and 5-fluorouracil, a pyrimidine antagonist), microtubule disruptors (e.g., vincristine, vinblastine, paclitaxel), DNA intercalators (e.g., doxorubicin, daunomycin, cisplatin), and immunosuppressant (e.g., prednisone), each of which may be administered simultaneously, sequentially or in a common dosage regimen with antibodies of the invention (see, for e.g., Withrow & MacEwen's, *Small Animal Clinical Oncology*, Saunders Elsevier, 4th ed. (2007)).

[0079] Antibodies (mAbs) that can be subjected to the techniques set forth herein include monoclonal and polyclonal mAbs, and antibody fragments such as Fab, Fab', F(ab')₂, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments derived from various sources. An antibody is obtained from a sequence donor species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of the donor species antibody has specificity for a desired antigen. The donor species is any species which was used to generate the antibodies or antibody libraries, e.g., mouse, rat, rabbit, cat, dogs, goat, chicken, bovine, horse, llama, camel, dromedaries, sharks, non-human primates, human, humanized, recombinant sequence, engineered sequence, etc. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art.

[0080] After sequencing the antibody obtained from the donor species or from a library, the variable regions (VH and VL) are separated into discrete regions such as leader sequences, frameworks (FRs) and CDRs using any published definition of CDRs and frameworks (e.g., Kabat, Chothia, AbM, contact definition and any combination thereof, and any others known to those skilled in the art). In a particular embodiment, FRs and CDRs are identified with reference to the Kabat definitions.

[0081] Whenever it appears herein, a numerical range such as "1 to 100" refers to each integer in the given range; e.g., "1 to 100 nucleotides" means that the nucleic acid can contain only 1 nucleotide, 2 nucleotides, 3 nucleotides, etc., up to and including 100 nucleotides.

[0082] With respect to the constant domains of heavy chains, a constant domain or fragment thereof of any subclass from the target species may be fused to the heavy chain heterochimeric variable domains.

[0083] The engineering of the recombinant antibody of the claimed invention can be created by introducing modifications, additions or deletions into a nucleic acid encoding the antibody by any method known in the art including recombination, error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, site-specific mutagenesis, gene reassembly, synthetic ligation reassembly or a combination thereof.

[0084] Further envisioned within the scope of this invention is the usage of the recombinant nucleic acids or proteins described herein, or fragments or derivatives thereof, for the treatment of all companion animal diseases and/or conditions that are mediated or associated with the onset of inflammation, as well as companion animal diseases and/or conditions that are mediated or associated with autoimmunity. Such diseases and/or conditions are referred to herein as inflammatory disorders and include but are not restricted to inflammation, autoimmune disease and immune-mediated.

[0085] In a further aspect, the invention features pharmaceutical compositions in which antibodies of the present invention are provided for therapeutic or prophylactic uses. The invention features a method for treating a dog subject having a particular antigen, e.g., one associated with disease. The method includes administering a therapeutically effective amount of a recombinant antibody specific for the particular antigen, with the recombinant antibody described herein.

[0086] The amount of antibody useful to produce a therapeutic effect can be determined by standard techniques well known to those of ordinary skill in the art. The antibodies will generally be provided by standard technique within a pharmaceutically acceptable buffer, and may be administered by any desired route. The route of administration of the antibody or antigen-binding moiety of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration.

[0087] Antibodies produced in the manner described above, or by equivalent techniques, can be purified by a combination of affinity and size exclusion chromatography for characterization in functional biological assays. These assays include determination of specificity and binding affinity as well as effector function associated with the expressed isotype, e.g., ADCC, apoptosis, or complement fixation. Such antibodies may be used as passive or active therapeutic agents against a number of diseases, including B cell lymphoma, T cell lymphoma, autoimmune diseases, inflammatory diseases, infectious diseases, and transplantation.

[0088] “**Antibody-dependent cell-mediated cytotoxicity**” and “**ADCC**” refer to a cell-mediated reaction in which nonspecific cytotoxic cells such as Natural Killer (NK) cells, neutrophils, and macrophages recognize bound antibody on a target cell and subsequently cause lysis of the target cell (see, for e.g., Janeway et al., *Immuno Biology*: Elsevier Science Ltd., 4th ed., (1999)).

[0089] “**Complement dependent cytotoxicity**” and “**CDC**” refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen.

[0090] An “**enhanced**” or “**reduced**” ADCC or CDC activity, as used herein, generally refers to a heavy chain that confers more activity or less activity than a reference heavy chain. As would be

understood in the art, amount of an activity may be determined quantitatively or qualitatively in parallel or in separate runs according to any assay or technique known in the art.

[0091] In certain embodiments of the above aspects, the antigen is a tumor antigen, an antigen involved in an immune disorder, an antigen involved in an autoimmune response, a receptor expressed on a host cell or available in blood circulation or secreted by a cell and the recombinant antibody is able to either deplete undesired cells or to block or stimulates receptor functions, or neutralizes active soluble products.

[0092] The antibodies (or fragments thereof) of this invention may also be useful for treating tumors in companion animals. More specifically, they should be useful for reducing tumor size, inhibiting tumor growth and/or prolonging the survival time of tumor-bearing animals. Accordingly, this invention also relates to a method of treating tumors in a dog or other animals by administering an effective dose. An effective dose is expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day. It is further contemplated that an effective dose may also be from about: 0.05, 0.10, 0.50, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0 and 100 milligrams per kilogram body weight per day.

[0093] In a particular embodiment, the invention provides antibodies to CD20. The canine CD20 is a non-glycosylated integral membrane phosphoprotein expressed on the surface of almost all normal and malignant B cells. It has four membrane spanning hydrophobic regions and a short extracellular loop between the third and fourth transmembrane domain.

[0094] The CD20 protein is predicted to contain domains of amino acid sequences consisting of two extracellular domains, four transmembrane domains, and three intracellular domains as human CD20.

[0095] The amino acid sequence of canine CD20 shows sequence similarities with those of human and mice. The amino acid sequences of canine CD20 exhibit a high degree of similarity with the human gene, suggesting a similar biological function. Despite the sequence homology between the canine and human CD20 sequence, Rituximab, a monoclonal antibody to the human CD20 antigen does not react with canine B cells probably due to the lack of homology between humans and dogs in the epitope of the extracellular domain of CD20 recognized by Rituximab (Veterinary Journal, 2006, vol 171, 556).

There are several reported versions of canine CD20. In one embodiment, the canine CD20 is of SEQ ID NO: 1:

MTTPRNSMSGTLPVDPMKSPTAMYYPVQKIIPKRMPSVVGPTQNFFMRESKTLGAVQIMNGLF
 HIALGSLMIHTDVYAPICITMWYPLWGGIMFIISGSLLAADKNPRKSLVKGKMIMNSLSLF
 AAISGIIFLIMDIFNITISHFFKMENLNLIKAPMPYVDIHNCDPANPSEKNSLSIQYCGSIRSVFLG
 VFAVMVIFTFFQKLVTAGIVENEWKKLCSKPKSDVVVLLAAEEKKEQPIETTEEMVELTEIAS
 QPKKEEDIEIIPVQEEEELEINFAEPPQEQUSSPIENDSIP

[0096] Canine antibody against the CD20 antigen expressed by normal and malignant B lymphocytes. The antibody is produced in mammalian cells (CHO or Per.C6) and meets manufacturing and purification specifications. The product is a sterile, clear, colorless, preservative free liquid concentrate for parenteral administration.

[0097] The invention thus provides: heterochimeric antibodies and/or fragments thereof that include (i) hypervariable region sequences wholly or substantially identical to sequences found in antibodies from a donor species; (ii) constant region sequences wholly or substantially identical to sequences found in antibodies from a target species which is different from the donor species; and (iii) heavy and/or light chain variable framework sequences which contain at least three contiguous non-CDR residues corresponding to sequences found in antibodies from a target species and at least three contiguous non-CDR residues corresponding to sequences found in antibodies from a donor species.

[0098] In certain embodiments antibodies of the present invention target antigens associate with a particular disease or disorder, such as acute inflammation, rheumatoid arthritis, transplant rejection, asthma, allergic inflammation, restenosis, arterial restenosis, inflammatory bowel disease, uveitis, multiple sclerosis, psoriasis, wound healing, lupus erythematosus, allergic rhinitis, atopic dermatitis, food allergies, diabetes mellitus, dermatitis, thrombotic thrombocytopenic purpura, encephalitis, leukocyte adhesion deficiency, rheumatic fever, psoriatic arthritis, osteoarthritis, ocular inflammatory disorders, progressive systemic sclerosis, primary biliary cirrhosis, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, ischemic heart disease, atherosclerosis, post-dialysis syndrome, leukemia, acquired immune deficiency syndrome, septic shock, lipid histiocytosis, and cancer.

[0099] Of particular interest is antigen CD20. The skilled artisan will appreciate that the antigen is preferably isolated or derived from the target species (e.g. canine, feline or equine), but suitable cross-reactive antibodies can in some cases be generated by using an antigen from a xenogenic species.

- 1.1. The antibody of any of the previous embodiments wherein the complementarity determining regions and framework regions are defined in accordance with Kabat.
- 1.2. The antibody of any of the previous embodiments wherein the constant region of the antibody is modified to enhance a cytotoxic effector functions selected from ADCC, antibody dependent cellular phagocytosis (ADCP), and complement dependent cytotoxicity (CDC).

[00100] In a further embodiment, the invention provides

2. Antibody 2, which is an antibody or antibody fragment that recognizes canine or feline or equine CD20,
 - 2.1. Antibody 2 wherein the antibody is to canine or feline or equine CD20.
 - 2.2. Antibody 2.1 wherein the antibody is derived from or has substantially the same hypervariable domain as an antibody raised against an immunogenic construct

- comprising or expressing a peptide containing the sequence of one or more extracellular loops of CD20.
- 2.3. Any of Antibodies 2 – 2.2 wherein the antibody induces apoptosis of cells expressing CD20.
 - 2.4. Any of Antibodies 2 – 2.3 wherein the antibody suppresses growth of cells expressing CD20.
 - 2.5. Any of Antibodies 2 – 2.4 wherein the antibody causes the death of cells expressing CD20 by antibody dependent cell-mediated cytotoxicity (ADCC).
 - 2.6. Any of Antibodies 2 – 2.5 wherein the antibody causes the death of cells expressing CD20 by complement-dependent cytotoxicity (CDC).
 - 2.7. Any of Antibodies 2 – 2.6 wherein the antibody is to feline CD20, e.g., of SEQ ID NO.:2.
 - 2.8. Any of Antibodies 2 – 2.6 wherein the antibody is to canine CD20, e.g. of SEQ ID NO.:1.
 - 2.9. Antibody 2.8 wherein the antibody is derived from or has substantially the same hypervariable domain as an antibody raised against an immunogenic construct comprising or expressing a peptide containing a sequence selected from one or more of the following sequences: SEQ ID NO.:1 and SEQ ID NO.:2.
 - 2.10. Antibody 2.8 or 2.9 wherein the antibody specifically recognizes an epitope on the extracellular loop of canine CD20, wherein the epitope comprises or is found within a region of the CD20 comprising or expressing a peptide containing a sequence selected from one or more of the sequences of residues 74-84, 178-188, 154-170, 140-146, 162-173, 148-159, 142-153, 148-169, 166-177, or 161-176 of SEQ ID NO:1.
 - 2.11. Any of Antibodies 2 – 2.6 wherein the antibody is to equine CD20.
 - 2.12. Any of Antibodies 2 – 2.11 wherein the antibody comprises hypervariable sequences from a donor species antibody and constant region sequences from a target species.
 - 2.13. Any of Antibodies 2 wherein the antibody is caninized.
 - 2.14. Any of Antibodies 2 wherein the antibody is felinized.
 - 2.15. Any of Antibodies 2 wherein the antibody is equinized.
 - 2.16. Any of Antibodies 2.23 to 2.26 wherein the antibody is a heterochimeric antibody of any of Antibodies 1 - 1.35.
 - 2.17. Any of Antibodies 2 wherein the antibody is monoclonal and is fully canine.
 - 2.18. Any of Antibodies 2 wherein the antibody is monoclonal and is fully feline.
 - 2.19. Any of Antibodies 2 wherein the antibody is monoclonal and is fully equine.
 - 2.20. Any of Antibodies 2 recognizing a canine or feline CD20, wherein the antibody comprises a sequence selected from SEQ ID NOS 17-43.

- 2.21. Any of Antibodies 2 recognizing canine or feline CD20 and comprising at least one of the CDR regions from SEQ ID NOS 17-43.
- 2.22. Any of Antibodies 2 having the binding characteristics of an antibody selected from mAb CD20-1, CD20-2, CD20-3, CD20-4, CD20-5, and CD20-6.
- 2.23. Any of Antibodies 2 according to any of claims 1-3 comprising a variable domain structure selected from AVD-1 through AVD-13.
- 2.24. Any of Antibodies 2 comprising a light chain selected from SEQ ID NOS: 20,21,22,24,25,26,28,32,33,36,37,38,39,41, and 43 and a heavy chain selected from SEQ ID NOS:17,18,19,23,27,29,30,31,34,35,38,40, and 42.
- 2.25. Any of Antibodies 2 which is a heterochimeric antibody.
- 2.26. The antibody or antibody fragment wherein the constant domain comprises a sequence selected for providing enhanced ADCC and/or CDC.
- 2.27. Any of Antibodies 2 which binds to canine CD20 and wherein the constant region is of canine origin.
- 2.28. Any of Antibodies 2 which binds to feline CD20 and wherein the constant region is of feline origin.

[00101] The invention further provides nucleic acid encoding any of Antibodies 1 or 2.

[00102] The invention further provides

- a. a method of treating a patient suffering from a disease or condition characterized by the presence of abnormal cells or abnormal levels of cells expressing a target antigen comprising administering a therapeutically effective amount of an antibody binding to such target antigen, wherein the antibody is selected from Antibody 1 or 2.
- b. a method of treating a patient suffering from a disease or condition characterized by the presence of abnormal cells or abnormal levels of cells expressing CD20 comprising administering a therapeutically effective amount of an antibody selected from Antibody 1 and 2.
- c. Method b) wherein the patient is a dog.
- d. Method c) wherein the condition to be treated is canine lymphoma.
- e. Method a) wherein the disease is selected from the group consisting of: acute inflammation, rheumatoid arthritis, transplant rejection, asthma, allergic inflammation, restenosis, arterial restenosis, inflammatory bowel disease, uveitis, multiple sclerosis, psoriasis, wound healing, lupus erythematosus, allergic rhinitis, atopic dermatitis, food allergies, diabetes mellitus, dermatitis, thrombotic thrombocytopenic purpura, encephalitis, leukocyte adhesion deficiency, rheumatic fever, psoriatic arthritis, osteoarthritis, ocular inflammatory disorders, progressive systemic sclerosis, primary biliary cirrhosis, CNS inflammatory disorder, antigen-

antibody complex mediated diseases, autoimmune hemolytic anemia, ischemic heart disease, atherosclerosis, post-dialysis syndrome, leukemia, acquired immune deficiency syndrome, septic shock, lipid histiocytosis, and cancer.

- f. Method a, b, c or d or e further comprising administration of chemotherapy.
- g. Method f wherein the chemotherapy comprises administration of one or more agents selected from cyclophosphamide, doxorubicin, vincristine, prednisone, L-asparaginase, cytoxan and adriamycin.
- h. Method f or g wherein the chemotherapy spares or enhances effector cells, e.g., so as to enhance or reduce interference with ADCC effects of antibody on cancer cells.
- i. Any of the foregoing methods further comprising administration of a corticosteroid, e.g., prednisone.
- j. Any of the foregoing methods further comprising administration of radiation.
- k. Any of the foregoing methods comprising co-administration of antibody to CD20 and CD52.

[00103] The invention further provides pharmaceutical compositions comprising any of antibodies 1 or 2, e.g., for use in any of methods a-k.

[00104] The invention further provides the use of any of antibodies 1 or 2 as pharmaceuticals, or in the manufacture of a medicament for use in any of the methods a-k.

[00105] The invention further provides a cell line stably expressing any of antibodies 1 or 2, for example a CHO cell line or PerC6 stably expressing any of antibodies 1 or 2.

[00106] The invention further provides a vector or vectors expressing at least one heavy chain and at least one light chain of any of antibodies 1 or 2.

[00107] The invention further provides a method of making an antibody comprising transforming a cell line with a vector or vectors expressing at least one heavy chain and at least one light chain of any of antibodies 1 or 2.

[00108] Antibodies to CD52, e.g. for use in Method K, are described for example in the co-pending U.S. Provisional Application Serial No. 61/310,450, and the US and PCT applications claiming priority therefrom, the contents of which are incorporated herein by reference.

[00109] In another embodiment the invention provides a method of diagnosing a disease or condition treatable with the antibodies of the invention, comprising obtaining a tissue sample and measuring binding by one of the antibodies of the invention, together with diagnostic kits for performing such a method comprising an antibody of the invention, e.g., any of antibodies 1 or 2.

[00110] Thus the invention provides the following antibodies, as well as functional fragments and conservative variants thereof:

SEQ ID NO.	Designation	Description
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SEQ ID NO. 1	CD20	Canine CD20
SEQ ID NO. 2	CD20	Feline CD20
SEQ ID NO. 3	CD20 FL-F	Primer
SEQ ID NO. 4	CD20 FL-R	Primer
SEQ ID NO. 5	CD20 Lp-F	Primer
SEQ ID NO. 6	CD20 Lp-R	Primer
SEQ ID NO. 7	FCD20R	Primer
SEQ ID NO:8	VET200	Canine HC
SEQ ID NO:9	VET201	Canine HC
SEQ ID NO:10	VET202	Canine HC
SEQ ID NO:11	VET203	Canine HC
SEQ ID NO:12	VET204	Canine HC
SEQ ID NO:13	VET205	Canine HC
SEQ ID NO:14	VET206	Canine HC
SEQ ID NO:15	VET100	Canine LC
SEQ ID NO:16	VET101	Canine LC
SEQ ID NO.17	VET256	Mab CD20-1 HC
SEQ ID NO.18	VET229	Mab CD20-1 HC FR4 _T
SEQ ID NO.19	VET230	Mab CD20-1 HC FR1 _T FR4 _T
SEQ ID NO.20	VET132	Mab CD20-1 LC
SEQ ID NO.21	VET119	Mab CD20-1 LC FR4 _T
SEQ ID NO.22	VET120	Mab CD20-1 LC FR1 _T FR4 _T
SEQ ID NO.23	VET259	Mab CD20-2 HC FR4 _T
SEQ ID NO.24	VET134	Mab CD20-2 LC
SEQ ID NO.25	VET138	Mab CD20-2 LC FR4 _T
SEQ ID NO.26	VET121	Mab CD20-3 LC FR4 _T
SEQ ID NO.27	VET235	Mab CD20-4 HC FR4 _T
SEQ ID NO.28	VET163	Mab CD20-3 LC
SEQ ID NO.29	VET287	Mab CD20-4 HC
SEQ ID NO.30	VET286	Mab CD20-5 HC
SEQ ID NO.31	VET268	Mab CD20-5 HC FR4 _T
SEQ ID NO.32	VET162	Mab CD20-5LC
SEQ ID NO.33	VET151	Mab CD20-5LC FR4 _T
SEQ ID NO.34	VET289	Mab CD20-6 HC
SEQ ID NO.35	VET281	Mab CD20-6 HC FR4 _T
SEQ ID NO.36	VET159	Mab CD20-6LC
SEQ ID NO.37	VET161	Mab CD20-6 LC FR4 _T
SEQ ID NO.38	VET305-Full Length HC	Mab CD20-2 HC
SEQ ID NO.39	VET305-Full Length LC	Mab CD20-2 LC
SEQ ID NO.40	VET308-Full Length HC	Mab CD20-5 HC
SEQ ID NO.41	VET308-Full Length LC	Mab CD20-5 LC
SEQ ID NO.42	VET309-Full Length HC	Mab CD20-6 HC
SEQ ID NO.43	VET309-Full Length LC	Mab CD20-6 LC
SEQ ID NO.44	VET246	Feline HC
SEQ ID NO.45	VET249	Feline HC
SEQ ID NO.46	VET131	Feline LC

[00111] Other features and advantages of the invention are apparent from the following description of the preferred embodiments thereof, and from the claims.

EXAMPLE 1. Cloning of Canine and Feline CD20***I. Cloning of Canine CD20.***

[00112] The canine CD20 gene can be cloned into a mammalian expression vector and the corresponding plasmid DNA transfected into mammalian cells. Cells expressing CD20 can be used for immunization and cell-screening based assays.

[00113] CD20 are isolated from canine peripheral blood mononuclear cells (PBMC). Total RNA is extracted from 1 million canine PBMC using the MasterPure™ RNA Purification Kit (Epicentre Biotechnology). The first-strand cDNA is synthesized from 2 µg of total RNA using the First-Strand Synthesis System for RT-PCR kit (Invitrogen) according to the manufacturer's instructions. The coding region is amplified with primers of SEQ ID NO:3 and SEQ ID NO:4 and a fragment thereof encompassing the large extracellular domain (loop) are amplified with primers of SEQ ID NO:5 and SEQ ID NO:6 by PCR. The samples are denatured at 94°C for 5 min followed by amplifications for 35 cycles (94°C for 30 s, 62°C for 20 s, 72°C for 45 s) and the PCR product is sequenced.

[00114] The amino-acid sequence of the canine CD20 isolated from canine PBMC is listed as SEQ ID NO 1.

II. Cloning of Feline CD20.

[00115] The feline CD20 coding region is isolated from 5 million feline PBMC fractionated from whole blood using the Mini RNA Isolation Kit (Zymo Research). The first-strand cDNA is synthesized from 2 µl of total RNA using First-Strand Synthesis System for RT-PCR kit according to the manufacturer's instructions (Invitrogen). The coding region is then amplified by PCR using the primers of SEQ ID NO:3 and SEQ ID NO:7 using GoTaq Green Master Mix according to manufacturer's instructions. The samples are then denatured at 94°C for 5 min followed by amplifications for 35 cycles (94°C for 30 s, 52°C for 30s, 72°C for 1 min). The PCR product is cloned and sequenced.

[00116] The amino-acid sequence of the feline CD20 isolated from feline PBMC is given as SEQ ID NO:2.

EXAMPLE 2. Immunization with CD20 and Generation of Murine Monoclonal Antibodies to Canine CD20

[00117] To generate monoclonal antibodies to canine CD20, CHO-DG44 (Chinese hamster ovary cells, *dihydrofolate reductase deficient* ATCC CRL-9096) and NIH:3T3 (ATCC CRL-1658) are transfected with an expression vector encoding the full-length canine CD20 protein. Magnetic Proteoliposome Particles (MPLs) containing CD20, such that the native conformation of the transmembrane receptor is maintained are prepared for immunizations and panning. In brief, recombinant canine CD20 that contains an epitope tag are solubilized from a transfected CD20-expressing cell line using the detergent CHAPSO and the protein is captured on magnetic beads via

the epitope tag. A lipid membrane is reconstituted during removal of the detergent, such that the native membrane conformation of CD20 is maintained, to create the CD20-MPLs.

[00118] Anti-CD20 monoclonal antibodies are generated by immunization of mice to raise immunoglobulins specific for canine CD20. Washed CHO-DG44 cells expressing canine CD20 (1×10^7 cells in 100 μ L) or 100 μ L of CD20-MPLs (1×10^9 beads/mL) are used as immunogens. Mice are immunized with antigen in Ribi adjuvant intraperitoneally three times, then boosted twice on consecutive days. The immune response is monitored by retro-orbital bleeds. The sera are screened by FACS staining of CD20-expressing cells (versus untransfected parental cells) and CD20-MPLs.

[00119] Spleen are harvested from mice with sufficient titers of anti-CD20 immunoglobulin. A murine antibody library are prepared from spleen cells of the mice and displayed on phage such that the phage are then screened for expression of antibodies with specificity for CD20. This combination approach is generally described in U.S. Application No. 6,092,098 the contents of which are incorporated herein by reference.

[00120] The phage display library are screened for library members having affinity for CD20 by panning with canine CD20 incorporated into magnetic proteoliposomes (CD20-MPL). Three rounds of panning of the phage display library on the CD20-MPLs leads to several fold enrichment of CD20-binders as compared to background. Variable region fragments of interest are recloned into a Fab expression vector and the Fab retested for antigen binding against transfected CD20-expressing cells.

[00121] Anti-CD20 antibodies with high affinity for the canine CD20 exhibiting efficacy are identified by testing them in a panel of assays using methodologies available to those in the art.

[00122] The specific binding of the newly generated anti-CD20 antibodies is assessed by FACS with cells expressing CD20. Since it is important to measure the relative binding affinity of the antibodies to native CD20, live cells expressing CD20 are used in FACS analysis. For cell-binding assay, CD20 expressing cells or canine lymphoma cells are washed with phosphate-buffered saline (PBS) and seeded in wells. After one hour at room temperature to allow cell attachment to the plate surface, the cells are washed with FBS to block non-specific binding sites on the plates. Supernatants from cells expressing the anti-canine CD20 antibodies are then added. After one hour incubation at room temperature, the plates are washed with PBS. The secondary antibody is then added and detected using standard procedures.

[00123] Immunohistochemistry (IHC) is performed on lymph node tissues collected from dogs with B-cell lymphoma. Cross-sections are stained using the ImPRESS reagent (Vector Laboratories) following manufacturer's instructions. Sections are evaluated under 40x objective and scored as positive or negative compared with control tissues. B-cells and T-cells are identified by using an anti-dog CD21 antibody (Serotec) and an anti-dog CD3 antibody (Serotec), respectively. Newly generated anti-canine CD20 antibodies exhibit a strong staining of B cells and some of them with a predominant staining of the membrane.

EXAMPLE 3. Heterochimeric Antibodies

[00124] The following **EXAMPLE** provides general representations of heterochimeric antibodies, which are constructed according to standard techniques using the sequences and general patterns illustrated below. In the examples listed below, the CDRs are defined using the Kabat nomenclature.

I. Antibody Variable Domains.

[00125] Illustrated in **Table 1**, are diagrammatic representations of the heterochimerization for the light chain (AVD1 to AVD10) and heavy chain (AVD11 to AVD13) antibodies, showing contiguous sequences of discrete immunoglobulin domains. Additional antibody variants are constructed by flanking the variable regions from the donor species with any of the constant domains from the target species.

Table 1.

AVD 1:	FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-Lambda} -C _{T-Lambda}
AVD 2:	FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-Kappa} -C _{T-Lambda}
AVD 3:	FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-Lambda} -C _{T-Kappa}
AVD 4:	FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-kappa} -C _{T-Kappa}
AVD 5:	FR1 _{T-Lambda} -CDR1-FR2-CDR2-FR3-CDR3-FR4-C _{T-Lambda}
AVD 6:	FR1 _{T-Kappa} -CDR1-FR2-CDR2-FR3-CDR3-FR4-C _{T-Lambda}
AVD 7:	FR1 _{T-Lambda} -CDR1-FR2-CDR2-FR3-CDR3-FR4-C _{T-Kappa}
AVD 8:	FR1 _{T-kappa} -CDR1-FR2-CDR2-FR3-CDR3-FR4-C _{T-Kappa}
AVD 9:	FR1 _{T-Lambda} -CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-Lambda} -C _{T-Lambda}
AVD 10:	FR1 _{T-kappa} -CDR1-FR2-CDR2-FR3-CDR3-FR4 _{T-kappa} -C _{T-Kappa}
AVD 11:	FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 _T -C _T
AVD 12:	FR1 _T -CDR1-FR2-CDR2-FR3-CDR3-FR4-C _T
AVD 13:	FR1 _T -CDR1-FR2-CDR2-FR3-CDR3- FR4 _T -C _T

AVD= Antibody Variable Domain; T= Target species; Lambda= lambda light chain; Kappa= kappa light chain; C= Constant domain; FR=Framework region; CDR= Complementarity Determining Region.

II. Framework Sequences.

[00126] Exemplary framework sequences used as a source to construct the light chain and heavy chain heterochimeric antibodies are generally described in the US Serial No. 12/584,390 and PCT/US2009/04997 which applications are incorporated herein by reference.

III. Constant Domain Sequences.

[00127] Exemplary constant domain sequences used as a source to construct the antibody variants and/or fragments thereof are generally described in the international publication WO 2010/110838, the contents of which are incorporated herein by reference.

EXAMPLE 4. Construction, Expression and Purification of Antibody Variants.

[00128] The anti-CD20 monoclonal antibodies are generated in a non-canine mammal and may not be suitable for repeated administration. Antibody variants are generated to include sequences from the target species. The antibody variants are then tested for a panel of properties.

I. Antibody Variants Derived from the Mouse Anti-canine CD20 Antibodies.

[00129] The mouse anti-dog CD20 antibodies are modified as described in **EXAMPLE 3**. Variable regions are prepared by assembling synthetic oligonucleotides and cloned into pSMART with HindIII and NheI as flanking restriction sites on the 5'- and 3'- end of the variable domains, respectively. Assembled products are then subcloned into an expression vector containing a promoter and the heavy chain constant domain or containing the lambda light chain constant domain. The entire expression cassette includes the human cytomegalovirus immediate-early (CMV) promoter, a kozak sequence and signal peptide sequence immediately upstream of the coding sequence and in frame with the variable region of both the light and heavy chains to direct the resulting antibody product towards the secretory pathway. The vectors also contain a lambda canine light chain constant domain and a canine heavy chain constant domain.

II. Expression, Purification and Quantitation of Antibody Variants.

[00130] These plasmids are transformed into *E. coli* chemically competent *E. coli* cells (Lucigen), grown in Luria Broth (LB) media and stocked in glycerol. Large scale plasmid DNA are prepared as described by the manufacturer (Zymo Research Corp.). The antibody variants are transiently expressed in the human embryonic kidney cell line 293F (Invitrogen) in serum-free condition. The heavy chain (VET200 series) and light chain (VET100 series) expression vectors are co-transfected using 293fectin (Invitrogen) and grown in 293F-FreeStyle culture medium (Invitrogen). The transfected 293 cultures expressed approximately 5-20 mg/L of recombinant antibody. Binding assays are performed with supernatants or with recombinant antibodies purified from supernatants.

[00131] The antibody titer is determined using a quantitative ELISA. Plates are coated with 100 μ L/well at 37°C for 1 hour with rabbit anti-dog IgG (H+L) antibody (Jackson Immuno-Research) diluted 1:100 in carbonate buffer (100mM NaHCO₃, 33.6 mM Na₂CO₃, pH 9.5). The plates are washed three times with TBS-T (50mM Tris, 0.14 M NaCl, 0.05% tween-20, pH 8.0) and blocked with 200 μ L/well TBS/BSA (50mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0) for 1 hour at 37°C. The

standard is prepared by diluting the reference antibody (Jackson Immuno-Research, Dog Gamma Globulin 10.0 mg) in TBS-T/BSA (TBS-T, 1% BSA) in a range of concentration from 0 to 500 ng/ml. After washing the plates twice with TBS-T, standard/samples preparation are added to each well and incubated at 37°C for 1 hour. The plates are then washed 3X with TBS-T and incubated for 1 hour at 37°C with HRP-rabbit anti-dog IgG antibody (Perodixase Rabbit Anti-Dog IgG (H+L) Jackson Immuno-Research) diluted 1:20,000 in TBS-T/BSA. The plates are washed twice with TBS-T and developed using 100 µL/well of TMB substrate. The reaction is stopped with 1M H₂SO₄ and the OD is measured at 450 nm. The standard curve is fitted using a four parameter equation and used to calculate the antibody concentration in the samples.

[00132] Antibodies are purified from culture supernatants using protein A affinity chromatography. Supernatants are diluted 1:1 with Binding Buffer (Pierce) and passed over a gravity-flow column (GE Healthcare), equilibrated with 20 resin-bed volumes of Binding Buffer. The antibody retained on the column is washed with 15 ml of binding buffer, eluted with low pH elution buffer (Pierce) and collected in 1 ml fractions containing 100 µL of Binding Buffer to neutralize the pH. Fractions with absorbance (280 nm) >0.1 are desalted using desalting columns (Pierce).

III. *Anti-canine CD20 Antibody Variant Sequences.*

[00133] The amino-acid sequence of the isolated antibody and the antibody variants is given as SEQ ID NO.:17 to SEQ ID NO.:43

IV. *Designation of Anti-canine CD20 Antibody Variant Sequences.*

[00134] Table 2 summarizes the designation and the parts of the various vectors.

Table 2.

Designation	Variable Domain	Constant Domain
VET124	VET121	VET101
VET133	VET132	VET101
VET135	VET134	VET101
VET237	VET235	VET201
VET257	VET256	VET201
VET258	VET256	VET205
VET260	VET259	VET201

EXAMPLE 4. Binding of Antibody Variants to Cells

I. *Antibody Variants to CD20 Bind Canine PBMCs.*

[00135] In the present example, the antibody variants are incubated with the CD20 positive cells and the amount of bound antibody is assessed following incubation with a fluorescent-labeled reporter reagent. The reporter is thereafter measured by FACS.

[00136] Briefly, for each assay, one million cells of Peripheral Blood Mononuclear Cell (PBMC) isolated from whole blood from a normal dog by standard techniques were resuspended in FACS buffer (PBS+2% FBS). Two μg of the primary antibody are added to the cells and the samples were incubated at 4°C for 1 h. The primary antibody is provided as supernatants from transfected cells with recombinant antibody constructs or from purified antibody preparation. The mouse anti-dog CD21 mAb (Serotec) recognizing B-lymphocytes is added to the cells as a control to estimate the percentage of B-lymphocytes in a given sample. One ml of FACS buffer are added and cells are spun down for 3 min at 800 x g in Eppendorf microcentrifuge. The cells are washed with 1 mL FACS buffer and spun down again. The secondary antibodies such as fluorescein-isothiocyanate (FITC) conjugated goat anti-mouse kappa (mFITC, Jackson ImmunoResearch), or the FITC-conjugated goat anti-dog IgG (H+L) (dFITC, Bethyl Laboratories) are added in 100 μL of FACS buffer supplemented with 1% BSA to appropriate tubes and the tubes were incubated at 4°C for 30 minutes. The wash steps are repeated. The cells are then resuspended in 500 μL FACS buffer and transferred into 12 x 75 mm polystyrene test tubes. The cells are analyzed by FACS with a FacScan cytometer using the CellQuest software (Becton-Dickenson). Analysis gates are set on the live lymphocyte population based on typical forward and side scatter characteristics. Several controls are utilized to determine the background fluorescence: (i) one tube of cells is incubated with the FITC-conjugated secondary antibody without the primary antibody, and (ii) one tube of cells is incubated with PBS only.

[00137] A typical staining profile is reported in **Table 3**. The overall percentage of B-cell subset in this sample is estimated to be approximately 12% based on the binding of the anti-dog CD21 mab control. The results of **Table 3** evidence effective binding of the antibody variants to lymphocytes.

Table 3. Binding of antibody variants to canine PBMCs.

	% Lymphocytes	Mean Fluorescence
PBMC	0.37	31.56
PBMC + dFITC	0.47	43.86
PBMC + VET 133 X VET 257 + dFITC	12.03	3165.67
PBMC + VET 135 X VET 257 + dFITC	8.33	2499.17
PBMC + VET 133 X VET 260 + dFITC	13.73	2906.29
PBMC + VET 135 X VET 260 + dFITC	12.72	2659.34
PBMC + VET 124 X VET 237 + dFITC	13.00	2656.60

II. Antibody Variants to CD20 Bind Cells Expressing Feline CD20.

[00138] The binding of the antibody variants of the present invention raised to canine CD20 can be assessed by FACS analysis for their binding to feline CD20 according to standard immunological techniques (see *Veterinary Immunology and Immunopathology* 2005,106:179–196; Brousseau et al., *Manual of Immunology Methods*, CRC Press, 1998).

[00139] In the present example, the antibody variants (2.5 µg/mL) are incubated with mammalian cells expressing the recombinant feline CD20 or canine CD20 or control cells. The amount of bound antibody is assessed by detection with a fluorescent-labeled reporter antibody reagent. Several controls are utilized to determine the background fluorescence: (i) CD20 expressing cells are incubated with the conjugated secondary antibody with an isotype antibody, and (ii) Control cells are incubated with the anti-CD20 antibodies.

[00140] A typical binding profile is reported in **Table 4**. The results reported as mean fluorescence units evidence effective binding of the antibodies CD20-2, CD20-5, and CD20-6 to the recombinant canine CD20 expressed in HEK cells. Only CD20-2 and CD20-5 antibodies bind feline CD20 expressed in mammalian cells.

Table 4.

Mab	Canine	Feline	Control
	CD20	CD20	Cells
CD20-2	2340	2209	49
CD20-5	2769	2048	215
CD20-6	2107	60	4
Isotype	55	50	14

III. Anti-CD20 Antibody Variants Alter Proliferation of Tumor Cells.

[00141] The antibody variants of the present invention were tested for their ability to alter proliferation of lymphoma cells.

[00142] Lymphoma cells are grown in RPMI medium with FBS 10% in 5% carbon dioxide (CO₂) at 37°C. Cells are seeded at 5,000 cells/well in 96-well plates in medium with 5% FBS. Cells are treated with the antibody variants or isotype controls (10 µg/ml) and incubated for 72h at 37°C in a CO₂ incubator. Ten (10) µL MTT solution is added to each well and incubated at 37°C for 4h according to the manufacturer's instruction (Trevigen). Optical density (OD) is then measured at 490nm and data is presented as percentage of reduction of cell proliferation of triplicate measurements. The data in **Table 5** illustrate that the antibody variants have an anti-proliferative effect on lymphoma cells. Furthermore, the antibody variants sensitize the lymphoma cell line to Doxorubicin, a cytotoxic drug commonly used in the treatment of cancer for companion animals.

Table 5. Effects of antibody variants on proliferation of lymphoma cells.

Mabs (10 ug/mL)	Doxorubicin		
	0 nM	6.6 nM	66 nM
Isotype Control	-0.30	12.20	38.69
VET 133 X VET 257	59.82	65.77	76.79
VET 135 X VET 260	40.48	58.63	64.88

[00143] The antibody variants of the present invention are further tested for their ability to alter proliferation of lymphoma cells. Cell assays are conducted as described in the example above. Data is presented in **Table 6** as percentage of reduction of cell proliferation of triplicate measurements. The data illustrate that the antibody variants at a concentration of 10 µg/mL with various affinities exhibit various level of anti-proliferative effect on lymphoma cells.

Table 6. Effects of antibody variants on proliferation of lymphoma cells.

Mabs	Reduction (%)	KD (nM)
CD20-2 (VET305)	55.0	8.845
CD20-3	22.4	76.28
CD20-4	17.5	77.37
CD20-5 (VET308)	60.1	1.21
CD20-6 (VET309)	27.6	63.41

IV. Affinity and Epitope of Anti-CD20 Antibody Variants.

[00144] The antibody variants of the present invention are compared for their binding affinity to canine CD20. Binding affinity is assessed by measuring binding to the recombinant canine CD20 expressed in CHO cells by FACS analysis as described above. The affinity is 1.0 nM, 8.845 nM, 76.28 nM, 77.37 nM, 1.2 nM, 63.41 nM for the anti-canine CD20 antibodies CD20-1, CD20-2, CD20-3, CD20-4, CD20-5, and CD20-6, respectively.

[00145] Competition experiments are performed using standard techniques. Briefly, biotinylated recombinant anti-canine CD20 antibodies are titrated on CHO cells expressing recombinant canine CD20. Cells are first incubated with an excess of unlabelled recombinant anti-canine CD20 antibody (25 µg/mL). Thereafter, cells are incubated with a biotin-labeled antibody at 2 µg/mL. After washing, cells are incubated with streptavidin- phycoerythrin and fluorescence is analyzed by flow cytometry. An irrelevant antibody is used as negative control for inhibition. Results are expressed as a ratio between fluorescence intensity of the cells pre-incubated with the unlabelled antibody and the fluorescence intensity obtained for each biotinylated antibody alone. The percentage of inhibition (PI) is calculated by the following formula: $PI = [1 - (\text{Experimental-Background}) / (\text{Biotinylated antibody alone} - \text{Background})] \times 100\%$.

[00146] The results reported in **Table 7** evidence that antibody CD20-1, CD20-3, and CD20-6 recognize different epitopes of canine CD20.

Table 7. Epitope mapping of anti-CD20 antibody variants using CHO cells expressing the recombinant canine CD20.

Competitor mab	Labeled Antibody (2 µg/mL)		
	CD20-1	CD20-2	CD20-5
CD20-1	111.65	-22.08	87.68

CD20-2	0.90	124.33	3.69
CD20-5	107.07	6.47	85.88
CD20-6	6.03	1.41	5.99

V. Creation of Anti-CD20 Producing Cell Line

[00147] The vectors harboring both the light chain gene and the heavy chain gene of the anti-canine CD20 antibody CD20-2 and CD20-5 are introduced into mammalian cells to create a cell line expressing the corresponding recombinant antibody. In this example, PER.C6 cells are used as mammalian cells. Cells are cultured in a chemically-defined, protein-free medium CDM4PerMab (Hyclone, Thermo-Scientific, Cat No. SH30871.02) supplemented with 3.0 mM Glutamine (Invitrogen, Gibco, Cat No. 25030-081). Four passages after thaw, the PER.C6 cells are transfected by electroporation using standard techniques with the linearized vector DNA. Cells which stably incorporate the vector are selected for by survival in the presence of 125.0 ug/mL Geneticin (Invitrogen, Cat No. 11811-023) by limited dilution at a seeding density of 0.3 cells per well in 96-well plates. When colonies became visible, single clones from single wells are first measured for titer and binding to the target and then scaled-up to larger wells. Selected clones are further evaluated in larger scale cultures for antibody titer, binding to CD20-expressing cells, cell doubling time, cell viability, and cell stability. Clones with optimal characteristics are frozen in the culture medium supplemented with 7.5 ± 0.5 % Dimethylsulphoxide (Sigma, Cat No. D2650).

VI. Half-life of the recombinant anti-canine CD20

[00148] The half-life of the recombinant anti-canine CD20 antibody are assessed. In this example, the half-life of antibody CD20-2 (VET305) is assessed by dosing beagle dogs intravenously. Blood is collected for analysis of CD20-2 in plasma samples harvested as whole blood treated with Ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. An enzyme linked immunosorbant assay (ELISA) method is utilized to determine the plasma antibody concentrations. In this assay, a 96-well plate is coated with a rabbit polyclonal antibody raised to the variable domain of the antibody CD20-2. The antibody CD20-2 in standards or in samples is captured by the polyclonal antibody and is detected by an enzyme conjugated anti-dog secondary antibody. A non-linear regression fit of the standards is used to determine the recombinant antibody concentrations in plasma.

[00149] A single dose of 2.4 mg/kg of the antibody CD20-2 shows that high plasma antibody concentrations are achieved in all animals and that its elimination half-life ranges between 56 to 67 hours. Multiple doses of the antibody CD20-2 ranging from 2.0 to 5.0 mg/kg show that plasma antibody concentrations increases overtime and persists at significant levels during the treatment intervals with elimination half life of longer than one week and the volume of distribution approximating plasma volume.

[00150] Interestingly, the half-life values of the antibody CD20-2 increase after consecutive administration. The half-life values are influenced by the number of target cells and depend on the size of the B lymphocyte pool at a given time point. Due to the binding to CD20 and eventual lysis of lymphocyte cells, the B-cell depleting effect of the antibody could explain half-life value increases.

VII. Depletion of B Cells in Vivo

[00151] Three beagle dogs receive three consecutive dosages of the antibody CD20-2 (VET305) ranging from 2.0 mg/kg to 5.0 mg/kg every 5 days. Blood samples drawn at several time points are centrifuged at 2000 RPM for 5 min. Plasma is removed for assay of the antibody levels. The pellet containing peripheral blood leukocytes and red blood cells is resuspended in a plasma equivalent volume of phosphate saline solution (Dulbecco's Phosphate-Buffered Saline, Mediatech, Cat No. 21-030-CM) for quantitation of lymphocyte populations by flow cytometry. A 0.1 mL volume of the cell preparation is distributed into micro-centrifuge tubes. Labeled monoclonal antibody with specificity for the canine lymphocyte surface marker CD21 is added to the vial to identify the B lymphocyte cell population. An additional sample is included with no reagents for determination of autofluorescence. Cells are incubated with the fluorescent antibody for 30 min. Red blood cells were then lysed for 15 min using a lysis buffer (Red Blood Cell Lysis Buffer, Biolegend, Cat No. 420301) and then washed prior to analysis on a Becton Dickinson FACS instrument.

[00152] Interestingly, a single dose of 2.4 mg/kg of the antibody CD20-2 triggers a rapid and sustained B-cell depletion ranging from 36 to 95% of the pre-dosing level. Three consecutive doses of 2.4 mg/kg to 5.0 mg/kg of the antibody CD20-2 show a decrease in B lymphocyte cell percentage after treatment across all tested dose ranges and depletion is maintained for at least 10 days after the last dose.

[00153] Weekly dose of the antibody CD20-2 over a period of 4 weeks is well tolerated locally and systemically and no adverse effects are noticed on clinical and behavioral observations or body weights.

EXAMPLE 5. Treatment with Anti-CD20 Antibody Variants

I. Treatment of Dogs.

[00154] A dog diagnosed with an immune condition including lymphoma, relapsed lymphoma, leukemia, mast cell tumor, hemolytic anemia, arthritis, atopic dermatitis is given therapy with the anti-CD20 monoclonal antibody. The dog is infused intravenously or subcutaneously with 1-5 mg/kg of antibody, and the treatment is repeated weekly for 4-8 weeks following the initial treatment. Two months after the final dose, the patient shows reduced levels of certain types of cells expressing CD20. The dog is then treated under a maintenance regimen with administration of the anti-CD20 antibody every 8-12 weeks. It is contemplated that a dog may be infused intravenously, subcutaneously, intramuscularly, or intraperitoneally. It is contemplated that a dog may be dosed at

1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5 mg/kg of antibody. It is contemplated that a dog may be given antibody at doses lower than 1mg/kg of body weight.

II. Treatment of Arthritis.

[00155] A dog with confirmed arthritis receives 1 mg/kg of anti-CD20 antibody treatment alone or in combination with standard treatment of care two to three times a week for an initial four weeks. Clinical response to treatment is assessed for improvement at the study end point. Improvement is defined as one of the following: (i) Reduction of at least 1 grade in lameness score at a walk or trot, and/or (ii) A combined reduction of at least 2 grades in scores for pain on palpation or manipulation, range of joint motion, and joint swelling. Overall lameness, pain on palpation or manipulation, range of motion, and joint swelling are observed at the scheduled times and scored as follows: (i) Overall Lameness Scoring (scored at a walk and a trot) [0 = No lameness, 1 = Mild lameness (dog touched toe to floor on all strides), 2 = Moderate lameness (dog touched toe to floor on all strides), 3 = Severe lameness (dog touched toe to floor on at least 50% of strides), 4 = Non-weight bearing lameness (dog touched toe to floor on less than 50% of strides)]; (ii) Pain on Palpation/Manipulation (most severely affected limb) [0 = No pain or not applicable, 1 = Slightly painful (scarcely withdrew limb), 2 = Moderately painful (definitely withdrew limb), 3 = Severely painful (prominently withdrew limb)]; (iii) Range of Motion (most severely affected limb) [0 = Normal range of motion, 1 = Slightly reduced (less than 25% reduction in range), 2 = Moderately reduced (25% to 50% reduction in range), 3 = Severely reduced (greater than 50% reduction in range)]; and (iv) Joint Swelling (most severely affected limb) [0 = No swelling or not applicable, 1 = Mild swelling (fibrosis or mild, palpable fluid distension), 2 = Moderate swelling (obvious, palpable fluctuant fluid distension), 3 = Severe swelling (pronounced, palpable fluctuant fluid distension)]. Two months after the final dose, the patient shows overall improvement. The dog is then treated under a maintenance regimen with administration of the anti-CD20 antibody every 8-12 weeks.

III. Treatment of Cats.

[00156] A cat diagnosed with an immune condition including lymphoma, relapsed lymphoma, leukemia, mast cell tumor, hemolytic anemia, arthritis, atopic dermatitis is given therapy with the anti-CD20 monoclonal antibody. The cat is infused intravenously or subcutaneously with 1-5 mg/kg of antibody, and the treatment is repeated weekly for 4-8 weeks following the initial treatment. Two months after the final dose, the patient shows reduced levels of certain types of cells expressing CD20. The cat is then treated under a maintenance regimen with administration of the anti-CD20 antibody every 8-12 weeks. It is contemplated that a cat may be infused intravenously, subcutaneously, intramuscularly, or intraperitoneally. It is contemplated that a cat may be dosed at 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5 mg/kg of antibody. It is contemplated that a cat may be given antibody at doses lower than 1mg/kg of body weight.

[00157] Alternative combinations and variations of the examples provided will become apparent based on this disclosure. It is not possible to provide specific examples for all of the many possible combinations and variations of the embodiments described, but such combinations and variations are nevertheless intended to be within the scope of the invention.

[00158] SEQUENCE LISTING

SEQ ID NO.	Sequence
SEQ ID NO. 1	MTTPRNSMSGTLPVDPMKSPAMYPVQKIIPKRMPSVVGPTQNFMMRESKTLGAVQIMNGLEFIALGSLMIHTDVYAPICITMWYPLWGGIMFIIISGSLAAADKNPRKSLVKGKMIMNSLSLFAISGIIIFLIMDIFNITISHFFKMENLNLIKAMPYVDIHNCDPANPSEKNLSIQYCGSIRS'FLGVFAVMVIFTFFQKLVTAGIVENEWKKLCSKPKSDVVLLAAEEKKEQPIETTEEMVELTIASQPKKEEDIEIIPVQEEEELEINFAEPPQEQESSPIENDSIP
SEQ ID NO. 2	MTTPRNSMSGTLPADAMKSPAMNPVQKIIPKRMPSVVGPTQNFMMKESKPLGAVQIMNGLFMALGGLMIHMEVYAPICMTVWYPLWGGIMYIISGSLVAAEKNPRKSLVKGKMIMNSLSLFAISGMILLIMDIFNIAISHFFKMENLNLLKSPKPYIDIHTCQPESEKNSLSIKYCDIRS'VFLSIFAVMVVFTLFQKLVTAGIVENEWKKLCSKPKADVLLAAEEKKEQLVEITEEAVEL'EVSSQPKNEEDIEIIPVQEEEEETEMNFPPEPPQDQEPSLIENDSIP
SEQ ID NO. 3	5' -TGAGATGACAACACCCAGAAA-3'
SEQ ID NO. 4	5' -TTAAGGGATGCTGTCGTTTTC-3'
SEQ ID NO. 5	5' -AATATTACCATTTCCCATTTTTTTTA-3'
SEQ ID NO. 6	5' -TATGCTGCCACAATATTGTATAG-3'
SEQ ID NO. 7	5' -GGATCCTTAAGGAATGCTATCGTTTT-3'
SEQ ID NO:8	ASTTAPSVFPLAPSCGSQSGSTVALACLVSgyIPEPVTVSWNSGSLTSGVHTFPSVLQSSGSLSSMVTVPSSRWPSETFTCNVAHPATNTKVDKPVVKECECKCNCNCPGCGLLGGPSVIFPPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQVQTANTQPREEQSNGTYRVVSLPIGHQDWLSGKQFKCKVNNKALPSPIEEIIISKT PGQAHQPNVYVLPSPRDEMKNVTTLTKVKDFFPPEIDVEWQSNQEQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMEALHNHYTQKSLSHSPGK
SEQ ID NO:9	ASTTAPSVFPLAPSCGSQSGSTVALACLVSgyFPEPVTVSWNSGSLTSGVHTFPSVLQSSGSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPFIFFPPKPKDILLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFNQTYR'SVLPIGHQDWLKGKQFTCKVNNKALPSPiERTISKARGQAHQPSVYVLPSPREELSKNTVS:CLIKDFFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICMHEALHNHYTQKSLSHSPGK
SEQ ID NO:10	ASTTAPSVFPLAPSCGSTSGSTVALACLVSgyFPEPVTVSWNSGSLTSGVHTFPSVLQSSGSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPFIFFPPKPKDILLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFNQTYR'SVLPIGHQDWLKGKQFTCKVNNKALPSPiERTISKARGQAHQPSVYVLPSPREELSKNTVS:CLIKDFFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICMHEALHNHYTQKSLSHSPGK
SEQ ID NO:11	ASTTAPSVFPLAPSCGSTSGSTVALACLVSgyFPEPVTVSWNSGSLTSGVHTFPSVLQSSGSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPFIFFPPKPKDILLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFNQTYR'SVLPIGHQDWLKGKQFTCKVNNKALPSPiERTISKARGQAHQPSVYVLPSPREELSKNTVS:CLIKDFFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICMHEALHNHYTQKSLSHSPGK
SEQ ID NO:12	ASTTAPSVFPLAPSCGSTSGSTVALACLVSgyFPEPVTVSWNSGSLTSGVHTFPSVLQSSG

	SLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSV FIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFNQTYRVV SVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLT CLIKDFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICAV MHEALHNHYTQKSLSHSPGK
SEQ ID NO:13	ASTTAPSVFPLAPSCGSQSGSTVALACLVSIGYIPEPVTVSWNSGSLTSGVHTFPPSILQSSGL SLSSMVTVPSSRWPSETFTCNVAHPATNTKVDKPVVKECECKCNCNCPGCGLLGGPSVF FPPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDGKQMOTAKTQPREEQFNQTYRVV LPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTC IKDFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICAVM ESLHNHYTQKSLSHSPGK
SEQ ID NO:14	ASTTAPSVFPLAPSCGSTSGSTVALACLVSIGYFPEPVTVSWNSGSLTSGVHTYPSVLQSSGL SLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKVPKRENGRVPRPPGCPKCPAPEMLGGPS FIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDSKQVQTANTQPREEQSNQTYRV SVLPIGHQDWLKGKQFKCKVNNKALPSPIEIIISKTFGQAHQPNVYVLPSSRDEMSTNTVTL CLVKDFPPPEIDVEWQSNQEQEPESKYRMTTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICA MHEALHNHYTQKSLSHSPGK
SEQ ID NO:15	NDAQPAVYLFQPSDQLHTGSASVCLLNSFYPKDINVKWKVDGVIQDTG IQESVTEQDKDS YLSSTLTMSSTEYLSHELSCETHKSLPSTLIKSFQRSECQRVD
SEQ ID NO:16	GQPKASPSVTLFPPSSEELGANKATLVCLISDFYPSGTVVAWKADGSPITQGVETTKPSKQS NKYAASSYLSLTPDKWKSHSSFSCLVTHEGSTVEKKVAPAACS
SEQ ID NO.17	EIQLOQSGAELVKPGASVKISCKASGYTFTDYYINWVKQRPGQGLEWIGKIGPGSGRTYYNE FKGKATLTADKSSSTAYIQISSLTSEDSAVYFCAVLSWGQGTTLTVSS
SEQ ID NO.18	EIQLOQSGAELVKPGASVKISCKASGYTFTDYYINWVKQRPGQGLEWIGKIGPGSGRTYYNE FKGKATLTADKSSSTAYIQISSLTSEDSAVYFCAVLSWGQGTTLTVSS
SEQ ID NO.19	EVQLVESGGDLVKPGGSLRLSCKASGYTFTDYYINWVKQRPGQGLEWIGKIGPGSGRTYYNE FKGKATLTADKSSSTAYIQISSLTSEDSAVYFCAVLSWGQGTTLTVSS
SEQ ID NO.20	DVQITQTPLTSLVTFGQPASISCKSSQSLKSDGRTYLNWLLQRPQGSPKRLLYLVSKLDS PDRFTGSGSGTDFTLTKISRVEAEDLGYYICWQGTHTFPQTFGGGKLEIK
SEQ ID NO.21	DVQITQTPLTSLVTFGQPASISCKSSQSLKSDGRTYLNWLLQRPQGSPKRLLYLVSKLDS PDRFTGSGSGTDFTLTKISRVEAEDLGYYICWQGTHTFPQTFGGGTHLTVL
SEQ ID NO.22	QSVLTQPASVSGSLGQRVTISCKSSQSLKSDGRTYLNWLLQRPQGSPKRLLYLVSKLDSG DRFTGSGSGTDFTLTKISRVEAEDLGYYICWQGTHTFPQTFGGGTHLTVL
SEQ ID NO.23	QVQLQQSRAELVRPGASVTLSCKPSGYTFTDYEVHWVKQTPVHGLEWIGAIIDPETGGTADN FKGKAILTADKSSSTAYMELRSLTSEDSAVYYCTNFVDVWGTGTTVTVSS
SEQ ID NO.24	DVVMSSQSPSSLAVSVGEKVTMSCKSSQSLLYSGNQKNYLAWYQQKPGQSPRLLIYWASTRE VPDRFTGSGSGTDFTLTISSVKAEDLAVFYCQQYNYPLTFGAGTKLELK
SEQ ID NO.25	DVVMSSQSPSSLAVSVGEKVTMSCKSSQSLLYSGNQKNYLAWYQQKPGQSPRLLIYWASTRE VPDRFTGSGSGTDFTLTISSVKAEDLAVFYCQQYNYPLTFGGGTHLTVL
SEQ ID NO.26	DIVTSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRE VPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYNYPLTFGGGTHLTVL
SEQ ID NO.27	EVQLQQSVAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLEWIGIIDEIDPETGGTAYN FKGKAILTADKSSSTAYMELRSLTSEDSAVYYCTEYAMDYWGQGTTLTVSS
SEQ ID NO.28	DIVTSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRE VPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYNYPLTFGAGTKLELK
SEQ ID NO.29	EVQLQQSVAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLEWIGIIDEIDPETGGTAYN FKGKAILTADKSSSTAYMELRSLTSEDSAVYYCTEYAMDYWGQGTSTVTVSS
SEQ ID NO.30	RVQLKQSGAELVKPGASVKISCKASGYTFTDYYINWVKQRPGQGLEWIGKIGPRSGSIYYN FKGKATLTADKSSSTAYMQLRSLTSEDSAVYFCAVLKVGQGTTLTVSS
SEQ ID NO.31	QVQLKQSGAELVKPGASVKISCKASGYTFTDYYINWVKQRPGQGLEWIGKIGPRSGSIYYN FKGKATLTADKSSSTAYMQLRSLTSEDSAVYFCAVLKVGQGTTLTVSS
SEQ ID NO.32	DAVMTQIPLTSLVTFGQPASISCKSSQSLHSDGKTYLNWLLQRPQGSPKRLIYLVSCLD PDRFTGSGSGTDFTLTKISRVEAEDLGYYICWQGTHTFPQTFGGGKLEIK
SEQ ID NO.33	DAVMTQIPLTSLVTFGQPASISCKSSQSLHSDGKTYLNWLLQRPQGSPKRLIYLVSCLD

	PDRFTGSGSGTDFTLTKISRVEAEDLGVYYCWQGFTHFPQTFGGGTHLTVL
SEQ ID NO.34	EIQLOQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLEWIGGIDPETGGTAYNQ FKGKAILTADKSSSTVYME LRSLTSEDSAVYYCTR DYGTSGYWGQGTLLTVSS
SEQ ID NO.35	EIQLOQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLEWIGGIDPETGGTAYNQ FKGKAILTADKSSSTVYME LRSLTSEDSAVYYCTR DYGTSGYWGQGTLLTVSS
SEQ ID NO.36	DVVVTQTPLSLPV SFGDQVSI SCRSSQSLANSYGNTYLSWYLHKPGQSPQLLIYGISNRFSG PDRFSGSGSGTDFTLTKISRVEAEDLGVYFCSQSTHVPWTFGGGKLEIK
SEQ ID NO.37	DVVVTQTPLSLPV SFGDQVSI SCRSSQSLANSYGNTYLSWYLHKPGQSPQLLIYGISNRFSG PDRFSGSGSGTDFTLTKISRVEAEDLGVYFCSQSTHVPWTFGGGTHLTVL
SEQ ID NO.38	QVQLQQSRAELVRPGASVTLSCKPSGYTFTDYEVHWVKQTPVHGLEWIG AIDPETGGTADNQ FKGKAILTADKSSSTAYMELRSLTSEDSAVYYCTNFVDVWGTGTTVTVSSASTTAPSVFPLA SCGSQSGSTVALACL VSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSR PSETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLL ARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFN GTYRVVSVLP IGHQDWL K KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFPDID EWQSNQEQEPESKYRTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQK LSHSPGK
SEQ ID NO.39	DVVMSSPSSLAVSVGEKVTMSCKSSQSLLYSGNQKNYLAWYQQKPGQSPRLLIYWASTRES VPDRFTGSGSGTDFTLTISSVKAEDLAVFYCQQYYNYPLTFGGGTHLTVLGQPKASPSVTLF PSSEELGANKATLVCLISDFYPSGVTVAWKADGSPITQGVETTKPSKQSNNKYAASSYLSLT DKWKSHSSFCLVTHEGSTVEKKVAPAEC S
SEQ ID NO.40	QVQLKQSGAELVKPGASVKISCKASGYTFTDYINWVKQRPGQGLEWIGKIGPRSGSIYYNE FKGKATLTADKSSSTAYMQLRSLTSEDSAVYFCAVLKWKQGTLLTVSSASTTAPSVFPLAPS GSQSGSTVALACL VSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWI ETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLLI TPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFN GTYRVVSVLP IGHQDWLKG FTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFPDIDV QSNQEQEPESKYRTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSI HSPGK
SEQ ID NO.41	DAMVTQIPLTLSVTIGQPASISCKSSQSLHSDGKTYLNWLLQRPQSPKRLIYLVSKLDS PDRFTGSGSGTDFTLTKISRVEAEDLGVYYCWQGFTHFPQTFGGGTHLTVLGQPKASPSVTLF SSEELGANKATLVCLISDFYPSGVTVAWKADGSPITQGVETTKPSKQSNNKYAASSYLSLT KWKSHSSFCLVTHEGSTVEKKVAPAEC S
SEQ ID NO.42	EIQLOQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLEWIGGIDPETGGTAYNQ FKGKAILTADKSSSTVYME LRSLTSEDSAVYYCTR DYGTSGYWGQGTLLTVSSASTTAPSV LAPSCGSQSGSTVALACL VSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV SRWPSETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPK LLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFN GTYRVVSVLP IGHQ LKKGQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFP IDVEWQSNQEQEPESKYRTPPQLDEDEGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHN QKSLSHSPGK
SEQ ID NO.43	DVVVTQTPLSLPV SFGDQVSI SCRSSQSLANSYGNTYLSWYLHKPGQSPQLLIYGISNRFSG PDRFSGSGSGTDFTLTKISRVEAEDLGVYFCSQSTHVPWTFGGGTHLTVLGQPKASPSVTLF SSEELGANKATLVCLISDFYPSGVTVAWKADGSPITQGVETTKPSKQSNNKYAASSYLSLT KWKSHSSFCLVTHEGSTVEKKVAPAEC S
SEQ ID NO.44	ASTTAPSVFPLAPSCGTTSGATVALACLVLGYFPEPVTVSWNSGAL TSGVHTFPSVLQASG SLSSMVTVPSSRWLSDTFTCNVAHPPSNTKVDKTVRKT DHPPGPKPCDCPKCPPPEMLGGP FI FPPKPKDTLSISRTPEVTCVVVDLGPDDSDVQITWFDNTQVYTAKTSPREEQFNSTYR SVLPILHQDWLKGKFKCKVNSKSLPSPIERTISKDKGQPHEPQVYVLP PAQEELSRNKVS CLIEGFYPSDIAVEWEITGQPEPENNYRTPPQLDSDGTYFLYRSLSVDRSRWQRGNTYTC SHEALSHHTQKSLTHSPGK
SEQ ID NO.45	ASTTAPSVFPLAPSCGTTSGATVALACLVLGYFPEPVTVSWNSGAL TSGVHTFPAVLQASG SLSSMVTVPSSRWLSDTFTCNVAHPPSNTKVDKTVRKT DHPPGPKPCDCPKCPPPEMLGGP FI FPPKPKDTLSISRTPEVTCVVVDLGPDDSDVQITWFDNTQVYTAKTSPREEQFNSTYR SVLPILHQDWLKGKFKCKVNSKSLPSPIERTISKDKGQPHEPQVYVLP PAQEELSRNKVS

	CLIKSFHPPDIAVEWEITGQPEPENNYRTTPPQLDSDGTYFVYSKLSVDRSHWQRGNTYTCSV SHEALSHHTQKSLTHSPGK
SEQ ID NO.46	RSDAQPSVFLFQPSLDELHTGSASIVCILNDFYPKEVNVKWKVDGTVVQNKGIQESTTEQNSKI STYSLSTLTMSSTEYQSHEKFSCEVTHKSLASTLVKSFNRSECQRE

CLAIMS

1. An antibody or antibody fragment thereof recognizing a canine or feline CD20 wherein the antibody reduces the percentage of CD20-expressing cells in a companion animal host.
2. The antibody or antibody fragment of claim 1 which reduces B-cell lymphoma cells in a companion animal host.
3. The antibody or antibody fragment of claim 1 which depletes B-cell lymphoma cells in a companion animal host.
4. An antibody or antibody fragment recognizing canine or feline CD20 and comprising at least one of the CDR regions from SEQ ID NOS 17-43.
5. An antibody or antibody fragment according to claim 4 recognizing a canine or feline CD20, and comprising a sequence selected from SEQ ID NOS 17-43.
6. An antibody or antibody fragment having the binding characteristics of an antibody selected from mAb CD20-1, CD20-2, CD20-3, CD20-4, CD20-5, and CD20-6.
7. An antibody or antibody fragment according to any of claims 1-6 comprising a variable domain structure selected from AVD-1 through AVD-13.
8. An antibody or antibody fragment comprising a light chain selected from SEQ ID NOS: 20,21,22,24,25,26,28,32,33,36,37,38,39,41,and 43 and a heavy chain selected from SEQ ID NOS:17,18,19,23,27,29,30,31,34,35,38,40, and 42.
9. The antibody or antibody fragment according to any of the preceding claims which is a heterochimeric antibody.
10. The antibody or antibody fragment according to any of the preceding claims which binds to canine CD20 and wherein the constant region is of canine origin.
11. The antibody or antibody fragment according to any of claims 1-9 which binds to feline CD20 and wherein the constant region is of feline origin.
12. The antibody or antibody fragment according to any of the preceding claims wherein the constant domain comprises a sequence selected for providing enhanced ADCC and/or CDC.
13. An antibody or antibody fragment according to claims 1-12 that binds to the same epitope as an antibody selected from the group consisting of mAb CD20-1, mAb CD20-2, mAb CD20-5, mAb CD20-6.
14. A nucleic acid encoding an antibody or antibody fragment according to any of the preceding claims.
15. A method of treating an animal suffering from a disease or condition characterized by overproliferation of cells expressing CD20 comprising administering an effective amount of an antibody or antibody fragment according to any of claims 1-13.
16. The method of claim 15 wherein the disease or condition is a cancer, e.g. lymphoma.
17. The method of claim 16 wherein the disease or condition is an inflammatory disease, e.g. arthritis.

18. The method of any of claims 15-17 comprising co-administration of second drug, e.g., a chemotherapeutic drug, or a second monoclonal antibody, e.g. mAb to CD52.
19. The method of any of claims 15-18 wherein the animal is diagnosed as suffering from a disease or condition characterized by overproliferation of cells expressing CD20 using a diagnostic assay comprising an antibody to CD20.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/00415

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395 ; C07K 16/00

USPC - 424/155.1 ; 530/388.8

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 39/395 ; C07K 16/00

USPC: 424/155.1 ; 530/388.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 424/133.1

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

PubWest (PGPB,USPT,USOC,EPAB,JPAB); NCBI: CD20, antibody, feline, canine, region, cell

GenCore 6.3: SEQ NOS: 17 and 20

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0271662 A1 (BEALL et al.) 8 December 2005 (08.12.2005) para [0003], [0004], [0010], [0090], [0092]	1-3 ----- 7/(1-3)
Y	US 2004/0181039 A1 (KRAH et al.) 16 September 2004 (16.09.2004) para [0027], [0082], [0152]	7/(1-3)
A	US 7,342,110 B2 (HOFFEE et al.) 11 March 2008 (11.03.2008) SEQ ID NO: 75	4-6, 7/(4-6), 8

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 July 2011 (22.07.2011)

Date of mailing of the international search report

10 AUG 2011

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/00415

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

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

- 1. [] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. [] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. [X] Claims Nos.: 9-19 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-8, drawn to an antibody or antibody fragment thereof recognizing a canine or feline CD20 wherein the antibody reduces the percentage of CD20-expressing cells in a companion animal host. The first invention is restricted to an antibody comprising a light chain of SEQ ID NO:20/21 and a heavy chain of SEQ ID NO: 17/18. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

***** See Supplemental Box to continue *****

- 1. [] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. [] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. [] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-8 limited to SEQ ID NOS: 17, 18, 20, 21

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

***** Supplemental Box *****

Box III. Observations where unity of invention is lacking:

The inventions of Group I+ share the technical feature of an antibody or antibody fragment thereof recognizing a canine or feline CD20 wherein the antibody reduces the percentage of CD20-expressing cells in a companion animal host. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 7,531,628 B2 to Beall (hereinafter 'Beall') (12 May 2009) that teaches a canine CD20 protein (para [0004]) and an antibody that specifically binds to said canine CD20 protein (para [0010]), said antibody suggested as effective for therapeutic administration, to measurably decrease numbers of CD20-positive cells in a test sample from an animal (para [0090]). As said antibody was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I+ is a heavy chain of the specific amino acid sequence recited therein. As no significant structural similarities can readily be ascertained among the amino acid sequences, the inventions do not share a special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

Finally, another special technical feature of the inventions listed as Group I+ is a light chain of the specific amino acid sequence recited therein. As no significant structural similarities can readily be ascertained among the amino acid sequences, the inventions do not share a special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.