# THERAPEUTIC AND DIAGNOSTIC METHODS USING ANTI-CD200 ANTIBODIES

## Cross-Reference to Related Applications

This application claims priority to and the benefit of U.S. provisional patent application serial nos.: 61/337,962 filed on February 11, 2010~~, the disclosure of which is incorporated herein by reference in its entirety~~.

## Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS-Web ~~and is hereby incorporated by reference in its entirety~~. Said ASCII copy, created on February 9, 2011, is named ALXN1520.txt, and is 19,592 bytes bytes in size.

## Technical Field

The field of the invention is medicine, immunology, molecular biology, and protein chemistry.

## Background

Human CD200 protein is a type 1a transmembrane glycoprotein that is normally expressed on thymocytes (e.g., T cells and B cells), neurons, and endothelial cells. Through engagement with its cognate receptor, CD200R, CD200 protein transduces an immunoregulatory signal that can suppress T-cell-mediated immune responses. CD200 knockout animal studies as well as experiments using antagonist anti-CD200 antibodies and recombinant CD200-Fc fusion proteins have demonstrated that CD200 protein is an immunosuppressive agent in autoimmune disorder and during transplantation. See, e.g., Hoek et al. (2000) *Science* 290:1768-1771 and Gorczynski et al. (1999) *J Immunol* 163:1654–1660. The interaction between CD200 and CD200R results in altered cytokine profiles and promotes a $T_H2$ T cell response over a $T_H1$ response. (See, e.g., Kretz-Rommel (2007) *J Immunol* 178:5595-5605.)

Percent (%) amino acid sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the amino acids in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

Amino acid sequences for exemplary human CD200 proteins and human CD20 proteins, as well as antigenic peptide fragments thereof are known in the art and are set forth below.

As used herein, the term "antibody" refers to a whole or intact antibody molecule (e.g., IgM, IgG (including IgGl, IgG2, IgG3, and IgG4), IgA, IgD, or IgE) or any antigen-binding fragment thereof. The term antibody includes, e.g., a chimerized or chimeric antibody, a humanized antibody, a deimmunized antibody, and a fully human antibody. Antigen-binding fragments of an antibody include, e.g., a single chain antibody, a single chain Fv fragment (scFv), an Fd fragment, an Fab fragment, an Fab' fragment, or an $F(ab')_2$ fragment. An scFv fragment is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is derived. In addition, intrabodies, minibodies, triabodies, and diabodies (see, e.g., Todorovska et al. (2001) *J Immunol Methods* 248(1):47-66; Hudson and Kortt (1999) *J Immunol Methods* 231(1):177-189; Poljak (1994) *Structure* 2(12):1121-1123; Rondon and Marasco (1997) *Annual Review of Microbiology* 51:257-283~~, the disclosures of each of which are incorporated herein by reference in their entirety~~) are also included in the definition of antibody and are compatible for use in the methods described herein. Bispecific antibodies (including DVD-Ig antibodies; see below) are also embraced by the term "antibody." Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens.

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 disclosure pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the presently disclosed methods and compositions. ~~All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.~~

Other features and advantages of the present disclosure, e.g., methods for treating a rituximab-resistant cancer (e.g., chronic lymphocytic leukemia), will be apparent from the following description, the examples, and from the claims.

## Brief Description of the Drawings

**Fig. 1** is a line graph depicting the delay in anti-mouse RBC autoantibody production in mice with autoimmune hemolytic disease treated with an anti-CD200 antibody. The Y-axis represents the incidence (%) of autoantibody production in the mice in each group. The X-axis represents the time in which the presence of autoantibodies in each mouse was detected. The seven groups of mice evaluated included: mice that were immunized with rat RBCs, but not treated with an antibody (No Rx); mice that were immunized with rat RBCs and treated with a control antibody (Cntrl Ab); mice that were immunized with rat RBCs and treated with an anti-CD200 antibody (Antibody 1); mice that were immunized with rat RBCs and treated with cyclosporine (CsA); mice that were immunized with rat RBCs and treated with the control antibody and cyclosporine A (Cntrl Ab + CsA); mice that were immunized with rat RBCs and treated with an anti-CD200 antibody and cyclosporine A (Antibody 1 + CsA); and mice that were neither immunized with rat RBCs nor treated with antibody or cyclosporine (No-imm No Rx).

**Fig. 2** is a line graph depicting the effect of an anti-CD200 antibody on anti-RBC antibody titer in a mouse model of autoimmune hemolytic disease. C57BL/6 mice were administered $2 \times 10^8$ rat RBCs intraperitoneally (i.p.) once on study day 0 and then once per week thereafter for the remainder of the study. The rat RBC-immunized mice were then treated with an anti-CD200 antibody that possessed effector function (Antibody 1; Ab 1) at 5 mg/kg or 1 mg/kg; an anti-CD200 antibody that did not possess effector function (Antibody 2; Ab 2) at 5 mg/kg; or a control antibody (Cntl) at 5 mg/kg. A group of mice was also treated with vehicle only. A

An "epitope" refers to the site on a protein (e.g., a human CD200 protein) that is bound by an antibody. "Overlapping epitopes" include at least one (e.g., two, three, four, five, or six) common amino acid residue(s).

In some embodiments, the anti-CD200 antibody specifically binds to a human CD200 protein (e.g., the human CD200 protein having the amino acid sequence depicted in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or the extracellular domains of the mature forms of the CD200 proteins). The terms "specific binding" or "specifically binds" refer to two molecules forming a complex (e.g., a complex between an anti-CD200 antibody and a CD200 protein) that is relatively stable under physiologic conditions. Typically, binding is considered specific when the association constant ($K_a$) is higher than $10^6$ $M^{-1}$. Thus, an anti-CD200 antibody can specifically bind to a CD200 protein with a $K_a$ of at least (or greater than) $10^6$ (e.g., at least or greater than $10^7$, $10^8$, $10^9$, $10^{10}$, $10^{11}$ $10^{12}$, $10^{13}$, $10^{14}$, or $10^{15}$ or higher) $M^{-1}$. Examples of antibodies that specifically bind to a human CD200 protein are described in, e.g., U.S. Patent Nos.: 7,408,041; 7,427,665; 7,435,412; and 7,598,353~~, the disclosures of each of which are incorporated herein by reference in their entirety~~.

The amino acid sequences for several exemplary anti-CD200 antibodies are described in, e.g., U.S. Patent No. 7,408,041. For example, the anti-CD200 antibody can comprise the amino acid sequence of the heavy and light chain variable regions of one of the Fab antibodies – d1B10, d1A5, d1B5, c2aB7, c1A10, or c2aA10 – depicted in Fig. 23 of U.S. Patent No. 7,408,041~~, the sequences depicted in Fig. 23 being incorporated herein by reference in their entirety~~. In some embodiments, an anti-CD200 antibody described herein contains a paired set of heavy chain CDRs and light chain CDRs of one of the Fab antibodies depicted in Fig. 23 of U.S. Patent No. 7,408,041. For example, an anti-CD200 antibody described herein contains the paired set of CDRs from the d1B10 Fab antibody: a heavy chain CDR1 (HCDR1) comprising the following sequence: GFTFSGFAMS (SEQ ID NO:4); a heavy chain CDR2 (HCDR2) comprising the following sequence: SISSGGTTYYLDSVKG (SEQ ID NO:5); a heavy chain CDR3 (HCDR3) comprising the following sequence: GNYYSGTSYDY (SEQ ID NO:6); a light chain CDR1 (LCDR1) comprising the following sequence: RASESVDSYGNSFMH (SEQ ID NO:7); a light chain CDR2 (LCDR2) comprising the following sequence: RASNLES (SEQ ID NO:8); and a light

human CD200 protein. The peptide fragment can be a fragment of a human CD200 protein having the amino acid sequence depicted in, e.g., any one of SEQ ID NOs:1 to 3. As used herein, the term "crossblocking antibody" refers to an antibody that lowers the amount of binding of anti-CD200 antibody to an epitope on a CD200 protein relative to the amount of binding of the anti-CD200 antibody to the epitope in the absence of the antibody. Suitable methods for determining whether a first antibody crossblocks binding of a second antibody to an epitope are known in the art.

Methods for identifying the epitope to which a particular antibody (e.g., an anti-CD200 antibody) binds are also known in the art. For example, the binding epitope of an anti-CD200 antibody can be identified by measuring the binding of the antibody to several (e.g., three, four, five, six, seven, eight, nine, 10, 15, 20, or 30 or more) overlapping peptide fragments of a CD200 protein (e.g., several overlapping fragments of a protein having the amino acid sequence depicted in, e.g., any one of SEQ ID NOs:1 to 3). Each of the different overlapping peptides is then bound to a unique address on a solid support, e.g., separate wells of a multi-well assay plate. Next, the anti-CD200 antibody is interrogated by contacting it to each of the peptides in the assay plate for an amount of time and under conditions that allow for the antibody to bind to its epitope. Unbound anti-CD200 antibody is removed by washing each of the wells. Next, a detectably-labeled secondary antibody that binds to the anti-CD200 antibody, if present in a well of the plate, is contacted to each of the wells, and unbound secondary antibody is removed by washing steps. The presence or amount of the detectable signal produced by the detectably-labeled secondary antibody in a well is an indication that the anti-CD200 antibody binds to the particular peptide fragment associated with the well. *See*, e.g., Harlow and Lane (*supra*), Benny K. C. Lo (*supra*), and U.S. Patent Application Publication No. 20060153836~~, the disclosure of which is incorporated by reference in its entirety~~. A particular epitope to which an antibody binds can also be identified using BIAcore chromatographic techniques (see, e.g., Pharmacia BIAtechnology Handbook, "Epitope Mapping," Section 6.3.2, (May 1994); and Johne et al. (1993) *J Immunol Methods* 160:20191-8).

In some embodiments, an anti-CD200 antibody, or a CD200-binding fragment thereof, described herein binds to a human CD200 polypeptide expressed on the surface of a cell. Methods for determining whether an antibody binds to a protein expressed on the surface of a cell are known in the art and described in, e.g.,

precursor human CD20 isoform A protein. The amino acid sequence for a full-length human CD20 polypeptide is also described in, e.g., Tedder et al. (1988) *Proc Natl Acad Sci USA* 85(1):208-212.

An anti-CD20 antibody described herein binds to an epitope within the extracellular portion of a CD20 protein. For example, in some embodiments, the anti-CD20 antibody can bind to CD20 protein at an epitope within or overlapping with: (i) amino acids 72 to 80 of the amino acid sequence depicted in SEQ ID NO:40; or (ii) amino acids 140 to 186 of the amino acid sequence depicted in SEQ ID NO:40. *See*, e.g., Teeling et al. (2006) *J Immunol* 177:362-367. That is, an anti-CD20 antibody described herein can bind to an epitope of human CD20 within or overlapping with the amino acid sequence IPAGIYAPI (SEQ ID NO:41) or NIKISHFLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQYCYSI (SEQ ID NO:42).

In some embodiments, the anti-CD20 antibody specifically binds to a human CD20 protein (e.g., the human CD20 protein having the amino acid sequence depicted in SEQ ID NO:40 or one or more of the extracellular loops of the human CD20 protein). Examples of antibodies that specifically bind to a human CD20 protein are described in, e.g., Teeling et al. (2006) at 363, *supra*; Levene et al. (2005) *J R Soc Med* 98:146-152; and U.S. patent nos. 7,435,803; 5,595,721; and 7,422,739~~, the disclosures of each of which are incorporated herein by reference in their entirety~~.

Exemplary therapeutic anti-CD20 antibodies, which are approved for clinical use or are in clinical development, that can be used in the methods described herein include, without limitation, rituximab (Biogen Idec), $^{90}$Y-ibritumomab tiuxetan (Biogen Idec), $^{131}$I-tositumomab (GlaxoSmithKline), ofatumumab (Genmab), TRU-015 (Trubion), veltuzumab (IMMU-106; Immunomedics), ocrelizumab (Roche), and AME-133v (Applied Molecular Evolution). *See*, e.g., Levene et al. (2005), *supra*; Burge et al. (2008) *Clin Ther* 30(10):1806-1816; Kausar et al. (2009) *Expert Opin Biol Ther* 9(7):889-895; Morschhauser et al. (2009) *J Clin Oncol* 27(20):3346-3353; and Milani and Castillo (2009) *Curr Opin Mol Ther* 11(2):200-207.

Methods for determining whether an antibody binds to CD20 and/or the affinity of an antibody for CD20 are known in the art. In some embodiments, the anti-CD20 antibody can crossblock binding of another antibody that binds to an epitope within, or overlapping with, a human CD20 protein. In some embodiments,

formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known in the art. (See, e.g., J.R. Robinson (1978) "Sustained and Controlled Release Drug Delivery Systems," Marcel Dekker, Inc., New York.)

In some embodiments, an antibody described herein can be formulated in a composition suitable for intrapulmonary administration (e.g., for administration via nebulizer) to a mammal such as a human. Methods for preparing such compositions are well known in the art and described in, e.g., U.S. Patent Application Publication No. 20080202513; U.S. Patent Nos. 7,112,341 and 6,019,968; and PCT Publication Nos. WO 00/061178 and WO 06/122257~~, the disclosures of each of which are incorporated herein by reference in their entirety~~. Dry powder inhaler formulations and suitable systems for administration of the formulations are described in, e.g., U.S. Patent Application Publication No. 20070235029, PCT Publication No. WO 00/69887; and U.S. Patent No. 5,997,848.

In some embodiments, an anti-CD200 antibody (and/or an anti-CD20 therapeutic agent such as an anti-CD20 antibody) described herein can be modified, e.g., with a moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, or other tissues. The stabilization moiety can improve the stability, or retention of, the antibody by at least 1.5 (e.g., at least 2, 5, 10, 15, 20, 25, 30, 40, or 50 or more) fold.

In some embodiments, an anti-CD200 antibody described herein can be formulated with one or more additional active agents useful for treating cancer or ameliorating a symptom thereof. For example, an anti-CD200 antibody can be formulated with an anti-CD20 therapeutic agent (e.g., an anti-CD20 antibody such as any of the anti-CD20 antibodies described herein), a genotoxic agent or a chemotherapeutic agent, or one or more kinase inhibitors. The genotoxic or chemotherapeutic agent can be, but is not limited to: carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, bisulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide, podophyllotoxin, taxol, satraplatinum, 5-fluorouracil, vincristin, vinblastin, methotrexate, ara-C, taxotere, gemcitabine,

cisplatin (CDDP), adriamycin (ADR), or an analog of any of the aforementioned. Kinase inhibitors include, e.g., one or more of: trastuzumab, gefitinib, erlotinib, imatinib mesylate, or sunitinib malate. Additional agents are known in the art and described herein.

When the anti-CD200 antibody is to be used in combination with a second active agent, or when two or more different anti-CD200 antibodies are to be used, the agents can be formulated separately or together. For example, the respective pharmaceutical compositions can be mixed, e.g., just prior to administration, and administered together or can be administered separately, e.g., at the same or different times (see below).

As described above, a composition can be formulated such that it includes a therapeutically effective amount of an anti-CD200 antibody or the composition can be formulated to include a sub-therapeutic amount of the antibody and a sub-therapeutic amount of one or more additional active agents such that the components in total are therapeutically effective for treating a cancer or an autoimmune disorder. In some embodiments, a composition can be formulated to include two or more anti-CD200 antibodies, each at sub-therapeutic doses, such that the antibodies in combination are at a concentration that is therapeutically effective for treating a cancer or an autoimmune disorder in a human. Methods for determining a therapeutically effective dose of an anti-CD200 antibody are known in the art and described herein.

Methods for Producing an Anti-CD200 or an Anti-CD20 Antibody

Suitable methods for producing an antibody (e.g., an anti-CD200 antibody or an anti-CD20 antibody) or antigen-binding fragments thereof, in accordance with the disclosure are known in the art (see, e.g., U.S. Patent Nos. 7,427,665; 7,435,412; and 7,408,041~~, the disclosures of each of which are incorporated herein by reference in their entirety~~) and described herein. For example, monoclonal anti-CD200 antibodies may be generated using human CD200-expressing cells, a human CD200 polypeptide, or an antigenic fragment of a human CD200 polypeptide as an immunogen, thus raising an immune response in animals from which antibody-producing cells and in turn monoclonal antibodies may be isolated. Similarly, a monoclonal anti-CD20 antibody can be generated using human CD20-expressing cells, a human CD20 polypeptide, or an antigenic fragment of the human CD20 polypeptide as an

immunogen in an animal. The sequence of such antibodies may be determined and the antibodies or variants thereof produced by recombinant techniques. Recombinant techniques may be used to produce chimeric, CDR-grafted, humanized and fully human antibodies based on the sequence of the monoclonal antibodies as well as polypeptides capable of binding to the antigen of interest (e.g., CD200 or CD20).

Moreover, anti-CD200 antibodies derived from recombinant libraries ("phage antibodies") may be selected using CD200-expressing cells, or polypeptides derived therefrom, as bait to isolate the antibodies or polypeptides on the basis of target specificity. The production and isolation of non-human and chimeric anti-CD200 antibodies are well within the purview of the skilled artisan. It is understood that anti-CD20 antibodies can be selected using merely routine adaptations of the methods described above.

Recombinant DNA technology can be used to modify one or more characteristics of the antibodies produced in non-human cells. Thus, chimeric antibodies can be constructed in order to decrease the immunogenicity thereof in diagnostic or therapeutic applications. Moreover, immunogenicity can be minimized by humanizing the antibodies by CDR grafting and, optionally, framework modification. See, U.S. Patent Nos. 5,225,539 and 7,393,648~~, the contents of each of which are incorporated herein by reference~~.

Antibodies can be obtained from animal serum or, in the case of monoclonal antibodies or fragments thereof, produced in cell culture. Recombinant DNA technology can be used to produce the antibodies according to established procedure, including procedures in bacterial or preferably mammalian cell culture. The selected cell culture system preferably secretes the antibody product.

In another embodiment, a process for the production of an antibody disclosed herein includes culturing a host, e.g. *E. coli* or a mammalian cell, which has been transformed with a hybrid vector. The vector includes one or more expression cassettes containing a promoter operably linked to a first DNA sequence encoding a signal peptide linked in the proper reading frame to a second DNA sequence encoding the antibody protein. The antibody protein is then collected and isolated. Optionally, the expression cassette may include a promoter operably linked to a polycistronic (e.g., bicistronic) DNA sequence encoding antibody proteins each individually operably linked to a signal peptide in the proper reading frame.

Multiplication of hybridoma cells or mammalian host cells *in vitro* is carried out in suitable culture media, which include the customary standard culture media (such as, for example Dulbecco's Modified Eagle Medium (DMEM) or RPMI 1640 medium), optionally replenished by a mammalian serum (e.g. fetal calf serum), or trace elements and growth sustaining supplements (e.g. feeder cells such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages, 2-aminoethanol, insulin, transferrin, low density lipoprotein, oleic acid, or the like). Multiplication of host cells which are bacterial cells or yeast cells is likewise carried out in suitable culture media known in the art. For example, for bacteria suitable culture media include medium LE, NZCYM, NZYM, NZM, Terrific Broth, SOB, SOC, 2 xYT, or M9 Minimal Medium. For yeast, suitable culture media include medium YPD, YEPD, Minimal Medium, or Complete Minimal Dropout Medium.

*In vitro* production provides relatively pure antibody preparations and allows scale-up production to give large amounts of the desired antibodies. Techniques for bacterial cell, yeast, plant, or mammalian cell cultivation are known in the art and include homogeneous suspension culture (e.g. in an airlift reactor or in a continuous stirrer reactor), and immobilized or entrapped cell culture (e.g. in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges).

Large quantities of the desired antibodies can also be obtained by multiplying mammalian cells *in vivo*. For this purpose, hybridoma cells producing the desired antibodies are injected into histocompatible mammals to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially mineral oils such as pristane (tetramethyl-pentadecane), prior to the injection. After one to three weeks, the antibodies are isolated from the body fluids of those mammals. For example, hybridoma cells obtained by fusion of suitable myeloma cells with antibody-producing spleen cells from Balb/c mice, or transfected cells derived from hybridoma cell line Sp2/0 that produce the desired antibodies are injected intraperitoneally into Balb/c mice optionally pre-treated with pristane. After one to two weeks, ascitic fluid is taken from the animals.

The foregoing, and other, techniques are discussed in, for example, Kohler and Milstein, (1975) *Nature* 256:495-497; U.S. Patent No. 4,376,110; Harlow and Lane, Antibodies: a Laboratory Manual, (1988) Cold Spring Harbor~~, the disclosures of which are all incorporated herein by reference~~. Techniques for the preparation of

recombinant antibody molecules are described in the above references and also in, e.g.:WO97/08320; U.S. Patent No. 5,427,908; U.S. Patent No. 5,508,717; Smith (1985) *Science* 225:1315-1317; Parmley and Smith (1988) *Gene* 73:305-318; De La Cruz et al. (1988) *Journal of Biological Chemistry* 263:4318-4322; U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409; WO88/06630; WO92/15679; U.S. Patent No. 5,780,279; U.S. Patent No. 5,571,698; U.S. Patent No. 6,040,136; Davis et al. (1999) *Cancer Metastasis Rev.* 18(4):421-5; and Taylor et al. (1992) *Nucleic Acids Research* 20: 6287-6295; Tomizuka et al. (2000) *Proc. Natl. Acad. Sci. USA* 97(2): 722-727~~, the contents of each of which are incorporated herein by reference in their entirety~~.

The cell culture supernatants are screened for the desired antibodies, preferentially by immunofluorescent staining of CD200-expressing cells, by immunoblotting, by an enzyme immunoassay, e.g. a sandwich assay or a dot-assay, or a radioimmunoassay.

For isolation of the antibodies, the immunoglobulins in the culture supernatants or in the ascitic fluid may be concentrated, e.g., by precipitation with ammonium sulfate, dialysis against hygroscopic material such as polyethylene glycol, filtration through selective membranes, or the like. If necessary and/or desired, the antibodies are purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose and/or (immuno-) affinity chromatography, e.g., affinity chromatography with one or more surface polypeptides derived from a CD200-expressing cell line or synthetic CD200 fragment peptides, or with Protein-A or -G.

Another embodiment provides a process for the preparation of a bacterial cell line secreting antibodies directed against a human CD200 protein or a human CD20 (depending on the antibody being generated) in a suitable mammal. For example a rabbit is immunized with pooled samples from CD200-expressing tissue or cells or CD200 polypeptide or fragments thereof. A phage display library produced from the immunized rabbit is constructed and panned for the desired antibodies in accordance with methods well known in the art (such as, e.g., the methods disclosed in the various references~~ incorporated herein by reference~~).

Hybridoma cells secreting the monoclonal antibodies are also disclosed. The preferred hybridoma cells are genetically stable, secrete monoclonal antibodies

phage libraries can be created which use randomized combinations of synthetic human antibody V-regions. By selection on antigen fully human antibodies can be made in which the V-regions are very human-like in nature. See, e.g., U.S. Patent Nos. 6,794,132, 6,680,209, 4,634,666, and Ostberg et al. (1983) *Hybridoma* 2:361-367~~, the contents of each of which are incorporated herein by reference in their entirety~~.

For the generation of human antibodies, also see Mendez et al. (1998) *Nature Genetics* 15:146-156, Green and Jakobovits (1998) *J Exp Med* 188:483-495~~, the disclosures of which are hereby incorporated by reference in their entirety~~. Human antibodies are further discussed and delineated in U.S. Patent Nos.: 5,939,598; 6,673,986; 6,114,598; 6,075,181; 6,162,963; 6,150,584; 6,713,610; and 6,657,103 as well as U.S. Patent Publication Nos. 20030229905 A1, 20040010810 A1, US 20040093622 A1, 20060040363 A1, 20050054055 A1, 20050076395 A1, 20050287630 A1. See also International Publication Nos. WO 94/02602, WO 96/34096, and WO 98/24893, and European Patent No. EP 0 463 151 B1. The disclosures of each of the above-cited patents, applications~~, and references are hereby incorporated by reference in their entirety~~.

In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more $V_H$ genes, one or more $D_H$ genes, one or more $J_H$ genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in, e.g., U.S. Patent Nos.: 5,545,807; 5,545,806; 5,625,825; 5,625,126; 5,633,425; 5,661,016; 5,770,429; 5,789,650; and 5,814,318; 5,591,669; 5,612,205; 5,721,367; 5,789,215; 5,643,763; 5,569,825; 5,877,397; 6,300,129; 5,874,299; 6,255,458; and 7,041,871~~, the disclosures of which are hereby incorporated by reference~~. See also European Patent No. 0 546 073 B1, International Patent Publication Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884~~, the disclosures of each of which are hereby incorporated by reference in their entirety~~. See further Taylor et al. (1992) *Nucleic Acids Res* 20: 6287; Chen et al. (1993) *Int Immunol* 5: 647; Tuaillon et al. (1993) *Proc Natl Acad Sci USA* 90: 3720-4;

Choi et al. (1993) *Nature Genetics* 4: 117; Lonberg et al. (1994) *Nature* 368: 856-859; Taylor et al. (1994) *International Immunology* 6: 579-591; Tuaillon et al. (1995) *J. Immunol.* 154: 6453-65; Fishwild et al. (1996) *Nature Biotechnology* 14: 845; and Tuaillon et al. (2000) *Eur J Immunol* 10: 2998-3005~~, the disclosures of each of which are hereby incorporated by reference in their entirety~~.

In certain embodiments, de-immunized anti-CD200 antibodies or antigen-binding fragments thereof are provided. De-immunized antibodies or antigen-binding fragments thereof are those modified so as to render the antibody or antigen-binding fragment thereof non-immunogenic, or less immunogenic, to a given species. De-immunization can be achieved by modifying the antibody or antigen-binding fragment thereof utilizing any of a variety of techniques known to those skilled in the art (see, e.g., PCT Publication Nos. WO 04/108158 and WO 00/34317). For example, an antibody or antigen-binding fragment thereof may be de-immunized by identifying potential T cell epitopes and/or B cell epitopes within the amino acid sequence of the antibody or antigen-binding fragment thereof and removing one or more of the potential T cell epitopes and/or B cell epitopes from the antibody or antigen-binding fragment thereof, for example, using recombinant techniques. The modified antibody or antigen-binding fragment thereof may then optionally be produced and tested to identify antibodies or antigen-binding fragments thereof that have retained one or more desired biological activities, such as, for example, binding affinity, but have reduced immunogenicity. Methods for identifying potential T cell epitopes and/or B cell epitopes may be carried out using techniques known in the art, such as, for example, computational methods (see e.g., PCT Publication No. WO 02/069232), *in vitro* or *in silico* techniques, and biological assays or physical methods (such as, for example, determination of the binding of peptides to MHC molecules, determination of the binding of peptide:MHC complexes to the T cell receptors from the species to receive the antibody or antigen-binding fragment thereof, testing of the protein or peptide parts thereof using transgenic animals with the MHC molecules of the species to receive the antibody or antigen-binding fragment thereof, or testing with transgenic animals reconstituted with immune system cells from the species to receive the antibody or antigen-binding fragment thereof, etc.). In various embodiments, the de-immunized antibodies (e.g., deimmunized anti-CD200 antibodies or deimmunized anti-CD20 antibodies) described herein include de-immunized antigen-binding

to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (scFv) dimers has also been reported. *See*, e.g., Gruber et al. (1994) *J Immunol* 152:5368. Alternatively, the antibodies can be "linear antibodies" as described in, e.g., Zapata et al. (1995) *Protein Eng* 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments ($V_H$-$C_H1$-$V_H$-$C_H1$) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The disclosure also embraces variant forms of bispecific antibodies such as the tetravalent dual variable domain immunoglobulin (DVD-Ig) molecules described in Wu et al. (2007) *Nat Biotechnol* 25(11):1290-1297. The DVD-Ig molecules are designed such that two different light chain variable domains (VL) from two different parent antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Methods for generating DVD-Ig molecules from two parent antibodies are further described in, e.g., PCT Publication Nos. WO 08/024188 and WO 07/024715~~, the disclosures of each of which are incorporated herein by reference in their entirety~~.

In some embodiments, anti-CD200 antibodies and/or anti-CD20 antibodies can be modified, e.g., with a moiety that improves the stabilization and/or retention of the antibodies themselves in circulation, e.g., in blood, serum, or other tissues. For example, an anti-CD200 antibody described herein can be PEGylated as described in, e.g., Lee et al. (1999) *Bioconjug Chem* 10(6): 973-8; Kinstler et al. (2002) *Advanced Drug Deliveries Reviews* 54:477-485; and Roberts et al. (2002) *Advanced Drug Delivery Reviews* 54:459-476. The stabilization moiety can improve the stability, or retention of, the antibody in a subject's body (e.g., blood or tissue) by at least 1.5 (e.g., at least 2, 5, 10, 15, 20, 25, 30, 40, or 50 or more) fold.

Modification of the Antibodies or Antigen-Binding Fragments Thereof

The anti-CD200 antibodies or anti-CD20 antibodies, or antigen-binding fragments thereof, can be modified following their expression and purification. The modifications can be covalent or non-covalent modifications. Such modifications can

What is claimed is:

1. An anti-CD200 antibody, or a CD200-binding fragment thereof, for use in treating a human afflicted with a cancer that is resistant~~, or is suspected of being resistant.~~ to ~~therapy with~~ an anti-CD20 antibody therapy~~therapeutic agent~~.

~~2. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to claim 1, wherein the cancer comprises cancer cells that express CD5.~~

~~3~~2. ~~An~~ The anti-CD200 antibody, or ~~a~~ CD200-binding fragment thereof, for use ~~in treating a human afflicted with a~~ according to claim 1, wherein the cancer is a chronic lymphocytic leukemia (CLL) comprising ~~cancer~~ CLL cells having reduced expression of CD20, relative to the expression of CD20 by normal cells of the same histological type as the cells from which the cancer cells are derived.

~~4~~3. ~~An~~ The anti-CD200 antibody, or a CD200-binding fragment thereof, for use ~~in treating a human afflicted with a cancer comprising cancer~~ according to claim 2, wherein the CLL comprises CLL cells that express CD5.

~~5. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-4, wherein the cancer is a solid tumor.~~

~~6~~4. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to ~~any one of~~ claims ~~1-~~4, wherein the cancer is a liquid tumor.

~~7~~5. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to claim ~~6~~4, wherein the liquid tumor is a chronic lymphocytic leukemia or multiple myeloma.

~~8~~6. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims ~~7~~2, 3, or 5, wherein the chronic lymphocytic leukemia is a B cell chronic lymphocytic leukemia.

~~9. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-4, wherein the anti-CD200 antibody or CD200-binding fragment thereof is to be administered to the human in conjunction with an anti-CD20 therapeutic agent.~~

~~10. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to claim 9, wherein the anti-CD20 therapeutic agent is an anti-CD20 antibody or a CD20-binding fragment thereof.~~

~~11~~7. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims ~~10~~1-6, wherein the anti-CD20 antibody ~~is~~ therapy comprises rituximab, ofatumumab, TRU-015, veltuzumab, ocrelizumab, or AME-133v.

~~12~~8. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims ~~10~~1-6, wherein the anti-CD20 antibody therapy comprises an anti-CD20 antibody, or CD20-binding fragment thereof, ~~is~~ conjugated to a toxin.

9. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to claim 8, wherein the toxin is selected from the group consisting of a small molecule drug, a toxic polypeptide, a bacterial toxin, a fungal toxin, a plant toxin, or a radioactive agent.

~~13~~10. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-49, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is a bispecific antibody comprising a first antigen combining site that binds to CD200 and a second antigen combining site that binds to CD20.

~~14~~11. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-49, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, inhibits the interaction between CD200 and CD200R.

~~15~~12. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-49, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, contains one of the following paired set of CDRs:

(i) a heavy chain CDR1 (HCDR1) comprising the amino acid sequence: GFTFSGFAMS (SEQ ID NO:4); a heavy chain CDR2 (HCDR2) comprising the amino acid sequence: SISSGGTTYYLDSVKG (SEQ ID NO:5); a heavy chain CDR3 (HCDR3) comprising the amino acid sequence: GNYYSGTSYDY (SEQ ID NO:6); a light chain CDR1 (LCDR1) comprising the amino acid sequence: RASESVDSYGNSFMH (SEQ ID NO:7); a light chain CDR2 (LCDR2) comprising the amino acid sequence: RASNLES (SEQ ID NO:8); and a light chain CDR3 (LCDR3) comprising the amino acid sequence: QQSNEDPRT (SEQ ID NO:9);

(ii) a HCDR1 comprising the amino acid sequence: GFNIKDYYMH (SEQ ID NO:10); a HCDR2 comprising the amino acid sequence: WIDPENGDTKYAPKFQG (SEQ ID NO:11); a HCDR3 comprising the amino acid sequence: KNYYVSNYNFFDV (SEQ ID NO:12); a LCDR1 comprising the amino acid sequence: SASSSVRYMY (SEQ ID NO:13); a LCDR2 comprising the amino acid sequence: DTSKLAS (SEQ ID NO:14); and a LCDR3 comprising the amino acid sequence: FQGSGYPLT (SEQ ID NO:15);

(iii) a HCDR1 comprising the amino acid sequence: GFNIKDYYIH (SEQ ID NO:16); a HCDR2 comprising the amino acid sequence: WIDPEIGATKYVPKFQG (SEQ ID NO:17); a HCDR3 comprising the amino acid sequence: LYGNYDRYYAMDY (SEQ ID NO:18); a LCDR1 comprising the amino acid sequence: KASQNVRTAVA (SEQ ID NO:19); a LCDR2 comprising the amino acid sequence: LASNRHT (SEQ ID NO:20); and a LCDR3 comprising the amino acid sequence: LQHWNYPLT (SEQ ID NO:21);

(iv) a HCDR1 comprising the amino acid sequence: GYSFTDYIIL (SEQ ID NO:22); a HCDR2 comprising the amino acid sequence: HIDPYYGSSNYNLKFKG (SEQ ID NO:23); a HCDR3 comprising the amino acid sequence: SKRDYFDY (SEQ ID NO:24); a LCDR1 comprising the amino acid sequence: KASQDINSYLS (SEQ ID NO:25); a LCDR2 comprising the amino acid sequence: RANRLVD (SEQ ID NO:26); and a LCDR3 comprising the amino acid sequence: LQYDEFPYT (SEQ ID NO:27);

(v) a HCDR1 comprising the amino acid sequence: GYTFTEYTMH (SEQ ID NO:28); a HCDR2 comprising the amino acid sequence: GVNPNNGGALYNQKFKG (SEQ ID NO:29); a HCDR3 comprising the amino acid sequence: RSNYRYDDAMDY (SEQ ID NO:30); a LCDR1 comprising the amino acid sequence: KSSQSLLDIDEKTYLN (SEQ ID NO:31); a LCDR2 comprising the amino acid sequence: LVSKLDS (SEQ ID NO:32); and a LCDR3 comprising the amino acid sequence: WQGTHFPQT (SEQ ID NO:33); or

(vi) a HCDR1 comprising the amino acid sequence: AFNIKDHYMH (SEQ ID NO:34); a HCDR2 comprising the amino acid sequence: WIDPESGDTEYAPKFQG (SEQ ID NO:35); a HCDR3 comprising the amino acid sequence: FNGYQALDQ (SEQ ID NO:36); a LCDR1 comprising the amino acid sequence: TASSSVSSSYLH (SEQ ID NO:37); a LCDR2 comprising the amino acid sequence: STSNLAS (SEQ ID NO:38); and a LCDR3 comprising the amino acid sequence: RQYHRSPPIFT (SEQ ID NO:39).

~~16~~13. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-4~~9~~, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is murine, chimeric, humanized, or fully human.

~~17~~14. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to any one of claims 1-4~~9~~, wherein the CD200-binding fragment is selected from the group consisting of

a Fab fragment, a $F(ab')_2$ fragment, a Fab' fragment, an scFv fragment, a minibody, a diabody, or a triabody.

~~18. An anti-CD200 antibody, or CD200-binding fragment thereof, for use, in conjunction with an anti-CD20 therapeutic agent, in treating a human afflicted with cancer.~~

~~19. The anti-CD200 antibody, or CD200-binding fragment thereof, for use according to claim 18, wherein the anti-CD20 therapeutic agent is an anti-CD20 antibody or a CD20-binding fragment thereof.~~

~~20. A bispecific antibody comprising a first antigen combining site that binds to a CD200 protein and a second antigen combining site that binds to a CD20 protein.~~

~~21. An anti-CD200 antibody, or CD200-binding fragment thereof, for use in treating a human afflicted with an autoimmune disorder, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is to be administered to the human in an amount and with a frequency sufficient to maintain in the human one or more of the following physiological conditions:~~

~~(i) a decreased concentration of at least one $CD200^+$ leukocyte subset as compared to a control concentration;~~

~~(ii) an increased concentration of $F4/80^+$ cells as compared to a control concentration; and~~

~~(iii) a decreased concentration of at least one bone marrow stem cell subset as compared to a control concentration.~~

~~22. An anti-CD200 antibody, or CD200-binding fragment thereof, for use in treating a human afflicted with an autoimmune disorder, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is to be administered to the human in an amount and with a frequency sufficient to reduce in the human the concentration of an autoantibody associated with the autoimmune disorder to thereby treat the autoimmune disorder.~~

15. Use of an anti-CD200 antibody, or a CD200-binding fragment thereof, in the manufacture of a medicament for treating a human afflicted which a cancer that is resistant to an anti-CD20 antibody therapy.

16. The use according to claim 15, wherein the cancer is a chronic lymphocytic leukemia (CLL) comprising CLL cells having reduced expression of CD20, relative to the expression of CD20 by normal cells of the same histological type as the cells from which the cancer cells are derived.

17. The use according to claim 16, wherein the CLL comprises CLL cells that express CD5.

18. The use according to claim 15, the cancer is a liquid tumor.

19. The use according to claim 18, wherein the liquid tumor is a chronic lymphocytic leukemia or multiple myeloma.

20. The use according to any one of claims 16, 17, or 19, wherein the chronic lymphocytic leukemia is a B cell chronic lymphocytic leukemia.

21. The use according to any one of claims 15-20, wherein the anti-CD20 antibody therapy comprises rituximab, ofatumumab, TRU-015, veltuzumab, ocrelizumab, or AME-133v.

22. The use according to any one of claims 15-20, wherein the anti-CD20 antibody therapy comprises an anti-CD20 antibody, or CD20-binding fragment thereof, conjugated to a toxin.

23. The use according to claim 22, wherein the toxin is selected from the group consisting of a small molecule drug, a toxic polypeptide, a bacterial toxin, a fungal toxin, a plant toxin, or a radioactive agent.

24. The use according to any one of claims 15-23, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is a bispecific antibody comprising a first antigen combining site that binds to CD200 and a second antigen combining site that binds to CD20.

25. The use according to any one of claims 15-23, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, inhibits the interaction between CD200 and CD200R.

26. The use according to any one of claims 15 to 23, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, contains one of the following paired set of CDRs:

(i) a heavy chain CDR1 (HCDR1) comprising the amino acid sequence: GFTFSGFAMS (SEQ ID NO:4); a heavy chain CDR2 (HCDR2) comprising the amino acid

sequence: SISSGGTTYYLDSVKG (SEQ ID NO:5); a heavy chain CDR3 (HCDR3) comprising the amino acid sequence: GNYYSGTSYDY (SEQ ID NO:6); a light chain CDR1 (LCDR1) comprising the amino acid sequence: RASESVDSYGNSFMH (SEQ ID NO:7); a light chain CDR2 (LCDR2) comprising the amino acid sequence: RASNLES (SEQ ID NO:8); and a light chain CDR3 (LCDR3) comprising the amino acid sequence: QQSNEDPRT (SEQ ID NO:9);

(ii) a HCDR1 comprising the amino acid sequence: GFNIKDYYMH (SEQ ID NO:10); a HCDR2 comprising the amino acid sequence: WIDPENGDTKYAPKFQG (SEQ ID NO:11); a HCDR3 comprising the amino acid sequence: KNYYVSNYNFFDV (SEQ ID NO:12); a LCDR1 comprising the amino acid sequence: SASSSVRYMY (SEQ ID NO:13); a LCDR2 comprising the amino acid sequence: DTSKLAS (SEQ ID NO:14); and a LCDR3 comprising the amino acid sequence: FQGSGYPLT (SEQ ID NO:15);

(iii) a HCDR1 comprising the amino acid sequence: GFNIKDYYIH (SEQ ID NO:16); a HCDR2 comprising the amino acid sequence: WIDPEIGATKYVPKFQG (SEQ ID NO:17); a HCDR3 comprising the amino acid sequence: LYGNYDRYYAMDY (SEQ ID NO:18); a LCDR1 comprising the amino acid sequence: KASQNVRTAVA (SEQ ID NO:19); a LCDR2 comprising the amino acid sequence: LASNRHT (SEQ ID NO:20); and a LCDR3 comprising the amino acid sequence: LQHWNYPLT (SEQ ID NO:21);

(iv) a HCDR1 comprising the amino acid sequence: GYSFTDYIIL (SEQ ID NO:22); a HCDR2 comprising the amino acid sequence: HIDPYYGSSNYNLKFKG (SEQ ID NO:23); a HCDR3 comprising the amino acid sequence: SKRDYFDY (SEQ ID NO:24); a LCDR1 comprising the amino acid sequence: KASQDINSYLS (SEQ ID NO:25); a LCDR2 comprising the amino acid sequence: RANRLVD (SEQ ID NO:26); and a LCDR3 comprising the amino acid sequence: LQYDEFPYT (SEQ ID NO:27);

(v) a HCDR1 comprising the amino acid sequence: GYTFTEYTMH (SEQ ID NO:28); a HCDR2 comprising the amino acid sequence: GVNPNNGGALYNQKFKG (SEQ ID NO:29); a HCDR3 comprising the amino acid sequence: RSNYRYDDAMDY (SEQ ID NO:30); a LCDR1 comprising the amino acid sequence: KSSQSLLDIDEKTYLN (SEQ ID NO:31); a LCDR2 comprising the amino acid sequence: LVSKLDS (SEQ ID NO:32); and a LCDR3 comprising the amino acid sequence: WQGTHFPQT (SEQ ID NO:33); or

(vi) a HCDR1 comprising the amino acid sequence: AFNIKDHYMH (SEQ ID NO:34); a HCDR2 comprising the amino acid sequence: WIDPESGDTEYAPKFQG (SEQ ID NO:35); a HCDR3 comprising the amino acid sequence: FNGYQALDQ (SEQ ID NO:36); a LCDR1 comprising the amino acid sequence: TASSSVSSSYLH (SEQ ID NO:37);

a LCDR2 comprising the amino acid sequence: STSNLAS (SEQ ID NO:38); and a LCDR3 comprising the amino acid sequence: RQYHRSPPIFT (SEQ ID NO:39).

27. The use according to any one of claims 15 to 24, wherein the anti-CD200 antibody, or CD200-binding fragment thereof, is murine, chimeric, humanized, or fully human.

28. The use according to any one of claims 15 to 24, wherein the CD200-binding fragment is selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, a Fab' fragment, an scFv fragment, a minibody, a diabody, or a triabody.