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(54) **Title:** REAGENTS AND TREATMENT METHODS FOR AUTOIMMUNE DISEASES

(57) **Abstract:** The invention concerns treatment methods using anti-CD22 monoclonal antibodies with unique physiologic properties. In particular, the invention concerns methods for the treatment of B-cell malignancies and autoimmune diseases by administering an effective amount of a blocking anti-CD22 monoclonal antibody specifically binding to the first two Ig-like domains, or to an epitope within the first two Ig-like domains of native human CD22 (hCD22).

REAGENTS AND TREATMENT METHODS FOR AUTOIMMUNE DISEASES

[0001] This application claims priority from U.S. Provisional Application Serial No. 60/359,419, filed February 21, 2002 and U.S. Provisional Application Serial No. 60/420,472, filed October 21, 2002, both of which applications are hereby incorporated by reference in their entireties. The present invention was made with the support of Grant No. CA 81776 from the National Institutes of Health. The United States government has certain rights in this invention.

Background of the Invention

Field of the Invention

[0002] The present invention concerns the therapeutic use of certain anti-CD22 monoclonal antibodies with unique physiologic properties. More specifically, the invention concerns methods of treating B-cell malignancies, such as lymphomas and leukemias, and autoimmune diseases with blocking anti-CD22 antibodies having unique pro-apoptotic properties.

Description of the Related Art

[0003] CD22 is a membrane glycophosphoprotein found on nearly all B lymphocytes and most B-cell lymphomas. Cross-linking CD22 triggers CD22 tyrosine phosphorylation and assembles a complex of effector proteins that activate the stress-activated protein kinase (SAPK) pathway. CD22 cross-linking provides a potent costimulatory signal in primary B-cells and pro-apoptotic signal in neoplastic B-cells. Structurally, CD22 is a member of the "sialoadhesin" subclass of the immunoglobulin (Ig) gene superfamily, having seven extracellular Ig domains with a single amino-terminal V-set Ig domain and six C-2 set Ig domains. Wilson *et al., J. Exp. Med.* 173:137-146 (1991); Engel *et al., J. Exp. Med.* 181:1581-1586 (1995); and Torres *et al., J. Immunol.* 149:2641-2649 (1992). It has been shown that CD22 is a critical lymphocyte-specific signal transduction molecule which negatively and positively regulates B lymphocyte antigen receptor (BCR) signaling by recruiting signaling effector molecules to physiologically pertinent sites. Tedder *et al., Annu. Rev. Immunol.* 15:481-504 (1997); Sato *et al., Immunology* 10:287-297 (1998).

[0004] Anti-CD22 antibodies have been described, for example in U.S. Patent Nos. 5,484,892; 6,183,744; 6,187,287; 6,254,868, and in Tuscano *et al., Blood* 94(4):1382-92 (1999). The use of monoclonal antibodies, including anti-CD22 antibodies, in the treatment of non-Hodgkin's lymphoma is reviewed, for example, by Renner *et al., Leukemia* 11(Suppl. 2):S55-9 (1997). A humanized anti-CD22 antibody, LymphoCide™ (empatuzumab, Immunomedics, Inc.) is in Phase III clinical trials for the treatment of indolent and aggressive forms of non-Hodgkin's lymphomas. An yttrium-90-labeled version of this antibody is currently in Phase I clinical trials for the same indication.

[0005] Despite recent advances in cancer therapy, B-cell malignancies, such as the B-cell subtype of non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, are major contributors of cancer-related deaths. Accordingly, there is a great need for further, improved therapeutic regimens for the treatment of B-cell malignancies.

Autoimmune diseases as a whole cause significant morbidity and disability. Based on incidence data collected from 1965 to 1995, it has been estimated that approximately 1,186,015 persons will develop a new autoimmune disease over the next 5 years. Jacobsen et al. (*Clin. Immunol. Immunopathol.* **84**:223 (1997)) evaluated over 130 published studies and estimated that in 1996, 8.5 million people in the United States (3.2% of the population) had at least one of the 24 autoimmune diseases examined in these studies. Considering the major impact of autoimmune diseases on public health, effective and safe treatments are needed to address the burden of these disorders. Thus, there is a need in the art for improved reagents and methods for treating autoimmune disease.

Summary of the Invention

[0006] The present invention concerns an improved clinical approach for the treatment of B-cell malignancies and autoimmune disease in human patients, taking advantage of the unique properties of certain blocking anti-CD22 monoclonal antibodies.

[0007] In one aspect, the invention concerns a method for treating a human patient diagnosed with a B-cell malignancy, comprising (1) administering to the patient an effective amount of a blocking anti-CD22 monoclonal antibody specifically binding to the first two Ig-like domains or to an epitope associated with the first two Ig-like domains of native human CD22 (hCD22) of SEQ ID NO: 1, and (2) monitoring the response of the malignancy to the treatment.

[0008] In a further aspect, the invention concerns a method for treating a subject (*e.g.*, a human patient) diagnosed with an autoimmune disease, comprising (1) administering to the subject an effective amount of a blocking anti-CD22 monoclonal antibody and, optionally, (2) monitoring the response of the autoimmune disease to the treatment.

[0009] As further aspects, the present invention provides methods of reducing B cell activity, reducing the number of B cells or B cell subsets or even essentially eliminating B cells or particular B cell subsets, increasing turnover of B cells and/or reducing antibody production by B cells by administering to a subject (*e.g.*, a human patient) an effective amount of a blocking anti-CD22 monoclonal antibody; and optionally, (2) monitoring the response to the treatment. By "reducing" it is meant at least about a 25%, 35%, 50% or 75% decrease or more. By "essentially eliminating" it is meant at least about a 90%, 95%, 985 or more decrease or more. By "increasing turnover", it is meant at least about a 25%, 35%, 50%, 75%, 100%, 150% or more elevation in turnover rate.

[0010] In a particular embodiment, the antibody used binds to essentially the same epitope as an antibody selected from the group consisting of HB22-7 (HB11347), HB22-23 (HB11349), HB22-33, HB22-5, HB22-13, and HB22-196, preferably HB22-7, HB22-23, or HB22-33, more preferably HB22-7 or HB22-33.

[0011] In a further embodiment, the antibody blocks CD22 binding to its ligand by at least 70%, preferably by at least 80%.

[0012] In another embodiment, the antibody comprises a heavy chain comprising a V_H sequence having at least about 95 % sequence identity with the sequence of amino acids 1 to 100 of SEQ ID NO: 9 (HB22-5 V_H sequence); or amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 13 (HB22-13 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 19 (HB22-196 V_H sequence).

[0013] In yet another embodiment, the antibody comprises a heavy chain comprising a V_H sequence having at least about 95 % sequence identity with the sequence of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

[0014] In a still further embodiment, the antibody comprises a V_H sequence selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

[0015] In a different embodiment, the antibody comprises a light chain comprising a V_κ sequence having at least about 95 % sequence identity with the amino acid sequence of SEQ ID NO: 21 (HB22-5 V_κ sequence); or SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 25 (HB22-13 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence); or SEQ ID NO: 31 (HB22-196 V_κ sequence).

[0016] In a particular embodiment, the antibody comprises a light chain comprising a V_κ sequence having at least about 95 % sequence identity with the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0017] In a further embodiment, the antibody comprises a V_κ sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); SEQ ID NO: 27 (HB22-23 V_κ sequence); and SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0018] In a preferred embodiment, the antibody comprises V_H and V_κ sequences selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence) and the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence) and the amino acid sequence of SEQ ID NO: 27 (HB22-23 V_κ sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence) and the amino acid sequence of SEQ ID NO: 29 (HB22-33 V_κ sequence).

[0019] In a different aspect, the invention concerns nucleic acid encoding any of the antibody heavy or light chain variable regions discussed above, or any portion thereof.

[0020] As a further aspect the present invention provides polypeptides comprising the heavy or light chain variable regions discussed above, or a portion thereof.

[0021] The targeted condition can be any type of autoimmune disease or B-cell malignancy, including but not limited to localized B-cell malignancies, or any other condition in which B cells or antibodies are implicated. Typical representatives of B-cell malignancies are B-cell subtype of non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, hairy cell leukemia, and prolymphocytic leukemia.

[0022] The treatment methods of the present invention may be performed without any further treatment of malignant B cells or autoimmune disease. With respect to B-cell malignancy, the treatment method of the present invention typically provides improved cure rate and/or increased survival and/or superior tumor volume reduction when compared to no treatment, combination treatment with the same antibody and radioimmunotherapy, or with radioimmunotherapy alone.

[0023] The antibody can be a complete antibody, or an antibody fragment, including, for example, Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. Thus, the antibody may have an additional antigen specificity, e.g. may be a bispecific antibody. The bispecific antibody may, for example, additionally bind to another epitope to CD22. In addition, the bispecific antibody may have binding specificity for other antigens, such as, CD19, CD20, CD52, CD3, CD28, or HLA-DR10 (Lym-1); or for Fc receptors, e.g. CD16, CD64 and CD89.

[0024] The antibody may be chimeric, humanized, primatized, or human.

[0025] The administration of the antibody may be performed by any conventional route, such as intravenous (i.v.) administration by repeated intravenous infusions.

[0026] The response to the treatment may be monitored by methods well known for a skilled practitioner, including monitoring shrinkage of a solid tumor, e.g. by magnetic resonance imaging (MRI), or by measuring improvement or stabilization in clinical indicia of autoimmune disease, as known by those skilled in the art.

Brief Description of the Drawings

[0027] Figure 1 shows the amino acid sequence of human CD22 (hCD22), where the boundaries of the Ig-like domains (domains 1-7) are indicated

[0028] Figure 2. Whole body autoradiography of Raji and Ramos tumor-bearing nude mice injected with ^{111}In-2IT-BAD-antiCD22 (HB22-7). Mice were sacrificed and autoradiographed 48 hours after injection. Upper image is Raji-tumored mouse, lower image is Ramos-tumored mouse.

[0029] Figure 3. The temporal assessment of tumor volume in Raji-xenografted mice that were untreated or treated with 125 uCi ^{90}Y-DOTA-peptide-Lym-1 (RIT) alone, anti-CD22 alone (HB22-7), or three different sequences of RIT and HB22-7 (CMRIT) in trial 081500. Tumor volume was assessed three times per week. Mouse numbers for each treatment group are tabulated (Table 2).

[0030] Figure 4. Summary analysis of tumor volume observed in all independent xenograft trials. The trials were conducted as described in Figure 2. Mouse numbers for each trial are tabulated (Table 2).

[0031] Figure 5. The response and cure rate for Raji-xenografted mice that were treated as described in Figure 2. The tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84-day study); CR, complete regression (tumor disappeared for at least 7 days but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew). The data represents results of all independent trials.

[0032] Figure 6. Overall survival was assessed for Raji xenografted mice that were treated as described in Figure 2. Mice were euthanized when the tumor burden exceeded 2000 mg or at the end of the 84 day trial. The data represents results of all independent trials.

[0033] Figures 7a, 7b and 7c. Hematologic toxicity was assessed by measuring white blood cell (WBC) (Figure 7b), red blood cell (RBC) (Figure 7c) and platelet counts (Figure 7a) twice weekly in the Raji-xenografted mice that were treated as described in Figure 2. When compared to RIT alone there was no difference in hematologic toxicity in the CMRIT groups. In addition, there was no hematologic toxicity observed in the mice treated with HB22-7 alone.

[0034] Figure 8. Non-hematologic toxicity was assessed by measuring body weights twice weekly in Raji xenografted mice that were treated as described in Figure 2. There were no significant differences in body weights in any of the treatment groups in all five xenograft trials.

[0035] Figure 9. RIT clearance was assessed by measuring radioactivity in whole body (WB) and blood daily for 5 days after initiation of treatment with RIT. The results were reported after adjusting for decay based on the $T_{1/2}$ of ^{90}Y. There were no significant differences in RIT clearance in any of the CMRIT treatment groups.

[0036] Figure 10. V_H amino acid sequence analysis of anti-CD22 antibodies (Abs) that block ligand binding. Amino acid numbering and designations of the origins of the coding sequence for each Ab is according to the convention of Kabat *et al.* (*Sequences of Proteins of Immunological Interest,* U.S. Government Printing Office, Bethesda, MD, 1991), where amino acid positions 1-94, CDR1 and 2, and FR1, 2, and 3 are encoded by a V_H gene. Sequences that overlap with the 5' PCR primers are not shown. A dot indicates a gap inserted in the sequence to maximize alignment of similar amino acid sequences. Gaps in the sequences were introduced between V_H, D and J segments for clarity. The rank order of sequences shown was based on relatedness to the HB22-5 sequence.

[0037] Figures 11-16. Nucleotide and encoded amino acid sequences for heavy chain V_H-D-J_H junctional sequences for anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 8 and 9), HB22-7 (SEQ ID NOS: 10 and 11); HB22-13 (SEQ ID NOS: 12 and 13); HB22-23 (SEQ ID NOS: 14 and 15); HB22-33 (SEQ ID NOS: 16 and 17); and HB22-196 (SEQ ID NOS: 18 and 19). Sequences that overlap with the 5' PCR primers are indicated by double underlining. D region sequences are underlined.

[0038] Figure 17. Light chain Vκ amino acid sequence analysis of anti-CD22 Abs that block ligand binding. Amino acid numbering and designation of origins of the coding sequence for each Ab is according to the convention of Kabat *et al., supra.* The amino acid following the predicted signal sequence cleavage site is numbered 1. A dot indicates a gap inserted in the sequence to maximize alignment of similar amino acid sequences. Gaps in the sequences were introduced between Vκ, J segments and κ constant region (double underlined) sequences for clarity.

[0039] Figures 18-23. Nucleotide and deduced amino acid sequences for kappa light chain V-J-constant region junctional sequences for anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 20 and 21); HB22-7 (SEQ ID NOS: 22 and 23): HB22-13 (SEQ ID NOS: 24 and 25): HB22-23 (SEQ ID NOS: 26 and 27); HB22-33 (SEQ ID NOS: 28 and 29); and HB22-196 (SEQ ID NOS: 30 and 31). Sequences that overlap with the 5' PCR primers are indicated by double underlining.

Detailed Description of the Preferred Embodiments

[0040] 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. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0041] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

A. Definitions

[0042] Unless defined otherwise, 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.

[0043] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0044] The term "immunoglobulin" (Ig) is used to refer to the immunity-conferring portion of the globulin proteins of serum, and to other glycoproteins, which may not occur in nature but have the same functional characteristics. The term "immunoglobulin" or "Ig" specifically includes "antibodies" (Abs). While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Native immunoglobulins are secreted by differentiated B cells termed plasma cells, and immunoglobulins without any antigen specificity are produced at low levels by the lymph system and at increased levels by myelomas. As used herein, the terms "immunoglobulin," "Ig," and grammatical variants thereof are used to include antibodies (as hereinabove defined), and Ig molecules without antigen specificity.

[0045] Native immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) 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 (V_H) followed by a number of constant domains. 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. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

[0046] The main Ig isotypes (classes) found in serum, and the corresponding Ig heavy chains, shown in parentheses, are listed below:

[0047] IgG (γ chain): the principal Ig in serum, the main antibody raised in response to an antigen, this antibody crosses the placenta;

[0048] IgE (ε chain): this Ig binds tightly to mast cells and basophils, and when additionally bound to antigen, causes release of histamine and other mediators of immediate hypersensitivity; plays a primary role in allergic reactions, including hay fever, asthma and anaphylaxis; and may serve a protective role against parasites;

[0049] IgA (α chain): this Ig is present in external secretions, such as saliva, tears, mucous, and colostrum;

[0050] IgM (μ chain): the Ig first induced in response to an antigen; it typically has lower affinity than other antibody isotypes produced later and is typically pentameric.

[0051] IgD (δ chain): this Ig is found in relatively high concentrations in umbilical cord blood, may be an early cell receptor for antigen, and is the main lymphocyte cell surface molecule.

[0052] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including, but not limited to, full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments as long as they exhibit the desired biological activity.

[0053] "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable (V) domain. 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.

[0054] 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, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

[0055] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins), as well as fragments of such antibodies, as long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Oi *et al., Biotechologies* 4(3):214-221 (1986); and Liu *et al., Proc. Natl. Acad Sci. USA* 84:3439-43 (1987)).

[0056] "Humanized" or "CDR grafted" forms of non-human (*e.g.*, murine) antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are also replaced by corresponding non-human residues (so called "back mutations"). Furthermore, humanized antibodies may be modified to comprise residues which are not found in the recipient antibody or in the donor antibody, in order to further improve antibody properties, such as affinity. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region

(Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); and Reichmann *et al.*, *Nature* 332:323-329 (1988).

[0057] "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 Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0058] 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 too 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. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

[0059] The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). 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.

[0060] Antibodies of the IgG, IgE, IgA, IgM, and IgD isotypes may have the same variable regions, i.e. the same antigen binding cavities, even though they differ in the constant region of their heavy chains. The constant regions of an immunoglobulin, e.g. antibody are not involved directly in binding the antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity (ADCC).

[0061] Some of the main antibody isotypes (classes) are divided into further sub-classes. IgG has four known subclasses: IgG1 ($\gamma1$), IgG2 ($\gamma2$), IgG3 ($\gamma3$), and IgG4 ($\gamma4$), while IgA has two known sub-classes: IgA1 ($\alpha1$) and IgA2 ($\alpha2$).

[0062] The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

[0063] Antibodies which bind to domain 1 and/or 2 within the amino acid sequence of native sequence human CD22, or to essentially the same epitope(s) bound by any of the monoclonal antibodies specifically disclosed herein, such as HB22-7, HB22-23, and HB22-33, can be identified by "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. According to the gene fragment expression assays, the open reading frame encoding the protein is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the protein with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. The latter approach is suitable to define linear epitopes of about 5 to 15 amino acids.

[0064] An antibody binds "essentially 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 (e.g. competition ELISA assays), which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.

[0065] The term amino acid or amino acid residue, as used herein, refers to naturally occurring L amino acids or to D amino acids as described further below with respect to variants. The commonly used one- and three-letter abbreviations for amino acids are used herein (Bruce Alberts et al., Molecular Biology of the Cell, Garland Publishing, Inc., New York (3d ed. 1994)).

[0066] As used herein, the term "polypeptide" encompasses peptides and proteins, including fusion proteins.

[0067] “Sequence identity” is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a native polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The % sequence identity values can be generated by the NCBI BLAST2.0 software as defined by Altschul *et al.,* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res., 25:3389-3402. The parameters are set to default values, with the exception of the Penalty for mismatch, which is set to –1.

[0068] As used herein, “treatment” or "treating" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” or "treating" can also mean prolonging survival as compared to expected survival if not receiving treatment. “Treatment” or "treating" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, “treatment” or "treating" 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. With respect to autoimmune disease, the treatment results in some improvement, amelioration, stabilization and/or delay in at least one clinical symptom of the autoimmune disease in the subject. In the context of B cell malignancies, the treatment may reduce the number of malignant cells; reduce the tumor size; inhibit (slow down or stop) the spread of malignant cells, including infiltration into peripheral organs, e.g. soft tissue or bone; inhibit (slow down or stop) metastasis; inhibit tumor growth; provide relief from symptoms associated with a B cell malignancy; reduce mortality; improve quality of life, etc. Treatment with the antibodies herein may result in cytostatic and/or cytotoxic effects.

[0069] The term "B cell malignancy," and grammatical variants thereof, are used in the broadest sense to refer to malignancies or neoplasms of B cells that typically arise in lymphoid tissues, such as bone marrow or lymph nodes, but may also arise in non-lymphoid tissues, such as thyroid, gastrointestinal tract, salivary gland and conjunctiva. The treatment methods of the present invention specifically concern CD22-positive B cell malignancies including, without limitation, B-cell subtype of non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, hairy cell leukemia, and prolymphocytic leukemia.

[0070] The term "autoimmune disease" refers to a condition which results from, or is aggravated by, the production of antibodies reactive with normal body tissues. It is a condition in which the immune system mistakenly attacks the body's own organs and tissues.

B. Detailed Description

1. Antibodies

[0071] Blocking anti-CD22 monoclonal antibodies designated HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and HB22-196 are known, and have been disclosed in U.S. Patent No. 5,484,892, Tuscano *et al., Eur. J. Immunol.* 26:1246 (1996), and Tuscano *et al., Blood* 94(4), 1382-1392 (1999). HB22-7 and HB22-23 are available from the American Type Culture Collection (ATCC), 12302 Parklawn Drive, Rockville, Md. 20852, under Accession Nos. HB22347 and HB11349, respectively. The preparation of these antibodies is also described in Example 1 below. Epitope mapping of CD22 has shown that these blocking monoclonal antibodies bind to the first two Ig-like domains or to epitopes which are associated with the first two Ig-like domains of human CD22 (U.S. Patent No. 5,484,892 and Tedder *et al., Annu. Rev. Immunol.* 15:481-504 (1997)). The heavy and light chain variable region sequences of the antibodies are also disclosed in the present application.

[0072] The present invention is based, in part, on the unexpectedly superior properties of blocking anti-CD22 antibodies having the overall characteristics of HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and HB22-196 in the treatment of B-cell malignancies, based on results obtained in a xenograft model of B-cell type non-Hodgkin's lymphoma (NHL). The invention is further based on the use of blocking anti-CD22 antibodies having the overall characteristics of HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and HB22-196 in the treatment of autoimmune disease.

[0073] The anti-CD22 monoclonal antibodies can be made by any standard method known in the art, such as, for example, by the hybridoma method (Koehler and Milstein, *Nature* 256:495-497 (1975); and Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, (Academic Press, 1986)), or by recombinant techniques, disclosed, for example, in U.S. Patent No. 4,816,567, and by Wood *et al., Nature* 314:446-9 (1985).

[0074] It is now also possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g. Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90, 2551-255 (1993); Jakobovits *et al.*, *Nature* 362, 255-258 (1993).

[0075] Mendez *et al.* (*Nature Genetics* 15: 146-156 (1997)) have further improved the technology and have generated a line of transgenic mice designated as "Xenomouse II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletions in the endogenous J_H segment as described above. The Xenomouse II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ, δ and χ), and also harbors 800 kb of human κ locus containing 32 Vκ genes, Jκ segments and Cκ genes. The antibodies produced in these mice closely resemble those seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletions in the endogenous J_H segment that prevents gene rearrangement in the murine locus.

[0076] Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348, 552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature* 352, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V-genes derived from the spleens of immunized mice. A repertoire of V-genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222, 581-597 (1991), or Griffith *et al.*, *EMBO J.* 12, 725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks *et al.*, *Bio/Technol.* 10, 779-783 **[0077]**). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V-region genes with repertoires of naturally occurring variants (repertoires) of V-domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse *et al.*, *Nucl. Acids Res.* 21, 2265-2266 (1993).

[0078] For further information concerning the production of monoclonal antibodies see also Goding, J.W., Monoclonal Antibodies: Principles and Practice, 3rd Edition, Academic Press, Inc., London, San Diego, 1996; Liddell and Weeks: Antibody Technology: A Comprehensive Overview, Bios Scientific Publishers: Oxford, UK, 1995; Breitling and Dubel: Recombinant Antibodies, John Wiley & Sons, New York, 1999; and Phage Display: A Laboratory Manual, Barbas *et al.*, editors, Cold Springs Harbor Laboratory, Cold Spring Harbor, 2001.

[0079] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *J. Biochem. Biophys. Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). In another embodiment, the $F(ab')_2$ is formed using the leucine zipper GCN4 to promote assembly of the $F(ab')_2$ molecule. According to another approach, Fv, Fab or $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

[0080] Heteroconjugate antibodies, composed of two covalently joined antibodies, are also within the scope of the present invention. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373). Heteroconjugate antibodies may be made using any convenient cross-linking methods, using well known, commercially available cross-linking agents.

[0081] The antibodies of the present invention, whether rodent, human, or humanized may also have a further antigen-specificity, to form bispecific antibodies. The second binding specificity may be directed, for example, against a further B cell antigen, such as CD19, CD20, CD52, and other CD antigens expressed on B cells, especially antigens associated with the targeted B cell malignancy. For example, CD20 is known to be expressed in more than 90% of non-Hodgkin's lymphomas. An anti-CD20 antibody (Rituxan®, IDEC Pharmaceuticals) is in clinical use for the treatment of non-Hodgkin's lymphoma. CAMPATH-1H (anti-CD52w) is another antibody developed for treating B cell malignancies. Bispecific antibodies including a binding specificity to the CD20 or CD52 antigen are specifically included within the scope herein. Another B cell antigen to which the bispecific antibodies of the present invention can bind is HLA-DR10 (Lym-1), a known marker of non-Hodgkin's lymphoma. Bispecific antibodies can be generated to enhance tumor localization as well as to recruit and/or augment the tumor-specific immune response. Examples of other antigen targets include, CD3, CD28, and the Fc receptors (CD16, CD64 and CD89). Bispecific antibodies are expected to have enhanced cytotoxicity and, as a result, improved remission rate and survival.

[0082] Antibodies binding to essentially the same epitope as HB22-7, HB22-23, HB22-33, HB22-5, HB22-13, and/or HB22-196 can be identified by epitope mapping. The simplest way to determine whether two different antibodies recognize the same epitope is a competition binding assay. This method determines if the antibodies are able to block each other's binding to the antigen, and works for both conformational and linear epitopes. The competition binding assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. In the most common version of this assay, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies to the antigen is then measured using radioactive or enzyme labels. For further details see, for example, Wagener *et al., J. Immunol.*, 130:2308-2315 (1983); Wagener *et al., J. Immunol. Methods,* 68:269-274 (1984); Kuroki *et al., Cancer Res.* 50:4872-4879 (1990); Kuroki *et al., Immunol. Invest.* 21:523-538 (1992); Kuroki *et al., Hybridoma* 11:391-407 (1992), and Using Antibodies: A Laboratory Manual, Ed Harlow and David Lane editors, Cold Springs Harbor Laboratory Press, Cold Springs Harbor, New York, 1999, pp. 386-389.

[0083] Alternatively, or in addition, epitope mapping can be performed by using a technique based on fragmentation of the antigen to which the antibody binds, either randomly or by specific genetic construction, and determining the reactivity of the fragments obtained with the antibody. Fragmentation can also be performed on the nucleic acid level, for example by PCR technique, followed by transcription and translation into protein *in vitro* in the presence of radioactive amino acids. For further details see, for example, Harlow and Lane, *supra*, pp. 390-392.

[0084] According to a further method of epitope mapping, a set of overlapping peptides is synthesized, each corresponding to a small linear segment of the protein antigen, and arrayed on a solid phase. The panel of peptides is then probed with the test antibody, and bound antibody is detected using an enzyme-labeled secondary antibody. (Harlow and Lane, *supra*, pp. 393-396.)

[0085] An additional method well known in the art for epitope mapping is antibody selection from a random synthetic or a phage display peptide library. Phage display libraries are constructed by cloning complex mixtures of peptide-encoding oligonucleotides into the amino terminus of the minor coat protein gene of the f1-type ssDNA phage. Such phage display libraries are commercially available, for example, from New England Biolabs. The libraries are amplified as stocks, and then an aliquot sufficient to represent multiple copies of each independent clone is mixed with the antibody of interest. Antibody-bound phage are collected by a procedure called "biopanning," and unbound phage are removed. The bound phage are eluted and used to infect bacteria, and the selected stock is amplified. Individual plaques of the final selected stock are growth and checked for specific antibody reactivity, e.g. by ELISA, and the DNA around the insert site is sequenced. Analysis of the sequence encoding the peptide to which the antibody binds defines the specificity of the antibody. For further details see, e.g. Smith and Scott, *Methods Enzymol.* 217:228-257 (1993), and Harlow and Lane, *supra*, pp. 397-398.

[0086] Non-human (rodent) antibodies can be further modified to make them more suitable for human clinical application. Chimeric antibodies are produced with mouse variable region gene segments of desired specificity spliced into human constant domain gene segments (see, e.g. U.S. Patent No. 4,816,567).

[0087] Non-human (rodent) antibodies can also be humanized in order to avoid issues of antigenicity when using the antibodies in human therapy. Generally, a humanized antibody has one or more amino acid residues introduced into it from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature,* 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Despite the relatively straightforward nature of antibody humanization, simple grafting of the rodent CDR's into human frameworks (FR) does not always reconstitute the binding affinity and specificity of the original rodent monoclonal antibody. Properties of a humanized antibody can be improved by suitable design, including, for example, substitution of residues from the rodent antibody into the human framework (backmutations). The positions for such backmutations can be determined by sequence and structural analysis, or by analysis of the variable regions' three-dimensional model. In addition, phage display libraries can be used to vary amino acids at chosen positions within the antibody sequence. The properties of a humanized antibody are also affected by the choice of the human framework. Early experiments used a limited subset of well-characterized human monoclonal antibodies, irrespective of the sequence identity to the rodent monoclonal antibody (the so-called fixed frameworks approach). More recently, some groups use variable regions with high amino acid sequence identity to the rodent variable regions (homology matching or best-fit method). According to another approach, consensus or germline sequences are used, or fragments of the framework sequences within each light or heavy chain variable region are selected from several different human monoclonal antibodies.

[0088] Amino acid variants of antibodies prepared by any technique discussed above or otherwise available can be prepared by introducing appropriate nucleotide changes into the anti-CD22 DNA, or, for example, by peptide synthesis. The amino acid changes also may alter post-translational processes of the humanized or variant anti-CD22 antibody, such as changing the number or position of glycosylation sites.

[0089] Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, *Chem. Immunol.* 65:111-128 (1997); Wright and Morrison, *TibTECH* 15:26-32 (1997)). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd *et al., Mol. Immunol.* 32:1311-1318 (1996); Wittwe and Howard, *Biochem.* 29:4175-4180 (1990)), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund, *supra*; Wyss and Wagner, *Current Opin. Biotech.* 7:409-416 (1996)). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra *et al., Nature Med.* 1:237-243 (1995)). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd *et al., Mol. Immunol.* 32:1311-1318 (1996)), while selective removal of sialic acid residues using neuraminidase resulted in no loss of CMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III

(GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al., Mature Biotech.* 17:176-180 (1999)).

[0090] Glycosylation variants of antibodies can be prepared by modifying the glycosylation sites in the underlying nucleotide sequence. In addition, the glycosylation of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse *et al., J. Biol. Chem.* 272:9062-9070 (1997)). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5.278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. made defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

[0091] The antibodies of the present invention may also be used by the antibody-directed enzyme prodrug therapy (ADEPT). ADEPT is a technology that utilizes the specificity of monoclonal antibodies targeting tumor antigens to target catalytic enzymes to the surface of cancer cells. There, the enzymes are in position to activate prodrug forms (*e.g.,* a peptidyl chemotherapeutic agent, see WO81/01145) of anti-cancer drugs to their fully active form. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

[0092] Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328:457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

[0093] Immunoconjugates of the antibodies herein are also specifically encompassed by this invention. Immunoconjugates comprise an antibody conjugated to a cytotoxic agent, such as chemotherapeutic agent, a toxin, or a radioisotope.

[0094] Specifically, the efficacy of the anti-CD22 antibodies herein can be further enhanced by conjugation to a cytotoxic radioisotope, to allow targeting a radiotherapy specifically to target sites (radioimmunotherapy). Suitable radioisotopes include, for example, I^{131} and Y^{90} , both used in clinical practice. Other suitable radioisotopes include, without limitation, In^{111}, Cu^{67}, I^{131}, As^{211}, Bi^{212}, Bi^{213}, and Re^{186}.

[0095] Chemotherapeutic agents useful in the generation of immunoconjugates include, for example, adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, *e.g.*, paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, Rnace), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), 5-FU, 6-thioguanine, 6-mercaptopurine, actinomycin D, VP-16, chlorambucil, melphalan, and other related nitrogen mustards.

[0096] Toxins to be used in the immunoconjugates herein include, for example, diphtheria A chain, exotoxin A chain, ricin A chain, enomycin, and tricothecenes. Specifically included are antibody-maytansinoid and antibody-calicheamicin conjugates. Immunoconjugates containing maytansinoids are disclosed, for example, in U.S. Patent Nos. 5,208,020, 5,416,020 and European Patent EP 0 425 235.

See also Liu *et al., Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996). Antibody-calicheamicin conjugates are disclosed, e.g. in U. S. Patent Nos. 5,712,374; 5,714,586; 5,739,116; 5,767,285; 5,770,701; 5,770,710; 5,773,001; and 5,877,296.

[0097] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science*, 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See*, WO94/11026.

[0098] Covalent modifications of the anti-CD22 antibodies are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the antibody, if applicable. Other types of covalent modifications of the antibody are introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. A preferred type of covalent modification of the antibodies comprises linking the antibodies to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner well known in the art.

2. <u>Pharmaceutical Formulations and Treatment Methods</u>

[0099] B-cell type Non-Hodgkin's Lymphoma is a term that is used to encompass a large group (over 29 types) of lymphomas caused by malignant (cancerous) B cell lymphocytes, and represents a large subset of the known types of lymphoma. B-cells are known to undergo many changes in their life cycle dependent on complex intracellular signaling processes, and apparently different types of B-cell malignancies can occur at different stages of the life cycle of B-cells. At the stem cell stage, acute lymphocytic leukemia (ALL) or lymphoblastic lymphoma/leukemia can typically develop.

Precursor B-cells can develop precursor B lymphoblastic lymphoma/leukemia. Typical malignancies of immature B-cells include small non-cleaved cell lymphoma and possibly Burkitt's/non-Burkitt's lymphoma. B cells before antigen exposure typically develop chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma, while after antigen exposure typically follicular lymphomas, large cell lymphoma and immunoblastic lymphoma are observed. There are also classification systems that characterize B-cell lymphomas by the rate of growth distinguishing aggressive (fast growing) and indolent (slow growing) lymphomas. For example, Burkitt's/non-Burkitt's lymphoma and LCL lymphoma belong in the aggressive group, while indolent lymphomas include follicular center cell lymphomas (FCCL), follicular large cell lymphomas, and follicular small cleaved cell lymphomas.

[0100] Non-Hodgkin's Lymphomas are also characterized by the stage of development. Stage I: cancer is found in only one lymph node area, or in only one area or organ outside the lymph nodes. Stage II: (1) Cancer is found in two or more lymph node areas on the same side of the diaphragm (the thin muscle under the lungs that helps breathing), or, (2) cancer is found in only one area or organ outside the lymph nodes and in the lymph nodes around it, or (3) other lymph node areas on the same side of the diaphragm may also have cancer. Stage III: Cancer is found in lymph node areas on both sides of the diaphragm. The cancer may also have spread to an area or organ near the lymph node areas and/or to the spleen. Stage IV: (1) Cancer has spread to more than one organ or organs outside the lymph system; cancer cells may or may not be found in the lymph nodes near these organs, or (2) cancer has spread to only one organ outside the lymph system, but lymph nodes far away from that organ are involved.

[0101] Current treatment options of B-cell malignancies, including non-Hodgkin's lymphomas depend on the type and stage of malignancy. Typical treatment regimens include radiation therapy, also referred to as external beam therapy, chemotherapy, immunotherapy, and combinations of these approaches. One promising approach is radioimmunotherapy (RIT). With external beam therapy, a limited area of the body is irradiated. With chemotherapy, the treatment is systemic, and often adversely affects normal cells, causing severe toxic side-effects. Targeted RIT is an approach in which a B-cell specific antibody delivers a toxic substance to the site of tumor. The therapeutic potential of RIT in patients with B-cell NHL has been shown using different targets, including CD20, CD19, CD22, and HLA-DR10 (Lym-1). More recently, combined modality therapy (CMT) has become an increasingly frequent maneuver for the treatment of solid tumors, and includes radiosensitization of cancer cells by drugs, and the direct cytotoxic effect of chemotherapy. The most common chemotherapy regiment for treating NHL is Cyclophosphamide-Hydroxydoxorubicin-Oncovin (vincristine)-Prednisone (CHOP) combination therapy. A randomized study of aggressive, but early stage NHL showed superior results with CHOP plus involved field radiation over treatment with CHOP alone. Despite its promise, the disadvantage of treatments involving external beam radiation is that external beam radiation can only be delivered in high doses to a limited region of the body, while NHL is mostly widespread. Accordingly, CMT has proven clinically useful for locally advanced malignancies.

[0102] Another current approach is combined modality radioimmunotherapy (CMRIT), which pairs the specific delivery of systemic radiation (e.g. ^{90}Y-DOTA-peptide-Lym-1) to NHL with the systemic radiation sensitizing effects of an additional chemotherapeutic agent. Because in CMRIT radiation is delivered continuously, cancer cells that are hypoxic may re-oxygenate, or pass through the radiosensitive G_2/M phase of the cell cycle during the course of treatment, making cure more likely. In addition, CMRIT provides specificity first, by the specific targeting of NHL by Lym-1, and second by timing. This allows the radiation sensitizer to potentially synergize only at the sites targeted by RIT, thus maximizing efficacy and minimizing toxicity. Several previous xenograft studies have demonstrated improved synergy when the radiation synthesizer (Taxol) was given 24-48 hours after RIT.

[0103] Although CMRIT is currently viewed as the most advanced therapeutic approach for the treatment of NHL, the antibodies of the present invention alone have been demonstrated to provide superior results both in terms of tumor volume reduction, cure rate and overall survival, when tested in the well accepted Raji and Ramos lymphoma xenograft models.

[0104] Autoimmune diseases are caused by a breakdown in self-tolerance leading to subsequent immune responses against self, including the production of autoantibodies and deposition of immunoglobulin in affected tissues. Autoantibodies form immune complexes that promote complement and Fc-receptor mediated tissue inflammation and destruction. Since B cells are the source of autoantibodies, they afford a rational target for treatment of these types of immune-mediated diseases. B cells also can present antigen and regulate the development of effector T cells. The pathologic mechanisms of these diseases are complex and often involve a combination of humoral and cellular immune mechanisms.

[0105] Most autoimmune diseases result from, or are aggravated by, the production of antibodies reactive with normal body tissues. Antibodies are produced by B cells following antigen stimulation and activation. Therefore, blocking CD22 function can inhibit the production of antibodies, including autoreactive antibodies. More than 80 autoimmune diseases have been identified. Autoimmune diseases, their etiology and treatment are discussed extensively in the Autoimmune Diseases Research Plan published by the Autoimmune Diseases Coordinating Committee of the National Institutes of Health. Autoimmune diseases that can be treated according to the present invention include, but are not limited to immune complex disorders such as those that result in glomerulonephritis, Goodspature's syndrome, necrotizing vasculitis, lymphadenitis, peri-arteritis nodosa and systemic lupus erythematosis. Other illustrative autoimmune diseases include but are not limited to rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosis, psoriasis, ulcerative colitis, systemic sclerosis, dermatomyositis/polymyositis, anti-phospholipid antibody syndrome, scleroderma, perphigus vulgaris, ANCA-associated vasculitis (e.g., Wegener's granulomatosis, microscopic polyangiitis), urveitis, Sjögren's syndrome, Crohn's disease, Reiter's syndrome, ankylosing spondylitis, Lyme arthritis, Guillain-Barre syndrome, Hashimoto's thyroiditis, and cardiomyopathy. Other diseases associated with antibody production include, but are not limited to multiple sclerosis, atopic dermatitis, thrombocytopenic purpura, agranulocytosis, autoimmune hemolytic anemias, immune reactions against foreign antigens such as fetal A-B-O blood groups during pregnancy, myasthenia gravis, Type I diabetes, Graves' disease, and allergic responses. The methods of the invention may be used to treat any other disorder or condition in which B cells or antibodies are implicated including, for example, transplant rejection.

[0106] The anti-CD22 antibodies herein are typically administered in the form of pharmaceutical formulations well known to all pharmaceutical chemists. See, e.g. Remington's Pharmaceutical Sciences, (15th Edition, Mack Publishing Company, Easton, Pa. (1975)), particularly Chapter 87, by Blaug, Seymour. These formulations include for example, powders, pastes, ointments, jelly, waxes, oils, lipids, anhydrous absorption bases, oil-in-water or water-in-oil emulsions, emulsions carbowax (polyethylene glycols of a variety of molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. A typical dosage form is a sterile, isotonic, water-based solution suitable for administration by the intravenous (i.v.) route. The concentration of the antibodies of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

[0107] The compositions of the invention may also be administered via liposomes. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the composition of the invention to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a desired target, such as an antibody, or with other therapeutic or immunogenic compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al. *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0108] The antibodies of the present invention can be administered alone or in combination with other therapeutic regimens. For example, in the case of B-cell malignancies, such regimes or therapies include chemotherapy, radioimmunotherapy (RIT), chemotherapy and external beam radiation (combined modality therapy, CMT), combined modality radioimmunotherapy (CMRIT), or cytokines alone or in combination, etc. Thus, the anti-CD22 antibodies of the present invention can be combined with CHOP (Cyclophosphamide-Hydroxydoxorubicin-Oncovin (vincristine)-Prednisolone), the most common chemotherapy regimen for treating non-Hodgkin's lymphoma. In addition, the anti-CD22 antibodies herein may be administered in combination with other antibodies, including anti-CD19, anti-CD20 and other anti-CD22 antibodies, such as LymphoCide™ (Immunomedics, Inc.) or LymphoCide Y-90. See, for example, Stein *et al., Drugs of the Future* 18:997-1004 (1993); Behr *et al., Clinical Cancer Research* 5:3304s-33314s, 1999 (suppl.); Juweid *et al., Cancer Res.* 55:5899s-5907s, 1995; Behr *et al., Tumor Targeting* 3:32-40 (1998), and U.S. Pat. Nos. 6,183,744, 6,187,287, and 6,254,868.

[0109] The inventive treatments may also be employed in combination with other therapies for autoimmune disorders. In particular embodiments, the subject is treated with the antibodies of the invention as well as with an anti-CD20 antibody (*e.g.*, Rituxan®, IDEC Pharmaceuticals) and/or an anti-inflammatory drug (*e.g.*, corticosteroids).

[0110] In particular embodiments, the patients to be treated in accordance with the present invention will have CD22 expressed on their malignant B cells. The presence of the CD22 antigen can be confirmed by standard techniques, such as immunohistochemistry, FACS, binding assay with labeled (e.g. radiolabeled) anti-CD22 antibody.

[0111] The antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intra-arterial, intraperitoneal, oral, intralymphatic, intramuscular, intradermal, subcutaneous, and intranasal administration. In particular embodiments, the route of administration is via bolus or continuous infusion over a period of time, such as continuous or bolus infusion, once or twice a week. In other particular embodiments, the route of administration is by subcutaneous injection. The dosage depends on the nature, form, and stage of the targeted B cell malignancy or autoimmune disease, the patient's sex, age, condition, prior treatment history, other treatments used, and other factors typically considered by a skilled physician. For example, non-Hodgkin's lymphoma patients or patients with autoimmune disease may receive from about 50 to about 1500 mg/m^2/week, specifically from about 100 to about 1000 mg/m^2/week, more specifically from about 150 to about 500 mg/m^2/week of an anti-CD22 antibody as described herein.

[0112] The patients can be monitored by standard techniques known in the art to follow clinical indicia of B-cell malignancy or the particular autoimmune disease. For example, in the case of B-cell malignancy, tumor regression (e.g. tumor size in the case of solid tumors), the phenotype of circulating B-cells or of biopsied tissues using anti-CD22 antibodies can be monitored.

[0113] While the invention has been discussed with reference to human therapy, it will be understood that the antibodies of the present invention also find use in veterinary medicine. For example, feline malignant lymphoma occurs frequently in domestic cats, and shows similar characteristics to human non-Hodgkin's lymphoma (Bertone *et al., Am. J. Epidemiol.*156:268-73 (2002)). Similarly, dogs are known to develop a variety of lymphomas. Accordingly, the antibodies herein can be used to treat feline and canine malignant lymphoma. Animal models of autoimmune disease are also known in the art. Dosages, and routes of administration depend on the animal species to be treated, and their determination is well within the skill of a veterinary of ordinary skill.

[0114] Further details of the invention are provided in the following non-limiting examples.

EXAMPLES

[0115] Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. In addition to production as disclosed in the following examples, hybridoma producing monoclonal antibody HB22-7 (ATCC Accession No. HB11349) may be obtained from the American Type Culture Collection, Rockville, MD.

EXAMPLE 1

Production of anti-CD22 monoclonal antibodies

[0116] Monoclonal antibodies (mAbs) HB22-7 (IgG2b), HB22-23 (IgG2a) HB22-33 (IgM), HB22-5 (IgG2a), HB22-13 (IgG2a), HB22-22 (IgA), and HB22-196 were produced according to the method of Engel *et al., J Immunol* 15:4710 (1993) and U.S. Pat. No. 5,484,892. See, also Tuscano *et al., Blood* 94:1382-1392 (1999). However, other methods may be used. Briefly, the HB22 mAbs were produced via hybridoma techniques using a mouse pre-B cell line 300.19, stably transfected with full length CD22 cDNA, as the immunogen. More specifically, thirty-three mAbs reactive with CD22 were generated by the fusion of NS-1 myeloma cell with spleen cells from Balb/c mice immunized three times with a mouse pre-B cell line, 300.19, stably transfected with a full-length CD22 cDNA. Hybridomas producing mAb reactive with mouse L cells transfected with CD22 cDNA, but not with untransfected cells, were cloned twice and used to generate supernatant or ascites fluid. mAb isotypes were determined using the Mouse Monoclonal Antibody Isotyping Kit (Amersham, Arlington Heights, Ill.). IgGmAb were purified using the Affi-Gel Protein A MAPS II Kit (Bio-Rad, Richmond, Calif.). The HB22-33 mAb (IgM) containing euglobulin fraction of ascites fluid was precipitated by extensive dialysis against distilled water and was shown to be essentially pure mAb by SDS-PAGE analysis. As disclosed in Table II of U.S. Pat. No. 5,484,892, mAbs HB22-7, HB22-22, HB22-23, and HB22-33 completely blocked (80-100%) the binding of Daudi, Raji and Jurkat cells to CD22 transfected COS cells. mAbs HB22-5, HB22-13, HB22-24, and HB22-28 partially blocked adhesion (20-80%).

[0117] The region(s) on CD22 that mediates ligand binding was characterized by mAb cross-inhibition studies using the "Workshop" CD22-blocking mAb and a panel of mAb that identify five different epitopes on CD22 (epitopes A, B, C, D, and E (Schwartz-Albiez *et al.,* "The carbohydrate moiety of the CD22 antigen can be modulated by inhibitors of the glycosylation pathway." The binding specificities of the Workshop mAb are depicted pictorially in Fig. 3. In Leukocyte Typing IV. White Cell Differentiation Antigens, Knapp *et al., eds.*, Oxford University Press, Oxford, p. 65 (1989)). It has been found that three of the monoclonal antibodies herein, HB22-7, HB22-22, and HB22-23, bind to very close or the same epitopes on CD22. Results of the epitope-mapping of these and other blocking antibodies are disclosed in Tedder *et al., Annu. Rev. Immunol.* 15:481-504 (1997). Unlike other anti-CD22 antibodies proposed for therapy, the blocking antibodies of the present invention bind to an epitope within the first two Ig-like domains of the hCD22 amino acid sequence.

EXAMPLE 2

Raji and Ramos Lymphoma Xenograft Trials

[0118] This example describes the results from our independent Raji and Ramos lymphoma xenograft trials. Nude mice xenografts are important tools for preclinical evaluations. Nude mice bearing human non-Hodgkin's lymphoma (NHL) xenografts utilizing the lymphoma cell lines Raji and Ramos have proven utility for evaluating efficacy for treatment of NHL . (Buchsbaum *et al., Cancer Res.* 52(23):6476-6481 (1992) and Flavell *et al., Cancer Res.* 57:4824-4829 (1997)).

Materials and Methods

[0119] *Reagents.* Carrier-free ^{90}Y (Pacific Northwest National Laboratory, Richland, WA) and ^{111}In (Nordion, Kanata, Ontario, Canada) were purchased as chlorides in dilute HCl. Lym-1 (Techniclone, Inc Tustin, CA) is an IgG_{2a} mAb generated in mice immunized with human Burkitt's lymphoma cell nuclei. Lym-1 recognizes a cell surface 31-35 kD antigen on malignant B cells, and reacts with greater than 80% of human B cell NHL. Lym-1 purity was assessed according to the specifications that required greater than 95% pure monomeric IgG by polyacrylamide gel electrophoresis. ^{90}Y-DOTA-peptide-Lym-1 was prepared as previously described (O'Donnell *et al., Cancer. Biother. Radiopharm.* 13:251-361 (1998)). Assessment by HPLC, TLC, and cellulose acetate electrophoresis revealed that ^{90}Y-DOTA-peptide-Lym-1 was prepared to 98% radiochemical purity with less than 5% aggregate content.

[0120] The anti-CD22 mAb, HB22-7, was prepared as previously described (Tuscano *et al., Blood* 94:1382-1392 (1999)), using a Protein A Sepharose Fast Flow column (Pharmacia). HB22-7 purity was determined by HPLC and flow cytometry, and found to be >95% pure. Physiologic properties were determined by flow cytometric-based analysis of apoptotic induction (Apo-Tag, Pharmacia) and found to be consistent with previous published results (Tuscano *et al., supra*). Endotoxin removal was achieved using an ActiClean ETOX column (Sterogene), with final endotoxin levels determined to be < 0.15 Endotoxin Units (EU)/mg mAb (Bio Whitaker). The Lym-1 and HB22-7 mAbs met MAP (mouse antibody production) guidelines for murine, viral, mycoplasma, fungal, and bacterial contamination, as well as endotoxin, pyrogen and DNA content and general safety testing in animals.

[0121] *Cell lines and Scatchard Analysis.* Raji and Ramos Burkitt lymphoma cell lines were purchased from American Type Culture Collection (ATCC, Gathersberg, MD). Both cell lines stained for CD22 expression by flow cytometric methods utilizing the HB22-7 mAb, as described previously (Tuscano *et al., supra*). The cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum at 0.5 x10^6 cells/ml. A Scatchard analysis using Raji and Ramos cells was performed as described previously (Scatchard, G., *Ann. of NY Acad Sci.* 51:660 (1947)). Briefly, HB22-7 was labeled with ^{125}I by the chloramine T method (specific activity of 1.1μCi/μg). A competitive binding assay was performed utilizing serially diluted, unlabeled HB22-7.

[0122] *Mouse studies.* Female athymic BALB/c nu/nu mice (Harlan Sprague-Dawley), 7-9 weeks of age were maintained according to University of California, Davis animal care guidelines on a normal diet ad libitum and under pathogen-free conditions. Five mice were housed per cage. Raji or Ramos cells were harvested in logarithmic growth phase; 2.5-5.0 X 10^6 cells were injected subcutaneously into both sides of the abdomen of each mouse. Studies were initiated 3 weeks after implantation, when tumors were 28-328 mm^3. Groups consisted of untreated, 125μCi of RIT alone, 1.4 mg of HB22-7 alone, or the combination of RIT and HB22-7, with HB22-7 being administered 24 hours prior, simultaneously, or 24 hours after RIT. To minimize ambient radiation, bedding was changed daily for 1 week after treatment with ^{90}Y-DOTA-peptide-Lym-1, and twice weekly thereafter.

[0123] *Tumoricidal Effect.* Tumor volume was calculated as described by the formula for hemiellipsoids (DeNardo *et al., Clin. Cancer Res.* 3:71-79 (1997)). Initial tumor volume was defined as the volume on the day prior to treatment. Mean tumor volume was calculated for each group on each day of measurement; tumors that had completely regressed were considered to have a volume of zero. Tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84 day study); CR, complete regression (tumor disappeared for at least 7 days, but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew).

[0124] *Statistical Analysis.* Differences in response among treatment groups were evaluated using the Kruskall Walis rank sum test with the response ordered as none, PR, CR, and Cure. Survival time was also evaluated using the Kruskall Walis test. Tumor volume was compared at 3 time points: month 1 (day 26-29), month 2 (day 54-57), and at the end of the study (day 84). If an animal was sacrificed due to tumor-related causes, the last volume was carried forward and used in the analysis of later time points. Analysis of variance was used to test for differences among treatment groups. P values are two-tailed and represent the nominal p-values. Protection for multiple comparisons is provided by testing only within subsets of groups found to be statistically significantly different.

Results

Scatchard Analysis

[0125] Scatchard analysis was utilized to assess the binding affinity of HB22-7 and the number of CD22 receptors on Ramos and Raji cells. The cells were assayed for maximum binding percentage (Bmax), disassociation constant (Ka) and number of antibodies bound per cell. The results shown in Table 1 are the average of two experiments.

Table 1

I. PARAMETER	Cell Lines	
Cell line	Raji	Ramos
Bmax	53.5 ±0.9%	21.0 ± 1.3%
R^2	0.954	0.926
Ka	1.3 ±0.08 X 10^9	5.95 ±1.0 X 10^8
Antibody/cell	118,000	43,000

[0126] The Scatchard analysis (Table 1) revealed a nearly 2.5 fold increase in the number of HB22-7 antibodies bound per cell, and Bmax, and a 2 fold increase in Ka for Raji cells versus Ramos cells, respectively.

Whole Body Autoradiography

[0127] In order to assess HB22-7-specific tumor targeting, whole body autoradiography of tumor-bearing nude mice injected with ^{111}In-2IT-BAD-anti-CD22 (HB22-7) was performed. Forty eight hours after injection mice were sacrificed, sectioned and autoradiographed (Figure 2), as previously described (DeNardo *et al., Cancer* 3:71-79 (1997)). Autoradiography revealed intense tumor localization in the Raji-tumored mice and moderate localization in the Ramos-tumored mice. This targeting study is consistent with the Scatchard analysis that revealed less HB22-7 bound per Ramos cells as compared to Raji. However the rapid growth of Ramos tumors, and likely central necrosis, may also contribute to the apparent inferior targeting of Ramos.

Efficacy of RIT and CMRIT

[0128] The initial trial (081500) utilized 125 uCi of ^{90}Y-DOTA-peptide-Lym-1 alone or in combination with HB22-7 (1.4 mg) given either 24 hours prior, simultaneously, or 24 hours after RIT, (Figure 3). In this trial there were 5 mice per group with the exception of the group treated with RIT alone, which had 9 mice and 5 untreated controls (mouse numbers are tabulated in Table 2).

Table 2

Trial	Treatment Groups					
	No Tx	HB22-7	RIT	-24	@RIT	+24
081500	5	4	9	5	5	5
101600	5	6	5	5	3	5
011601	_	5	4	_	9	7
032701	_	5	2	_	3	12
052401	3	_	3	_	_	_
060401	5	5	_	_	_	_
071701	7	5	_	_	_	4
092101	4	_	_	_	_	_
102401	13	_	_	_	_	_
Total	42	30	23	10	20	33

[0129] As predicted from similar Raji xenograft studies with ^{90}Y-2IT-BAD-Lym-1, RIT alone resulted in maximal mean tumor volume reduction by day 21, with increasing tumor volume thereafter. Xenografts treated with ^{90}Y-2IT-BAD-Lym-1(RIT) and HB22-7 (CMRIT) demonstrated greater and more sustained mean tumor volume reduction, which was greatest when HB22-7 was administered simultaneously, and 24 hours after RIT. Surprisingly, HB22-7 administered alone resulted in stabilization of mean tumor volume by 2-3 weeks, then a gradual and sustained tumor volume reduction.

[0130] Several additional replicate trials were conducted with highly reproducible results (Table 2). The data from all trials were compiled and, when compared graphically, revealed results highly consistent with the initial study, (Figure 4). The initial tumor volume reductions were again greatest at approximately day 21 when HB22-7 was administered simultaneously and 24 hours after RIT. In mice treated with HB22-7 alone, the stabilization in tumor growth that began 2 weeks after treatment followed by gradual sustained tumor volume reduction was also replicated in all subsequent trials. Using analysis of variance, when examining all treatment groups at day 30 the differences were highly significant ($p<0.001$). While analysis of volume reduction in all treatment groups at day 60 did not demonstrate significant differences ($p=0.39$), the differences at day 84 again were significant ($p=0.003$). The results observed graphically revealed that the difference in volume reduction in the RIT/CMRIT groups was highly reproducible and different from HB22-7 alone and untreated control, however, comparison of volume reduction only in only RIT treatment groups (including CMRIT) at all time points assessed (day 30, 60, and 84) did not reveal significant differences ($p\geq0.5$). Additional CMRIT trials were done with HB22-7 being administered 48 and 72 hours after RIT. The extended interval between the administration of RIT and HB22-7 did not result in improved tumor volume reduction when compared to trials in which HB22-7 was given simultaneously and 24 hours after RIT (data not shown).

[0131] Response and cure rates were consistent with the effects of treatment on tumor volume, (Figure 5). Treatment with ^{90}Y-DOTA-peptide-Lym-1 alone produced 48% PR, 13% CR, and a 13% cure rate. In the CMRIT groups, the overall response rate was maximized when HB22-7 and RIT were administered simultaneously generating 45% PR, 15% CR and 25% cure. However in the CMRIT groups the cure rate was the greatest (39%) when HB22-7 was administered 24 hours after RIT, which compared favorably to the cure rates observed in the untreated (29%), RIT alone (13%), 24 hours prior (10%) and simultaneous (25%) treatment groups. When examining the degree of response (ranking cure better than CR, better than PR) in all treatment groups using the Kruskal Walis test, the differences were statistically significant ($p=0.01$). Individual comparisons against untreated controls were all statistically significant ($p<0.05$), with the exception of RIT alone ($p=0.06$) and HB22-7 given 24 hours prior to RIT ($p=0.16$). While comparison of only active treatment groups (RIT alone, CMRIT, and HB22-7) was not significantly different ($p=0.18$), the CMRIT groups treated with HB22-7 simultaneously and after 24 hours had the best observed pattern of response. Interestingly the group treated with HB22-7 alone had the highest cure rate (47%) which was a significant improvement when compared to the untreated controls ($p<0.05$).

[0132] Tumor volume regression and cure rates translated into a similar pattern of survival. At the end of the 84 day study period 38 and 42% of the untreated and RIT alone groups were alive respectively, (Figure 6). In the CMRIT treatment groups, survival increased to 67 and 50% when HB22-7 was administered simultaneously and 24 hours after RIT, respectively. Analysis of survival using Kruskal Walis was significant ($p< 0.05$) for comparison of all groups. Similar to the response rate analysis, comparison of survival in the RIT groups only did not reveal significant differences ($p=0.41$), however the best survival in these groups was consistently observed when HB22-7 was administered either simultaneous or 24 hours after RIT.

[0133] The best overall survival, 76%, was observed in the group treated with HB22-7 alone, a significant difference when compared to untreated control ($p=0.02$).

Toxicity

[0134] Hematologic and non-hematologic toxicities were assessed by blood counts and mouse weights, respectively (Figure 7a-c). WBC and platelet nadirs in the RIT treatment groups were at 14-20, and 10-14 days respectively. WBC and platelet recovery was approximately 28 and 21 days after treatment, respectively. The WBC and platelet nadirs were consistent with observations in previous studies that utilized 150uCi of ^{90}Y-2IT-BAD-Lym-1. The hematologic toxicity of RIT was not altered by co-administration of HB22-7. No hematologic toxicity was detected in mice treated with HB22-7 alone. Analysis of mononuclear cell counts in all treatment groups revealed that HB22-7 had no effect on RIT-mediated mononuclear cell nadirs (data not shown). Non-hematologic toxicity as assessed by changes in mouse weight, and was found to be equivalent in all treatment groups (Figure 8). There were no deaths due to toxicity in any treatment groups.

^{90}Y-DOTA-peptide-Lym-1 Pharmacokinetics

[0135] Blood and whole body clearances of ^{90}Y-DOTA-peptide-Lym-1 in Raji-tumored mice with or without HB22-7 were similar (Figure 9). The blood biological $T_{1/2}\ \alpha$ was 1.4 hours for RIT alone, and 2.2, 2.4, and 2.0 hours for the 24 hour prior, simultaneous and 24 hour after groups respectively. The blood biological $T_{1/2}\ \beta$ was 127 hours for the RIT alone group and 133, 87, and 103 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The whole body $T_{1/2}$ was 246 hours for RIT alone and 207, 207, and 196 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The addition of HB22-7 to RIT did not change the pharmacokinetics of ^{90}Y-DOTA-peptide-Lym-1.

Discussion

[0136] Raji xenograft studies were designed to determine if the anti-CD22 mAb (HB22-7) would generate additive or synergistic effects when combined with RIT to enhance apoptosis and/or DNA damage induced by low dose-rate radiation. The Raji xenograft nude mouse model has proven useful when used to assess toxicity and efficacy of RIT using ^{90}Y-2IT-BAD-Lym-1 RIT alone (O'Donnell *et al., Cancer Biotherapy and Radiopharmaceuticals* 13:351-361 (1998)). Responses in this pre-clinical model translated into significant efficacy in human clinical trials (O'Donnell *et al., Anticancer Res.* 20:3647-55 (2000); O'Donnell *et al., J. Nucl. Med.* 40:216 (1999) (Abstract)).

[0137] In the studies described in this Example, the addition of the anti-CD22 mAb HB22-7 to ^{90}Y-DOTA-peptide-Lym-1(125uCi) enhanced the efficacy of RIT without any change in toxicity. Previous Raji xenograft studies with 150 and 200μCi of ^{90}Y-2IT-BAD-Lym-1 generated response and cure rates that were comparable to those observed in the present study (O'Donnel *et al.,* (1998), *supra*). The 125 μCi dose of ^{90}Y-DOTA-peptide-Lym-1 was chosen based on these previous studies with the 2IT-BAD linker. While the previous studies with 2IT-BAD demonstrated greatest efficacy with the 200 μCi dose, the choice of 125 μCi was based on the hypothesis that HB22-7 would be synergistic or additive with RIT and the lower dose would allow for better assessment of these effects. The studies of this Example utilized a novel linker (DOTA-peptide) that has not been previously examined in lymphoma xenograft models. The DOTA-peptide linker was designed for enhanced hepatic degradation of unbound radiopharmaceutical thereby leading to a more favorable biodistribution. While tumor-specific uptake was not assessed in detail in this study, the toxicity profile observed with 125 uCi of ^{90}Y-DOTA-peptide-Lym-1 alone was acceptable with no treatment-related mortality and predictable leukocyte and platelet nadirs.

[0138] HB22-7 was chosen based on *in vitro* studies demonstrating pro-apoptotic and signaling effects (Tuscano *et al., Blood* 94:1382-1392 (1999)). The treatment dose of HB22-7 utilized was empiric, however, it was based on the amount that was shown to be effective at inducing apoptosis *in vitro* and extrapolating this to the mouse model. In addition, when formulating the dose of HB22-7 consideration was given to the equivalent (when adjusted for body surface area differences in humans versus mice) dose of Rituximab® used in human clinical trials. The approximation to the Rituximab®

dose was utilized based on the fact that this is the only naked mAb available that has demonstrated efficacy for the treatment of lymphoma, granted, the optimal dose of Rituximab® is currently undefined.

[0139] The study was designed to assess the efficacy of HB22-7 alone, the combination of RIT and HB22-7 as well as the effect of three different sequence combinations. The tumor volume reduction observed with ^{90}Y-DOTA-peptide-Lym-1 alone was consistent with previous studies with ^{90}Y-2IT-BAD-Lym-1 in terms of timing, magnitude, and duration of response (O'Donnel *et al.,* 1998, *supra*). RIT alone resulted in approximately 50% reduction in tumor volume 14 days after therapy. When assessing at the approximate point of maximal volume reduction (day 21-30) the addition of HB22-7 to RIT significantly enhanced the magnitude of response in a sequence specific manner. It appears that the addition of HB22-7 was most effective when administered simultaneously or 24 hours after RIT. The distinctive pattern of volume reduction was highly reproducible. Independent replicate trials demonstrated similar patterns and magnitude of tumor volume reduction. The improved reductions in tumor volume translated into superior response rates and survival. RIT alone generated 13% CR and 13% cures, the addition of HB22-7 increased the cure rate to 25% when administered simultaneously with RIT, and to 39% when HB22-7 was administered 24 hours after RIT.

[0140] This is the first time that a second monoclonal antibody has been combined with RIT, and demonstrates the potential of utilizing monoclonal antibodies or other agents with well defined physiologic properties that may augment efficacy without increasing toxicity.

[0141] Surprisingly the mice treated with HB22-7 alone had impressive tumor volume reduction and superior cure and survival rates when compared to all other treatment groups. Again, several independent trials generated highly consistent results with a delayed initial tumor volume stabilization, and then tumor volume reduction beginning approximately 14 days after treatment. This translated into the best cure and overall survival rates observed in any of the treatment groups.

[0142] In conclusion, the antibodies of the present invention, when administered alone, have been demonstrated to provide superior results in terms of tumor volume reduction, cure rate and overall survival when compared to other treatment regimens, including CMRIT, which is currently viewed as the most advanced therapeutic approach for the treatment of NHL.

EXAMPLE 3

Sequence Analysis of anti-CD22 Antibodies

V_H and Light Chain Gene Utilization

[0143] Cytoplasmic RNA was extracted from 1-10 x 10^5 hybridoma cells using the RNeasy Mini Kit (Qiagen Chatsworth, CA). First strand cDNA was synthesized from cytoplasmic RNA using oligo-dT primers (dT_{18}) and a Superscript Kit (Gibco BRL, Gaithersburg, MD). One µl of cDNA solution was used as template for PCR amplification of V_H genes. PCR reactions were carried out in a 100-µl volume of a reaction mixture composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTP (Perkin Elmer, Foster City, CA), 50 pmol of each primer, and 5 U of Taq polymerase (ISC Bioexpress, Kaysville, UT). Amplification was for 30 cycles (94°C for 1 min, 58° for 1 min, 72°C for 1 min; Thermocycler, Perkin Elmer). V_H genes were amplified using a promiscuous sense 5' V_H primer (Ms V_HE: 5' GGG AAT TCG AGG TGC AGC TGC AGG AGT CTG G 3'; SEQ ID NO: 2) as previously described (Kantor *et al., J. Immunol.* 158:1175-86 (1996)), and antisense primers complementary to the Cµ coding region (primer Cµ-in: 5' GAG GGG GAC ATT TGG GAA GGA CTG 3'; SEQ ID NO: 3) or the Cγ region (Primer Cγ1: 5' GAG TTC CAG GTC ACT GTC ACT GGC 3'; SEQ ID NO: 4).

[0144] Light chain cDNA was amplified using a sense Vκ primer [5' ATG GGC (AT)TC AAG ATG GAG TCA CA(GT) (AT)(CT)(CT) C(AT)G G 3'; SEQ ID NO: 5] and a Cλ antisense primer (5' ACT GGA TGG TGG GAA GAT G 3'; SEQ ID NO: 6).

[0145] HB22-33 light chain sequences were amplified using a different sense Vκ primer (5' ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG 3'; SEQ ID NO: 7).

[0146] Amplified PCR products were purified from agarose gels using the QIAquick gel purification kit (Qiagen) and were sequenced directly in both directions using an ABI 377 PRISM DNA sequencer after amplification using the Perkin Elmer Dye Terminator Sequencing system with AmpliTaq DNA polymerase and the same primers for initial PCR amplification. All V_H and light chain regions were sequenced completely on both the sense and anti-sense DNA strands.

[0147] The alignment of the V_H and $V\kappa$ amino acid sequences for anti-CD22 monoclonal antibodies HB22-5, HB22-7, HB22-13, HB22-23, HB22-33, and HB22-196 are shown in Figures 10 and 17, respectively. Figures 11-16 show the nucleotide and amino acid sequences for heavy chain V_H-D-J_H junctions of anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 8 and 9), HB22-7 (SEQ ID NOS: 10 and 11); HB-22-13 (SEQ ID NOS: 12 and 13); HB-22-23 (SEQ ID NOS: 14 and 15); HB-22-33 (SEQ ID NOS: 16 and 17); and HB-22-196 (SEQ ID NOS: 18 and 19). Figures 18-23 show the nucleotide and deduced amino acid sequences for kappa light chain V-J-constant region junctions of anti-CD22 Abs from hybridomas HB22-5 (SEQ ID NOS: 20 and 21); HB22-7 (SEQ ID NOS: 22 and 23); HB22-13 (SEQ ID NOS: 24 and 25) HB22-23 (SEQ ID NOS: 26 and 27); HB22-33 (SEQ ID NOS: 28 and 29); and HB22-196 (SEQ ID NOS: 30 and 31).

WHAT IS CLAIMED IS:

1. A method for treating a human patient diagnosed with an autoimmune disease, comprising (1) administering to said human patient an effective amount of a blocking anti-CD22 monoclonal antibody wherein said antibody comprises a heavy chain comprising a V_H sequence having at least about 95 % sequence identity with the sequence of amino acids 1 to 100 of SEQ ID NO: 9 (HB22-5 V_H sequence); or amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 13 (HB22-13 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 19 (HB22-196 V_H sequence); and (2) monitoring the response of said autoimmune disease to said treatment.

2. The method of claim 1 wherein said antibody comprises a heavy chain comprising a V_H sequence having at least about 95 % sequence identity with the sequence of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

3. The method of claim 2 wherein said antibody comprises a V_H sequence selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

4. A method for treating a human patient diagnosed with an autoimmune disease, comprising (1) administering to said human patient an effective amount of a blocking anti-CD22 monoclonal antibody wherein said antibody comprises a light chain comprising a V_κ sequence having at least about 95 % sequence identity with the amino acid sequence of SEQ ID NO: 21 (HB22-5 V_κ sequence); or SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 25 (HB22-13 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence); or SEQ ID NO: 31 (HB22-196 V_κ sequence); and (2) monitoring the response of said autoimmune disease to said treatment.

5. The method of claim 4 wherein said antibody comprises a light chain comprising a V_κ sequence having at least about 95 % sequence identity with the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence).

6. The method of claim 5 wherein said antibody comprises a V_κ sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); SEQ ID NO: 27 (HB22-23 V_κ sequence); and SEQ ID NO: 29 (HB22-33 V_κ sequence).

7. The method of claim 1 wherein said antibody comprises V_H and V_κ sequences selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence) and the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence) and the amino acid sequence of SEQ ID NO: 27 (HB22-23 V_κ sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence) and the amino acid sequence of SEQ ID NO: 29 (HB22-33 V_κ sequence).

8. The method of claim 1 wherein said treatment is unaccompanied by any other treatment for the autoimmune disease.

9. The method of claim 1 wherein said antibody is a fragment of a complete antibody.

10. The method of claim 9 wherein said antibody is selected from the group consisting of Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

11. The method of claim 1 wherein said antibody comprises antigen-specificity and is effective to bind to the first two Ig-like domains of a CD22 molecule, or to bind to an epitope within the first two Ig-like domains of native human CD22 (hCD22) of SEQ ID NO: 1, and further has an additional antigen-specificity.

12. The method of claim 10 wherein said antibody is a bispecific antibody.

13. The method of claim 12 wherein said antibody additionally binds to another epitope of CD22.

14. The method of claim 1 wherein said antibody is chimeric.

15. The method of claim 1 wherein said antibody is humanized.

16. The method of claim 1 wherein said antibody is human.

17. The method of claim 1 wherein said antibody is administered intravenously.

18. The method of claim 17 wherein said antibody is administered by weekly intravenous infusions.

19. The method of Claim 1, wherein said human patient is further administered an anti-CD20 antibody.

20. The method of claim 7 wherein said antibody is chimeric.

21. The method of claim 7 wherein said antibody is humanized.

22. The method of claim 7 wherein said antibody is human.

23. The method of claim 4 wherein said treatment is unaccompanied by any other treatment for the autoimmune disease.

24. The method of claim 4 wherein said antibody is a fragment of a complete antibody.

25. The method of claim 24 wherein said antibody is selected from the group consisting of Fab, Fab', $F(ab')_2$, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

26. The method of claim 4 wherein said antibody comprises antigen-specificity and is effective to bind to the first two Ig-like domains of a CD22 molecule, or to bind to an epitope within the first two Ig-like domains of native human CD22 (hCD22) of SEQ ID NO: 1, and further has an additional antigen-specificity.

27. The method of claim 25 wherein said antibody is a bispecific antibody.

28. The method of claim 27 wherein said antibody additionally binds to another epitope of CD22.

29. The method of claim 4 wherein said antibody is chimeric.

30. The method of claim 4 wherein said antibody is humanized.

31. The method of claim 4 wherein said antibody is human.

32. The method of claim 4 wherein said antibody is administered intravenously.

33. The method of claim 32 wherein said antibody is administered by weekly intravenous infusions.

34. The method of claim 4, wherein said human patient is further administered an anti-CD20 antibody.

35. An isolated nucleic acid molecule comprising nucleic acid encoding an antibody heavy chain variable region comprising a V_H sequence having at least about 95 % sequence identity with the sequence of amino acids 1 to 100 of SEQ ID NO: 9 (HB22-5 V_H sequence); or amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); or

amino acids 1 to 100 of SEQ ID NO: 13 (HB22-13 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); or amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence); or amino acids 1 to 100 of SEQ ID NO: 19 (HB22-196 V_H sequence).

36. An isolated nucleic acid molecule comprising nucleic acid encoding an antibody light chain variable region comprising a V_κ sequence having at least about 95 % sequence identity with the amino acid sequence of SEQ ID NO: 21 (HB22-5 V_κ sequence); or SEQ ID NO: 23 (HB22-7 V_κ sequence); or SEQ ID NO: 25 (HB22-13 V_κ sequence); or SEQ ID NO: 27 (HB22-23 V_κ sequence); or SEQ ID NO: 29 (HB22-33 V_κ sequence); or SEQ ID NO: 31 (HB22-196 V_κ sequence).

37. An isolated nucleic acid molecule comprising nucleic acid encoding a V_H sequence selected from the group consisting of amino acids 1 to 97 of SEQ ID NO: 11 (HB22-7 V_H sequence); amino acids 1 to 100 of SEQ ID NO: 15 (HB22-23 V_H sequence); and amino acids 1 to 98 of SEQ ID NO: 17 (HB22-33 V_H sequence).

38. An isolated nucleic acid molecule comprising nucleic acid encoding a V_κ sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 23 (HB22-7 V_κ sequence); SEQ ID NO: 27 (HB22-23 V_κ sequence); and SEQ ID NO: 29 (HB22-33 V_κ sequence).

Figure 1

→ domain 1

1 MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPC

45 TYRALDGDLESFILFHNPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQ

95 LGDKNKNCTLSIHPVHLNDSGQLGLRMESKTEKWM

domain 1 ← → domain 2

130 ERIHLNVSE | RPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPIQL

175 QWLLEGVPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTC

domain 2 ← → domain 3

220 QLQDADGKFLSNDTVQLNVKH | TPKLEIKVTPSDAIVREGDSVTMT

265 CEVSSSNPEYTTVSWLKDGTSLKKQNTFTLNLREVTKDQSGKYCC

domain 3 ← → domain 4

310 QVSNDVGPGRSEEVFLQVQY | APEPSTVQILHSPAVEGSQVEFLCM

355 SLANPLPTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVAE

domain 4 ← → domain 5

400 NILGTGQRGPGAELDVQY | PPKKVTTVIQNPMPIREGDTVTLSCNY

445 NSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGWDNTTIACARCNS

domain 5 ← → domain 6

490 WCSWASPVALNVQY | APRDVRVRKIKPLSEIHSGNSVSLQCDFSSS

535 HPKEVQFFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQ

domain 6 ← → domain 7

580 TASKAWTLEVLY | APRRLRVSMSPGDQVMEGKSATLTCESDANPPV

625 SHYTWFDWNNQSLPHHSQKLRLEPVKVQHSGAYWCQGTNSVGKGR

domain 7 ←

670 SPLSTLTVYY | SPETIGRRVAVGLGSCLAILILAICGLKLQRRWKR

715 TQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDG

760 ISYTTLRFPEMNIPRTGDAESSEMQRPPRTCDDTVTYSALHKRQV

805 GDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH"

(SEQ ID NO: 1)

Figure 2

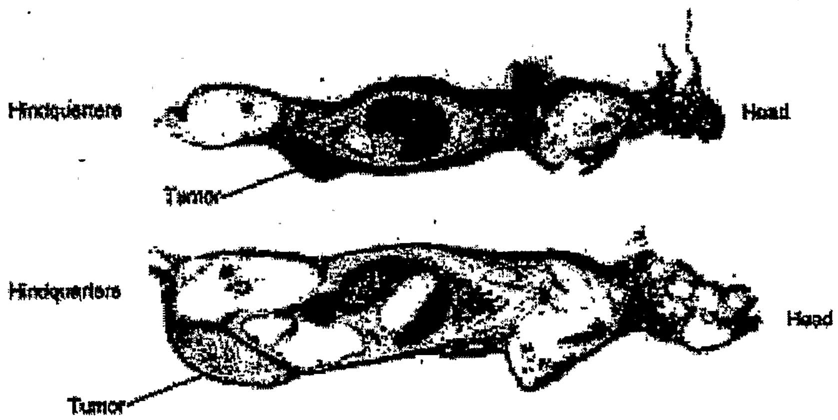


Figure 3

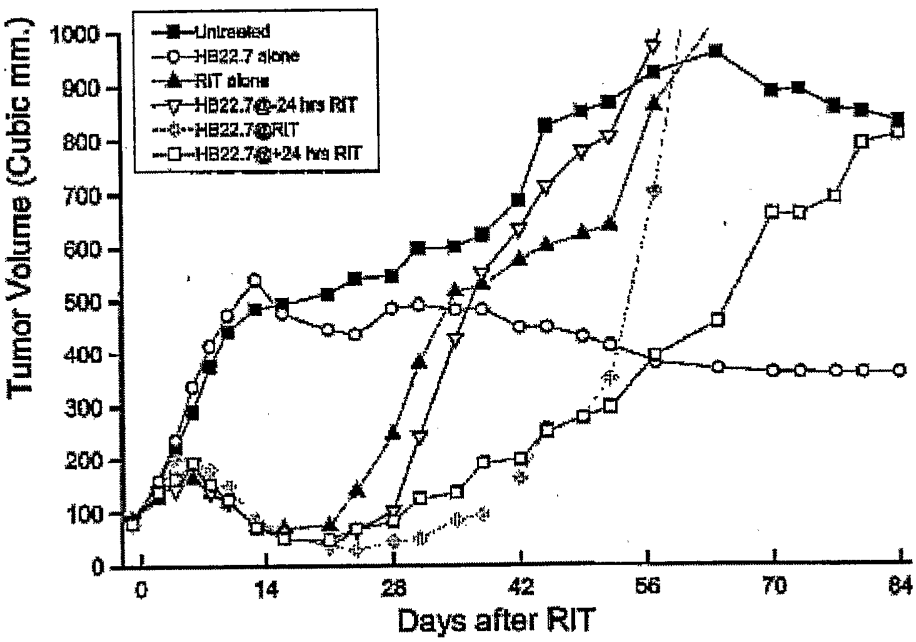
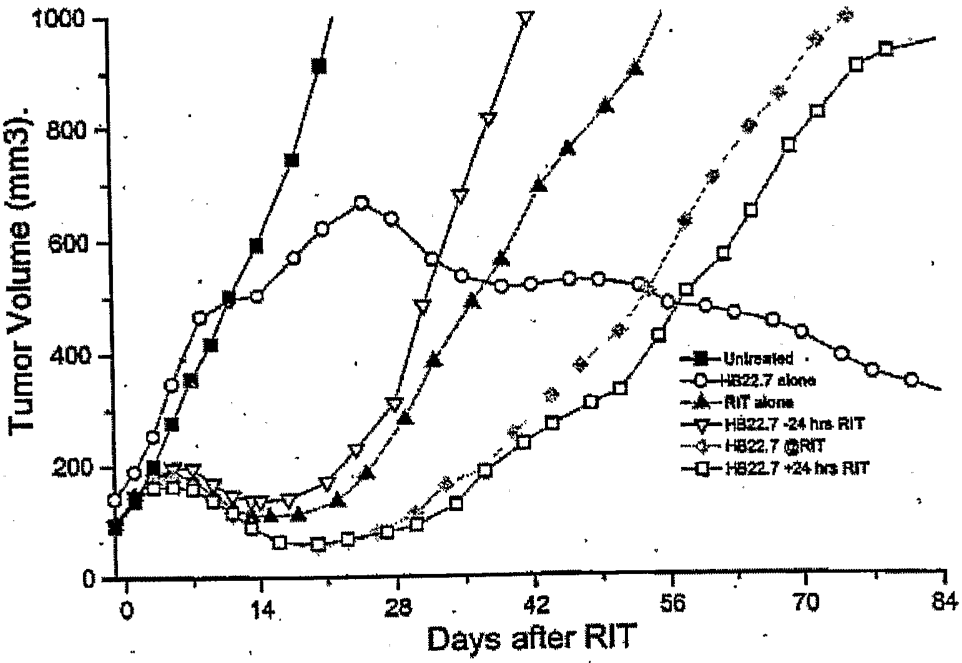

Untreated
HB22.7 alone
RIT alone
HB22.7@-24 hrs RIT
HB22.7@RIT
HB22.7@+24 hrs RIT
Tumor Volume (Cubic mm.)
1000
900
800
700
600
500
400
300
200
100
0
0
14
28
42
56
70
84
Days after RIT

Figure 4


1000
800
600
400
200
0
Tumor Volume (mm3).
0
14
28
42
56
70
84
Days after RIT
Untreated
HB22.7 alone
RIT alone
HB22.7 -24 hrs RIT
HB22.7 @RIT
HB22.7 +24 hrs RIT

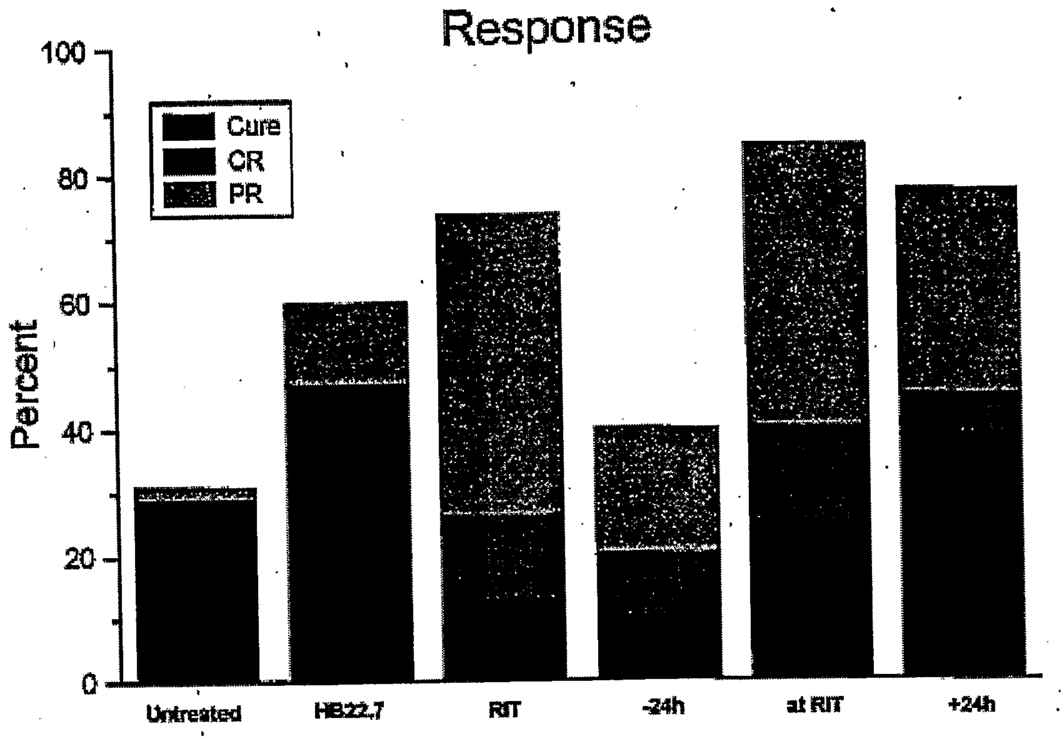

Response
100
80
60
40
20
0
Percent
Cure
CR
PR
Untreated
HB22.7
RIT
-24h
at RIT
+24h

Figure 5

Figure 6

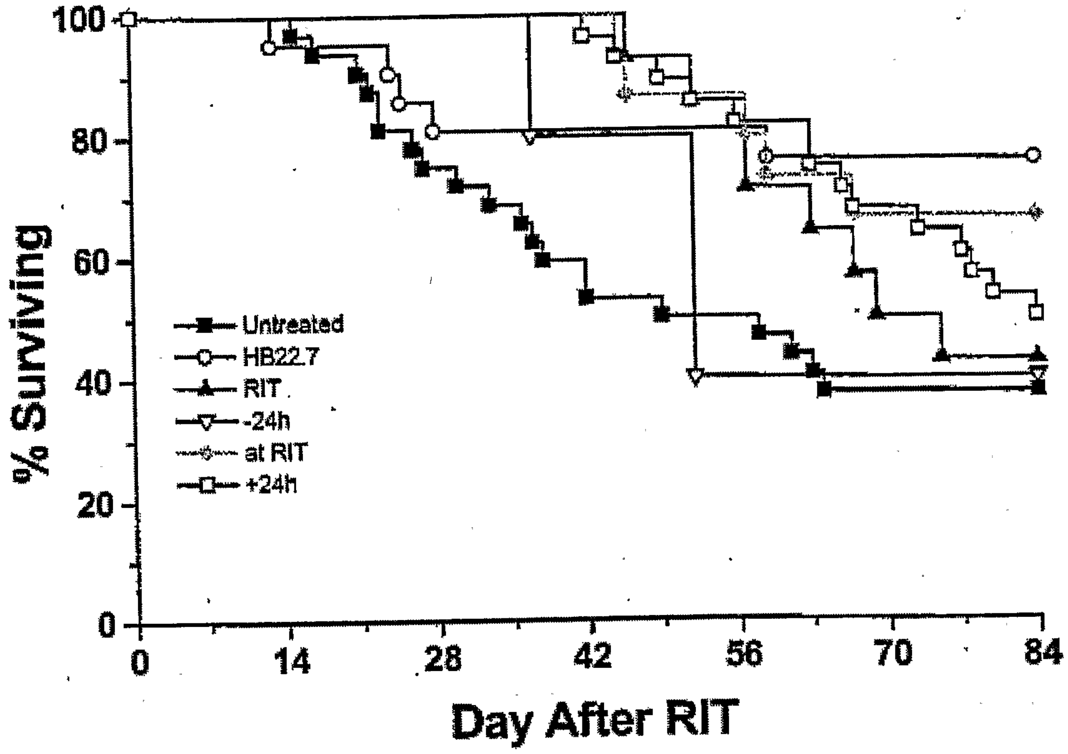

100
80
60
40
20
0
% Surviving
0
14
28
42
56
70
84
Day After RIT
Untreated
HB22.7
RIT
-24h
at RIT
+24h

Figure 7

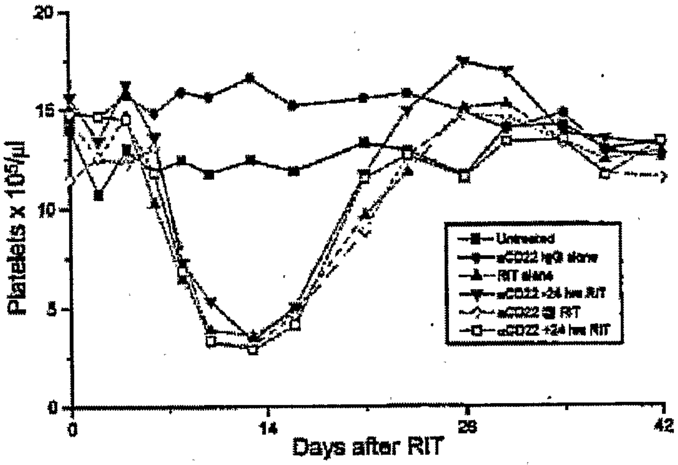

20
15
10
5
0
Platelets x 10⁵/µl
0
14
28
42
Days after RIT
Untreated
RIT alone

Figure 8

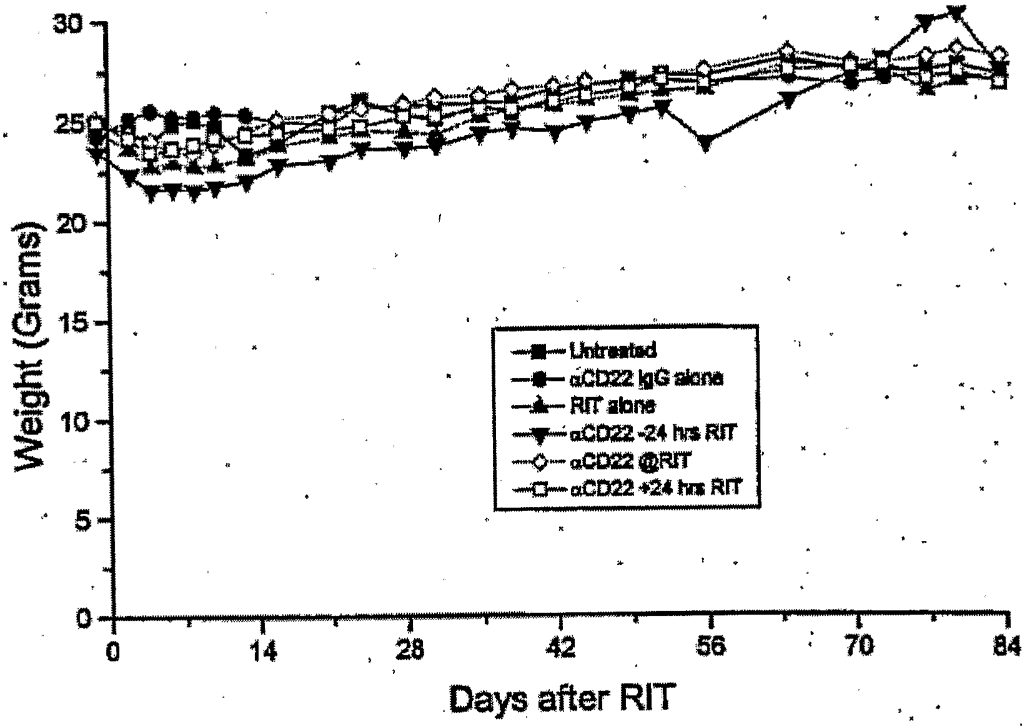

30
25
20
15
10
5
0
Weight (Grams)
0
14
28
42
56
70
84
Days after RIT
Untreated
αCD22 IgG alone
RIT alone
αCD22 -24 hrs RIT
αCD22 @RIT
αCD22 +24 hrs RIT

Figure 9

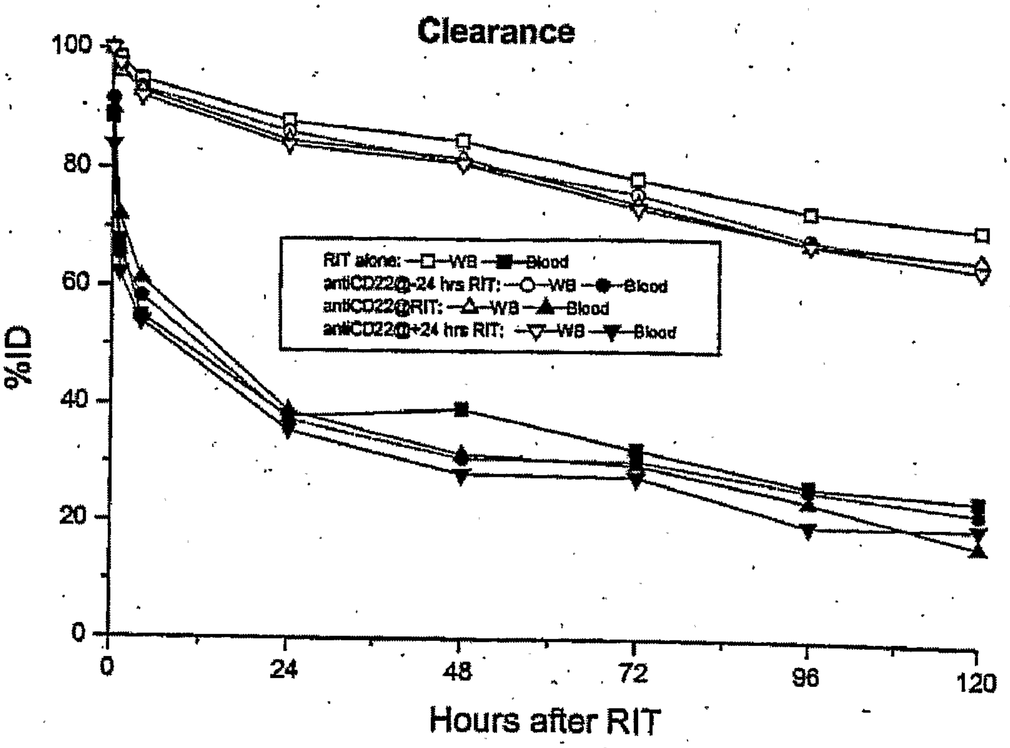

Clearance
RIT alone: WB Blood
antiCD22@-24 hrs RIT: WB Blood
antiCD22@RIT: WB Blood
antiCD22@+24 hrs RIT: WB Blood
%ID
100
80
60
40
20
0
0
24
48
72
96
120
Hours after RIT

Figure 10

HB22 Hybridoma Antibody Heavy Chair Sequences

Residue	10 20 30 CDR1 40 50 CDR2 60 70 80 90 94	CDR3	
HB22-5	GPELVKPGASMKISCKASGYSF.TDYTMNWVKQSHGKNLEWIGLLH.PFNG.GTSYNQKFKGKATLSVDKSSSTAYMELLSLTSEDSAVYFCAR	GTGRH	YAMDYWGQGTSVTVSS
HB22-196	GPDLVKPGASVKISCKASGYSF.IGYYMHWLKQSHGKSLEWIGRVN.PNTA.GLTYNQRFKDKAILTVDKSSNTAYMELRSLTSEDSAVYYCSR	VDYDDYG	YWFFDVWGAGTTVTVSS
HB22-7	GPGLVAPSQSLSITCTVSGFSL.SDYGVNWVRQIPGKGLEWLGIIW..GDG.RTDYNSALKSRLNISKDNSKSQVFLKMNSLKADDTARYYCAR	APGNR	AMEYWGQGTSVTVSS
HB22-33	GPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYIR..YDG.SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAR	GGITV	AMDYWGQGTSVTVSS
HB22-13	GGGLVQPGGSLRLSCATSGFTF.IDYYMNWVRQPPGKALEWLGFIKNKFNGYTTEYNTSVKGRFTISRDNSQSILYLQMNTLRAEDSATYYCAR	GLGRS	YAMDYWGQGTSVTVSS
HB22-23	GGGLGATWRSMKLSCVASGFTF.SYYWMNWVRQSPEKGLEWIAEIRLKSNNYATHYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCTR	YDGSSR	DYWGQGTTLTVSS
	V_H Sequences	D Region	J Sequences

Figure 11

HB22-5 VH Sequence

```
1                                           10                                          20
E   V   Q   L   Q   E   S   G   P   E   L   V   K   P   G   A   S   M   K   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGA GCT TCA ATG AAG ATA    60

21                                          30                                          40
S   C   K   A   S   G   Y   S   F   T   D   Y   T   M   N   W   V   K   Q   S
TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GAC TAC ACC ATG AAC TGG GTG AAG CAG AGC   120

41                                          50                                          60
H   G   K   N   L   E   W   I   G   L   L   H   P   F   N   G   G   T   S   Y
CAT GGA AAG AAC CTT GAG TGG ATT GGA CTT CTT CAT CCT TTC AAT GGT GGT ACT AGC TAC   180

61                                          70                                          80
N   Q   K   F   K   G   K   A   T   L   S   V   D   K   S   S   S   T   A   F
AAC CAG AAG TTC AAG GGC AAG GCC ACA TTA TCT GTA GAC AAG TCA TCC AGC ACA GCC TTC   240

81                                          90                                          100
M   E   L   L   S   L   T   S   E   D   S   A   V   Y   F   C   A   R   G   T
ATG GAG CTC CTC AGT CTG ACA TCT GAG GAC TCT GCA GTC TAT TTC TGT GCA AGA GGG ACA   300

101                                         110                                     120
G   R   N   Y   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S   S
GGT CGG AAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA       357
```

Figure 12

HB22-7 VH Sequence

```
1                                                         10                                                          20
E   V   Q   L   Q   E   S   G   P   G   L   V   A   P   S   Q   S   L   S   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC    60

21                                                        30                                                          40
T   C   T   V   S   G   F   S   L   S   D   Y   G   V   N   W   V   R   Q   I
ACA TGC ACC GTC TCA GGG TTC TCA TTA AGC GAC TAT GGT GTA AAC TGG GTT CGC CAG ATT   120

41                                                        50                                                          60
P   G   K   G   L   E   W   L   G   I   I   W   G   D   G   R   T   D   Y   N
CCA GGA AAG GGT CTG GAG TGG CTG GGA ATA ATA TGG GGT GAT GGA AGG ACA GAC TAT AAT   180

61                                                        70                                                          80
S   A   L   K   S   R   L   N   I   S   K   D   N   S   K   S   Q   V   F   L
TCA GCT CTC AAA TCC AGA CTG AAC ATC AGC AAG GAC AAC TCC AAG AGC CAA GTT TTC TTG   240

81                                                        90                                                          100
K   M   N   S   L   K   A   D   D   T   A   R   Y   Y   C   A   R   A   P   G
AAA ATG AAC AGT CTG AAA GCT GAT GAC ACA GCC AGG TAC TAC TGT GCC AGA GCC CCC GGT   300

101                                                       110                                         117
N   R   A   M   E   Y   W   G   Q   G   T   S   V   T   V   S   S
AAT AGG GCT ATG GAG TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA               351
```

Figure 13

HB22-13.VH Sequence

```
1                                          10                                         20
E   V   Q   L   Q   E   S   G   G   G   L   V   Q   P   G   G   S   L   R   L
GAG GTG CAG CTG CAG GAG TCT GGA GGA GGC TTG GTA CAG CCT GGG GGT TCT CTG AGA CTC     60

21                                         30                                         40
S   C   A   T   S   G   F   T   F   I   D   Y   Y   M   N   W   V   R   Q   P
TCC TGT GCA ACT TCT GGG TTC ACC TTC ATT GAT TAC TAC ATG AAC TGG GTC CGC CAG CCT    120

41                                         50                                         60
P   G   K   A   L   E   W   L   G   F   I   K   N   K   F   N   G   Y   T   T
CCA GGA AAG GCA CTT GAG TGG TTG GGT TTT ATT AAA AAC AAA TTT AAT GGT TAC ACA ACA    180

61                                         70                                         80
E   Y   N   T   S   V   K   G   R   F   T   I   S   R   D   N   S   Q   S   I
GAA TAC AAT ACA TCT GTG AAG GGT CGG TTC ACC ATC TCC AGA GAT AAT TCC CAA AGC ATC    240

81                                         90                                        100
L   Y   L   Q   M   N   T   L   R   A   E   D   S   A   T   Y   Y   C   A   R
CTC TAT CTT CAA ATG AAC ACC CTG AGA GCT GAG GAC AGT GCC ACT TAT TAC TGT GCA AGA    300

101                                       110                                        120
G   L   G   R   S   Y   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S
GGG CTG GGA CGT AGC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC    360

121
S
TCA                                                                                363
```

Figure 14

HB22-23 VH Sequence

```
1                                           10                                          20
E   V   Q   L   Q   E   S   G   G   G   L   G   A   T   W   R   S   M   K   L
GAG GTG CAG CTG CAG GAG TCT GGA GGA GGG CTT GGT GCA ACC TGG AGA TCC ATG AAA CTC    60

21                                          30                                          40
S   C   V   A   S   G   F   T   F   S   Y   Y   W   M   N   W   V   R   Q   S
TCC TGT GTT GCC TCT GGA TTC ACT TTC AGT TAC TAC TGG ATG AAC TGG GTC CGC CAG TCT   120

41                                          50                                          60
P   E   K   G   L   E   W   I   A   E   I   R   L   K   S   N   N   Y   A   T
CCA GAG AAG GGG CTT GAG TGG ATT GCT GAA ATT AGA TTG AAA TCT AAT AAT TAT GCA ACA   180

61                                          70                                          80
H   Y   A   E   S   V   K   G   R   F   T   I   S   R   D   D   S   K   S   S
CAT TAT GCG GAG TCT GTG AAA GGG AGG TTC ACC ATC TCA AGA GAT GAT TCC AAA AGT AGT   240

81                                          90                                          100
V   Y   L   Q   M   N   N   L   R   A   E   D   T   G   I   Y   Y   C   T   R
GTC TAC CTG CAA ATG AAC AAC TTA AGA GCT GAA GAC ACT GGC ATT TAT TAC TGT ACC AGG   300

101                                         110                                     120
Y   D   G   S   S   R   D   Y   W   G   Q   G   T   T   L   T   V   S   S
TAT GAT GGT TCC TCC CGG GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA       357
```

Figure 15

HB22-33 VH Sequence

```
1                                       10                                       20
E   V   Q   L   Q   E   S   G   P   G   L   V   K   P   S   Q   S   L   S   L
GAG GTG CAG CTG CAG GAG TCT GGA CCT GGC CTC GTG AAA CCT TCT CAG TCT CTG TCT CTC    60

21                                      30                                       40
T   C   S   V   T   G   Y   S   I   T   S   G   Y   Y   W   N   W   I   R   Q
ACC TGC TCT GTC ACT GGC TAC TCC ATC ACC AGT GGT TAT TAC TGG AAC TGG ATC CGG CAG   120

41                                      50                                       60
F   P   G   N   K   L   E   W   M   G   Y   I   R   Y   D   G   S   N   N   Y
TTT CCA GGA AAC AAA CTG GAA TGG ATG GGC TAC ATT AGG TAC GAC GGT AGC AAT AAC TAC   180

61                                      70                                       80
N   P   S   L   K   N   R   I   S   I   T   R   D   T   S   K   N   Q   F   F
AAC CCA TCT CTC AAA AAT CGA ATC TCC ATC ACT CGT GAC ACA TCT AAG AAC CAG TTT TTC   240

81                                      90                                       100
L   K   L   N   S   V   T   T   E   D   T   A   T   Y   Y   C   A   R   G   G
CTG AAG TTG AAT TCT GTG ACT ACT GAG GAC ACA GCT ACA TAT TAC TGT GCA AGA GGG GGG   300

101                                     110                                  118
I   T   V   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S   S
ATT ACG GTT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA           360
```

Figure 16

HB22-196 VH Sequence

```
1                                       10                                      20
E   V   Q   L   Q   E   S   G   P   D   L   V   K   P   G   A   S   V   K   I
GAG GTG CAG CTG CAG GAG TCT GGA CCT GAC CTG GTG AAG CCT GGG GCT TCA GTG AAG ATA   60

21                                      30                                      40
S   C   K   A   S   G   Y   S   F   I   G   Y   Y   M   H   W   L   K   Q   S
TCC TGT AAG GCT TCT GGT TAC TCA TTC ATT GGC TAT TAC ATG CAC TGG CTG AAG CAG AGC  120

41                                      50                                      60
H   G   K   S   L   E   W   I   G   R   V   N   P   N   T   A   G   L   T   Y
CAT GGA AAG AGC CTT GAG TGG ATT GGA GCT GTT AAT CCT AAC ACT GCT GGT CTT ACC TAC  180

61                                      70                                      80
N   Q   R   F   K   D   K   A   I   L   T   V   D   K   S   S   N   T   A   Y
AAC CAG AGG TTC AAG GAC AAG GCC ATA TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAT  240

81                                      90                                      100
M   E   L   R   S   L   T   S   E   D   S   A   V   Y   Y   C   S   R   V   D
ATG GAG CTC CGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT TCA AGA GTG GAC  300

101                                     110                                     120
Y   D   D   Y   G   Y   W   F   F   D   V   W   G   A   G   T   T   V   T   V
TAT GAT GAC TAC GGG TAC TGG TTC TTC GAT GTC TGG GGC GCA GGG ACC ACG GTC ACC GTC  360

121
S   S
TCC TCA                                                                          366
```

Figure 17

HB22 Hybridoma Antibody V kappa light chain sequences

```
Antibody -20       -10        1       10       20 CDR1 ABCDEFG     35  40          50 CDR2  60         70        80        90 CDR3  J Segment   Constant
HB22-5    MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSTGDRVTITCKASQTVT      NDLAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTINTVQAEDLAVYFCQQDYSSP LTFGAGTKLELK RADAAPTV
HB22-7    MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSAGDRITLTCKASQSVT      NDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYRSP WTFGGGTKLEIK RADAAPTV
HB22-13   MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSAGDRVSITCKASQSVT      NDVTWYQQKPGQSPKLLIYFASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSP LTFGAGTKLELK RADAAPTV
HB22-23   MESQTQVFVFLLLCVSGAHGSIVMTQTPKFLLVSAGDRVSITCKASQSVT      NDVTWYQQKPGQSPKLLIYFASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSP LTFGAGTKLELK RADAAPTV
HB22-33    MKLPVRLLVLMFWIPASSSDVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVP YTFGGGTKLEIK RADAAPTV
HB22-196  MESQTQVFISLLLWLYGADGNIVMTQSPKSMSMSVGERVTLTCKASENVV      TYVSWYQQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTISSVQAEDLADYHCGQGYSYP YTFGGGTKLEIK RADAAPTV
```

Figure 18

HB22-5 Vk Sequence

```
    M   E   S   Q   T   Q   V   F   V   F   L   L   L   C   V   S   G   A   H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT     60

G   S   I   V   M   T   Q   T   P   K   F   L   L   V   S   T   G   D   R   V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA ACA GGA GAC AGG GTT    120

T   I   T   C   K   A   S   Q   T   V   T   N   D   L   A   W   Y   Q   Q   K
ACC ATT ACC TGC AAG GCC AGT CAG ACT GTG ACT AAT GAT TTA GCT TGG TAC CAA CAG AAG    180

P   G   Q   S   P   K   L   L   I   Y   Y   A   S   N   R   Y   T   G   V   P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT    240

D   R   F   T   G   S   G   Y   G   T   D   F   T   F   T   I   N   T   V   Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAC TTC ACT TTC ACC ATC AAC ACT GTG CAG    300

A   E   D   L   A   V   Y   F   C   Q   Q   D   Y   S   S   P   L   T   F   G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCT CTC ACG TTC GGT    360

A   G   T   K   L   E   L   K   R   A   D   A   A   P   T   V
GCT GGG ACC AAG CTG GAA CTG AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                 410
```

Figure 19

HB22-7 Vk Sequence

```
 M   E   S   Q   T   Q   V   F   V   F   L   L   L   C   V   S   G   A   H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT    60

 G   S   I   V   M   T   Q   T   P   K   F   L   L   V   S   A   G   D   R   I
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG ATT   120

 T   L   T   C   K   A   S   Q   S   V   T   N   D   V   A   W   Y   Q   Q   K
ACC TTA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT GTA GCT TGG TAC CAA CAG AAG   180

 P   G   Q   S   P   K   L   L   I   Y   Y   A   S   N   R   Y   T   G   V   P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT   240

 D   R   F   T   G   S   G   Y   G   T   D   F   T   F   T   I   S   T   V   Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG   300

 A   E   D   L   A   V   Y   F   C   Q   Q   D   Y   R   S   P   W   T   F   G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGG TCT CCG TGG ACG TTC GGT   360

 G   G   T   K   L   E   I   K   R   A   D   A   A   P   T   V
GGA GGC ACC AAG CTG GAA ATC AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                410
```

Figure 20

HB22-13 Vk Sequence

```
    M   E   S   Q   T   Q   V   F   V   F   L   L   L   C   V   S   G   A   H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT    60

 G   S   I   V   M   T   Q   T   P   K   F   L   L   V   S   A   G   D   R   V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTT   120

 S   I   T   C   K   A   S   Q   S   V   T   N   D   V   T   W   Y   Q   Q   K
TCC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT GTA ACT TGG TAC CAA CAG AAG   180

 P   G   Q   S   P   K   L   L   I   Y   F   A   S   N   R   Y   T   G   V   P
CCA GGG CAG TCT CCT AAA TTG CTG ATA TAC TTT GCA TCC AAT CGC TAC ACT GGA GTC CCT   240

 D   R   F   T   G   S   G   Y   G   T   D   F   T   F   T   I   S   T   V   Q
GAT CGC TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG   300

 A   E   D   L   A   V   Y   F   C   Q   Q   D   Y   S   S   P   L   T   F   G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG CTC ACG TTC GGT   360

 A   G   T   K   L   E   L   K   R   A   D   A   A   P   T   V
GCT GGG ACC AAG CTG GAG CTG AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                410
```

Figure 21

HB22-23 Vk Sequence

```
     M   E   S   Q   T   Q   V   F   V   F   L   L   L   C   V   S   G   A   H
AAG ATG GAG TCA CAG ACC CAG GTC TTC GTA TTT CTA CTG CTC TGT GTG TCT GGT GCT CAT     50

 G   S   I   V   M   T   Q   T   P   K   F   L   L   V   S   A   G   D   R   V
GGG AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTA TCA GCA GGA GAC AGG GTC    100

 T   I   S   C   K   A   S   Q   S   V   S   N   D   V   A   W   Y   Q   Q   K
ACC ATA AGC TGC AAG GCC AGT CAG AGT GTG AGT AAT GAT GTA GCT TGG TAC CAA CAG AAG    150

 P   G   Q   S   P   K   L   L   I   Y   Y   A   S   K   R   Y   T   G   V   P
CCA GGG CAG TCT CCT AAA CTG CTG ATA TAC TAT GCA TCC AAG CGC TAT ACT GGA GTC CCT    200

 D   R   L   T   G   S   G   Y   G   T   D   F   T   F   T   I   S   T   V   Q
GAT CGC CTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG    250

 A   E   D   L   A   V   Y   F   C   Q   Q   D   H   S   Y   P   W   T   F   G
GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT CAT AGC TAT CCG TGG ACG TTC GGT    300

 G   G   T   K   L   E   I   K   R   A   D   A   A   P   T   V
GGA GGC ACC AAG CTG GAG ATC AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                 350
```

Figure 22

HB22-33 Vk Sequence

M K L P V R L L V L M F W I P A S S S D

ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GCT TCC AGC AGT GAT 60

V V M T Q T P L S L P V S L G D Q A S I

GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA GCC TCC ATC 120

S C R S S Q S L V H S N G N T Y L H W Y

TCT TGC AGA TCT AGT CAG AGC CTT GTA CAC AGT AAT GGA AAC ACC TAT TTA CAT TGG TAC 180

L Q K P G Q S P K L L I Y K V S N R F S

CTG CAG AAG CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT 240

G V P D R F S G S G S G T D F T L K I S

GGG GTC CCA GAT AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC AAG ATC AGC 300

R V E A E D L G V Y F C S Q S T H V P Y

AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGC TCT CAA AGT ACA CAT GTT CCG TAC 360

T F G G G T K L E I K R A D A A P T V

ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TC 419

Figure 23

HB22-196 Vk Sequence

```
     M   E   S   Q   T   Q   V   F   I   S   I   L   L   W   L   Y   G   A   D
AAG ATG GAG TCA CAG ACC CAG GTC TTC ATA TCC ATA CTG CTC TGG TTA TAT GGA GCT GAT     60

 G   N   I   V   M   T   Q   S   P   K   S   M   S   M   S   V   G   E   R   V
GGG AAC ATT GTA ATG ACC CAA TCT CCC AAA TCC ATG TCC ATG TCA GTA GGA GAG AGG GTC    120

 T   L   T   C   K   A   S   E   N   V   V   T   Y   V   S   W   Y   Q   Q   K
ACC TTG ACC TGC AAG GCC AGT GAG AAT GTG GTT ACT TAT GTT TCC TGG TAT CAA CAG AAA    180

 P   E   Q   S   P   K   L   L   I   Y   G   A   S   N   R   Y   T   G   V   P
CCA GAG CAG TCT CCT AAA CTG CTG ATA TAC GGG GCA TCC AAC CGG TAC ACT GGG GTC CCC    240

 D   R   F   T   G   S   G   S   A   T   D   F   T   L   T   I   S   S   V   Q
GAT CGC TTC ACA GGC AGT GGA TCT GCA ACA GAT TTC ACT CTG ACC ATC AGC AGT GTG CAG    300

 A   E   D   L   A   D   Y   H   C   G   Q   G   Y   S   Y   P   Y   T   F   G
GCT GAA GAC CTT GCA GAT TAT CAC TGT GGA CAG GGT TAC AGC TAT CCG TAC ACG TTC GGA    360

 G   G   T   K   L   E   I   K   R   A   D   A   A   P   T   V
GGG GGG ACC AAG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TC                 410
```