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(54) Title: ANTIBODIES AND METHODS FOR MAKING AND USING THEM

(57) **Abrégé/Abstract:**

The invention provides antibodies, including chimeric human antibodies, recombinant antibodies, synthetic antibodies, and the nucleic acids encoding them, and methods for making and using these immunoglobulins. The invention provides recombinant and synthetic polypeptide and nucleic acid embodiments of these polypeptides and/or antibodies. The invention also provides polypeptides comprising, or consisting of, consensus human framework regions, or "Independently Consensused Frameworks (ICFs)", nucleic acids encoding them, and libraries and kits comprising these ICFs and/or antibodies of the invention, individually and in combinatorial libraries and combinations.

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(54) **Title:** ANTIBODIES AND METHODS FOR MAKING AND USING THEM

(57) **Abstract:** The invention provides antibodies, including chimeric human antibodies, recombinant antibodies, synthetic anti-
bodies, and the nucleic acids encoding them, and methods for making and using these immunoglobulins. The invention provides
recombinant and synthetic polypeptide and nucleic acid embodiments of these polypeptides and/or antibodies. The invention also
provides polypeptides comprising, or consisting of, consensus human framework regions, or "Independently Consensused Frame-
works (ICFs)", nucleic acids encoding them, and libraries and kits comprising these ICFs and/or antibodies of the invention, indi-
vidually and in combinatorial libraries and combinations.

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ANTIBODIES AND METHODS FOR MAKING AND USING THEM

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FIELD OF THE INVENTION

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This invention relates generally to genetic engineering, molecular immunology and medicine. In one aspect, the invention provides antibodies, such as chimeric human antibodies (chimeric antibodies with human components), the nucleic acids encoding them, and methods for making and using these immunoglobulins. The invention provides recombinant and synthetic polypeptide and nucleic acid embodiments of these polypeptides. The invention also provides polypeptides comprising, or consisting of, consensus human framework regions, or “Independently Consensused Framework regions (ICFs)”, nucleic acids encoding them, and libraries and kits comprising these ICFs and/or antibodies of the invention, individually and in combinatorial libraries and combinations.

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BACKGROUND

Antibodies (or Immunoglobulins, Igs) are proteins produced by the immune system in response to the presence of a foreign substance in the body. Immunoglobulins also serve and mediate other functions of the immune system. The nucleic acid sequences that encode

immunoglobulins are initially derived from several genes in the genome (germline), which are subsequently rearranged and mutated during maturation to further increase the diversity of the immunoglobulins in their final, mature form. IgG, a typical immunoglobulin, has a Y-shaped structure formed by four chains: two heavy and two light chains, each with a variable and constant region. The variable regions can be further divided into various subregions, such as the framework (FR) and complementarity-determining regions (CDRs).

Immunoglobulins have been used to treat various diseases and conditions, for example allergies, transplant rejection, cancer, and host-versus-graft disease. However, when administering therapeutic antibody preparations to human patients, the antibodies sometimes provoke an undesired and potentially dangerous immune response by the patient to the antibodies themselves (“immunogenicity”), especially after repeated administrations. Immunogenicity can pose a particular problem when the antibody is from a nonhuman source, such as from an animal. When the antibody is derived from mouse, as is frequently used in therapeutic models, the patient may develop a human anti-murine antibody (HAMA) response. To reduce undesired immunogenicity such as HAMA, certain regions of an animal antibody can be replaced with corresponding regions of human antibodies, in essence “humanizing” the antibody. Modified antibodies, such as “chimeric” antibodies and CDR-grafted antibodies, have been developed to reduce immunogenic responses. However, such replacement strategies may not sufficiently minimize immunogenicity and can reduce the therapeutic efficacy of the immunoglobulin. Thus, there is a need for modified immunoglobulins that reduce or eliminate immunogenicity while maintaining or even improving therapeutic efficacy.

SUMMARY OF THE INVENTION

The present invention provides antibodies having a framework derived from one species and sequences responsible for binding to antigen derived from another species. In alternative aspects these antibodies are in isolated, recombinant or synthetic form. In alternative embodiments, at least one, some, or all of the framework segments (or “framework regions”, or FRs) of the antibodies of the invention are encoded by nucleic acid sequences derived from germline sequences; and in one aspect, at least one, some, or all of the framework segments are “consensus sequences”, as described herein. In one aspect, the antibody framework segments are derived from the animal, e.g., a human, into which the antibody of the invention is to be administered, e.g., as an *in vivo* immunotherapeutic or immunodiagnostic reagent. The antibody sequences fragments responsible for binding to

antigen, also called “complementarity determining regions” (or CDRs), are derived from a non-human animal used to generate a desired antigen specific antibody; in alternative aspects, the antigen can be artificially administered to this animal, or the antigen can be the result of natural or accidental environmental exposure, such as infection or toxin or poison exposure, or by purposeful administration of antigen. In alternative embodiments, the FRs are encoded by “consensus sequences” derived from human genomic polynucleotides, and the CDRs are from a murine source, such as a mouse.

The present invention provides methods (“Human Framework Reassembly” or HuFR) for designing and providing the antibodies of the invention, including the recombinant antibodies, e.g., the recombinant humanized antibodies of the invention, that are more similar in character to antibodies native to the subject to be treated. The method can entail deducing consensus sequences for framework subregions (such as FR1, FR2, FR3, and FR4) of heavy chain (HC) and light chain (LC) variable regions, where the consensus sequence for each subregion is obtained and selected independently of the other framework subregions. Thus, a diverse collection of nucleic acids or polypeptides can be generated from a combinatorial library of independently selected consensus sequences for each framework subregion, which can subsequently be used to make recombinant antibodies, including the recombinant humanized antibodies of the invention. These consensus sequences can be derived from sequences of mature immunoglobulins or germline sequences of particular organisms, such as human, non-human primate, dog, cat, and horse, thus generating antibodies that have reduced immunogenicity in that particular organism, animal and/or human.

The invention provides recombinant heavy or light chain variable region polypeptides, and nucleic acids encoding them, where the variable region can comprise at least three “Independently Consensus’ed Framework” domains (ICF): ICF1, ICF2, and ICF3. The recombinant variable region polypeptide can further comprise an Independently Consensused Framework 4 domain (ICF4).

In one embodiment, each of the ICF domains comprises an amino acid consensus sequence determined from a plurality of amino acid sequences, translated from germline nucleic acid sequences, that each encode at least a portion of a corresponding Kabat framework region (KF) domain, such as KF1, KF2, or KF3. In one embodiment, each of the ICF domains comprises amino acid consensus sequences determined from mature KF domain amino acid sequences. In one embodiment, the process for obtaining such consensus sequences (“consensusing”) comprises: aligning a set of amino acid or nucleic acid sequences encoding at least a portion of one Kabat framework subregion (such as KF1, KF2, KF3, or

KF4) by inspection or using sequence alignment programs in the art; determining the frequency at which a residue (such as a nucleotide or amino acid) appears at each position for that specific subregion; and synthesizing highly frequent residues into a set of consensus sequences for that subregion, thus generating ICF1, ICF2, ICF3, or ICF4 consensus sequences. Exemplary ICFs are provided for heavy chain ICFs (see Tables 1 and 2) and light chain ICFs (see Tables 3 and 4).

The invention also provides Ig polypeptides comprising a heavy and light chain variable region of the invention, such as a full-length antibody, single chain antibody, bivalent antibody, Fab fragment, or single chain Fv. The ICF1, 2, and 3 domains can be derived from a first animal species and the CDR1, 2, and 3 domains can be derived from a second animal species. Exemplary antibodies are provided that bind to antigens such as CD20 or CD3.

The invention further provides methods for producing polypeptides and nucleic acids of the invention and their combinatorial libraries. Combinatorial libraries of the polypeptides of the invention can combine different ICF1s, ICF2s, and ICF3s in different combinations. Further association of pairs of individual members of heavy chain and light chain libraries can yield libraries of greater than 30,000 antibodies. The combinatorial libraries can be screened for desired properties, such as binding to a desired antigen or reduced immunogenicity.

The invention provides antibody or antigen-binding fragment thereof comprising at least one variable region having a combination of:

- (1) light chain BD22084 (SEQ ID NO:225) and heavy chain BD20332 (SEQ ID NO:138);
- (2) light chain BD22085 (SEQ ID NO:232) and heavy chain BD20335 (SEQ ID NO:143);
- (3) light chain BD22086 (SEQ ID NO:227) and heavy chain BD20335 (SEQ ID NO:143);
- (4) light chain BD22088 (SEQ ID NO:229) and heavy chain BD20337 (SEQ ID NO:148);
- (5) light chain BD22087 (SEQ ID NO:240) and heavy chain BD20335 (SEQ ID NO:143);
- (6) light chain BD22089 (SEQ ID NO:243) and heavy chain BD20335 (SEQ ID NO:143);
- (7) light chain BD22090 (SEQ ID NO:234) and

- heavy chain BD20337 (SEQ ID NO:148);
- (8) light chain BD22095 (SEQ ID NO:244) and
heavy chain BD20337 (SEQ ID NO:148);
- (9) light chain BD22091 (SEQ ID NO:242) and
heavy chain BD20337 (SEQ ID NO:148);
- (10) light chain BD22108 (SEQ ID NO:230) and
heavy chain BD20337 (SEQ ID NO:148);
- (11) light chain BD22092 (SEQ ID NO:235) and
heavy chain BD20338 (SEQ ID NO:149);
- (12) light chain BD22094 (SEQ ID NO:231) and
heavy chain BD20337 (SEQ ID NO:148);
- (13) light chain BD22096 (SEQ ID NO:241) and
heavy chain BD20337 (SEQ ID NO:148);
- (14) light chain BD22092 (SEQ ID NO:235) and
heavy chain BD20337 (SEQ ID NO:148);
- (15) light chain BD22102 (SEQ ID NO:248) and
heavy chain BD20337 (SEQ ID NO:148);
- (16) light chain BD22097 (SEQ ID NO:246) and
heavy chain BD20335 (SEQ ID NO:143).
- (17) light chain BD22104 (SEQ ID NO:239) and
heavy chain BD20337 (SEQ ID NO:148);
- (18) light chain BD22085 (SEQ ID NO:232) and
heavy chain BD20339 (SEQ ID NO:150);
- (19) light chain BD22107 (SEQ ID NO:226) and
heavy chain BD20339 (SEQ ID NO:150);
- (20) light chain BD22100 (SEQ ID NO:236) and
heavy chain BD20335 (SEQ ID NO:143);
- (21) light chain BD22103 (SEQ ID NO:228) and
heavy chain BD20337 (SEQ ID NO:148);
- (22) light chain BD22105 (SEQ ID NO:237) and
heavy chain BD20337 (SEQ ID NO:148);
- (23) light chain BD22101 (SEQ ID NO:247) and
heavy chain BD20335 (SEQ ID NO:143);
- (24) light chain BD22106 (SEQ ID NO:245) and

- heavy chain BD20333 (SEQ ID NO:142);
- (25) light chain BD22108 (SEQ ID NO:230) and
heavy chain BD20338 (SEQ ID NO:149);
- (26) light chain BD22109 (SEQ ID NO:233) and
heavy chain BD20341 (SEQ ID NO:154); or
- (27) light chain BD22111 (SEQ ID NO:238) and
heavy chain BD20336 (SEQ ID NO:144).

The invention provides antibodies or antigen-binding fragments thereof comprising at least a portion of a heavy chain variable region and at least a portion of a light chain variable region, wherein the light chain portion and the heavy chain portion is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or complete sequence identity to the respective light and heavy chains of at least one of the following combinations:

- (1) light chain BD22084 (SEQ ID NO:225) and
heavy chain BD20332 (SEQ ID NO:138);
- (2) light chain BD22085 (SEQ ID NO:232) and
heavy chain BD20335 (SEQ ID NO:143);
- (3) light chain BD22086 (SEQ ID NO:227) and
heavy chain BD20335 (SEQ ID NO:143);
- (4) light chain BD22088 (SEQ ID NO:229) and
heavy chain BD20337 (SEQ ID NO:148);
- (5) light chain BD22087 (SEQ ID NO:240) and
heavy chain BD20335 (SEQ ID NO:143);
- (6) light chain BD22089 (SEQ ID NO:243) and
heavy chain BD20335 (SEQ ID NO:143);
- (7) light chain BD22090 (SEQ ID NO:234) and
heavy chain BD20337 (SEQ ID NO:148);
- (8) light chain BD22095 (SEQ ID NO:244) and
heavy chain BD20337 (SEQ ID NO:148);
- (9) light chain BD22091 (SEQ ID NO:242) and
heavy chain BD20337 (SEQ ID NO:148);
- (10) light chain BD22108 (SEQ ID NO:230) and
heavy chain BD20337 (SEQ ID NO:148);
- (11) light chain BD22092 (SEQ ID NO:235) and
heavy chain BD20338 (SEQ ID NO:149);

- (12) light chain BD22094 (SEQ ID NO:231) and heavy chain BD20337 (SEQ ID NO:148);
- (13) light chain BD22096 (SEQ ID NO:241) and heavy chain BD20337 (SEQ ID NO:148);
- 5 (14) light chain BD22092 (SEQ ID NO:235) and heavy chain BD20337 (SEQ ID NO:148);
- (15) light chain BD22102 (SEQ ID NO:248) and heavy chain BD20337 (SEQ ID NO:148);
- 10 (16) light chain BD22097 (SEQ ID NO:246) and heavy chain BD20335 (SEQ ID NO:143).
- (17) light chain BD22104 (SEQ ID NO:239) and heavy chain BD20337 (SEQ ID NO:148);
- (18) light chain BD22085 (SEQ ID NO:232) and heavy chain BD20339 (SEQ ID NO:150);
- 15 (19) light chain BD22107 (SEQ ID NO:226) and heavy chain BD20339 (SEQ ID NO:150);
- (20) light chain BD22100 (SEQ ID NO:236) and heavy chain BD20335 (SEQ ID NO:143);
- 20 (21) light chain BD22103 (SEQ ID NO:228) and heavy chain BD20337 (SEQ ID NO:148);
- (22) light chain BD22105 (SEQ ID NO:237) and heavy chain BD20337 (SEQ ID NO:148);
- (23) light chain BD22101 (SEQ ID NO:247) and heavy chain BD20335 (SEQ ID NO:143);
- 25 (24) light chain BD22106 (SEQ ID NO:245) and heavy chain BD20333 (SEQ ID NO:142);
- (25) light chain BD22108 (SEQ ID NO:230) and heavy chain BD20338 (SEQ ID NO:149);
- 30 (26) light chain BD22109 (SEQ ID NO:233) and heavy chain BD20341 (SEQ ID NO:154); or
- (27) light chain BD22111 (SEQ ID NO:238) and heavy chain BD20336 (SEQ ID NO:144)

and wherein the at least a portion of a light chain, the at least a portion of the heavy chain, or both are derived at least in part from sequences made by the method comprising:

(1) providing an Independently Consensused Framework 1 (ICF1) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from at least a portion of a Kabat framework region 1 (KF1) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;

(2) providing at least a portion of a complementarity determining region 1 (CDR1) derived from the variable region of a 1F5 antibody;

(3) providing an Independently Consensused Framework 2 (ICF2) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from at least a portion of a Kabat framework region 2 (KF2) domain, wherein the plurality of amino acid sequences translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;

(4) providing at least a portion of a complementarity determining region 2 (CDR2) derived from the variable region of a 1F5 antibody;

(5) providing an Independently Consensused Framework 3 (ICF3) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from at least a portion of a Kabat framework region 3 (KF3) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;

(6) providing at least a portion of a complementarity determining region 3 (CDR3) derived from the variable region of a 1F5 antibody; and

(7) optionally providing an Independently Consensused Framework 4 (ICF4) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from at least a portion of a Kabat framework region 4 (KF4) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;

wherein at least one ICF is derived from a genomic nucleic acid sequence,

(8) joining, in a 5'-to-3' orientation, nucleic acids encoding the ICF1-CDR1-ICF2-CDR2-ICF3-CDR3 and optionally ICF4 domains.

The invention provides antigen binding antibody fragments of the invention, wherein the antibody fragment is an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, a single-chain antibody, an Fv fragment, an scFv fragment, an antibody mimetic, an Fd fragment, or an Fd' fragment; or alternatively an antigen binding antibody fragment of the invention is, or comprises, an antibody fragment fused to an Fc.

The invention provides recombinant, synthetic or isolated antibodies having a structure comprising at least one variable region combination of the invention.

The invention provides chimeric antibodies or antigen binding fragments thereof comprising at least one variable region combination of the invention.

The invention provides chimeric antigen binding antibody fragments of the invention, wherein the chimeric antibody fragment is a chimeric Fab, a chimeric Fab', a chimeric F(ab')₂, a chimeric single-chain antibody, a chimeric Fv, a chimeric scFv, an antibody mimetic, a chimeric Fd, or a chimeric Fd'.

The invention provides antibodies or antigen binding fragments thereof that specifically bind to a CD20 antigen and comprise a light chain variable region comprising (a) an ICF1 comprising an amino acid sequence of SEQ ID NOS:43-49; (b) a CDR1 comprising an amino acid sequence of SEQ ID NO:163; (c) an ICF2 comprising an amino acid sequence of SEQ ID NOS:58-61; (d) a CDR2 comprising an amino acid sequence of SEQ ID NO:164; (e) an ICF3 comprising an amino acid sequence of SEQ ID NOS:67-71, 73, or 74; (f) a CDR3 comprising an amino acid sequence of SEQ ID NO:165; and/or (g) an ICF4 comprising the amino acid sequence of SEQ ID NO:83.

The invention provides antibodies or antigen binding fragments thereof that specifically binds to a CD20 antigen and comprises a heavy chain variable region comprising (a) an ICF1 comprising an amino acid sequence of SEQ ID NOS:6 or 7; (b) a CDR1 comprising an amino acid sequence of SEQ ID NO:151; (c) an ICF2 comprising an amino acid sequence of SEQ ID NOS:9, 10, or 11; (d) a CDR2 comprising an amino acid sequence of SEQ ID NO:152; (e) an ICF3 comprising an amino acid sequence of SEQ ID NOS:13, 17, 19, or 20; (f) a CDR3 comprising an amino acid sequence of SEQ ID NO:153; and/or (g) an ICF4 comprising an amino acid sequence of SEQ ID NO:21.

The invention provides pharmaceutical compositions or formulations comprising: (a) an antibody or antigen binding fragment thereof of the invention; and in one aspect, the pharmaceutical composition or formulation further comprises a pharmaceutically acceptable carrier or excipient.

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The invention provides methods for treating or ameliorating a disease, infection, condition or toxic exposure comprising: (a) providing a composition comprising an antibody or an antigen binding fragment thereof of the invention; and, (b) administering a sufficient amount of said antibody or antigen binding fragment thereof to an individual in need thereof.

5 The invention provides methods for suppressing or abrogating an immune response comprising: (a) providing an antibody or antigen binding fragment thereof of the invention; and (b) administering a sufficient amount of said antibody or antigen binding fragment thereof to an individual in need thereof.

10 The invention provides methods for suppressing or abrogating a B-cell mediated immune response comprising: (a) providing an antibody or antigen binding fragment thereof of the invention; and, (b) administering a sufficient amount of said antibody or antigen binding fragment thereof to an individual in need thereof.

The invention provides methods of treating B-cell lymphoma comprising: (a) providing an antibody or antigen binding fragment thereof of the invention; and, (b) administering a sufficient amount of said antibody or fragment thereof to an individual (e.g., a human) in need thereof.

15 The invention uses of an antibody or antigen binding fragment thereof of the invention for the manufacture of a pharmaceutical composition for treating a subject (e.g., a human) having a B-cell mediated disease or condition by a method comprising administering an effective amount of said antibody or fragment thereof to said subject; and in one aspect, the disease is B-cell lymphoma.

20 The details of one or more aspects of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

30 Figure 1 is a schematic of the components of an exemplary heavy or light chain (amino acid sequences or nucleic acids encoding them), illustrating Human Framework Reassembly. A starting murine chain is shown, from which the sequences for three CDRs

(underlined) are obtained. Various Independently Consensused Framework domains (ICFs) are provided for each of the positions corresponding to FR1, FR2, FR3, and optionally FR4. Preferred ICFs are independently selected for each position and assembled with the murine CDRs, optionally with a constant domain (double-underlined), to obtain a recombinant HuFR immunoglobulin chain.

Figure 2 shows exemplary amino acid sequences derived from genes for human germline kappa light chain variable regions.

Figure 3 shows exemplary amino acid sequences derived from genes for human germline lambda light chain variable regions.

Figure 4 illustrates a graph of data from an anti-CD20 ELISA assay demonstrating the specific activity of the anti-CD20 HuFR clones in the anti-CD20 cellular ELISA, as discussed in detail in Example 3, below.

Figure 5 illustrates a bar graph of data comparing the specific activity of the top anti-CD20 HuFR clones in the anti-CD20 ELISA, as discussed in detail in Example 3, below.

Figure 6 is a bar graph of an apoptosis assay, which demonstrates that several of the top HuFR hits have activities equal to or better than reference antibody and DVSA-CD20, as discussed in detail in Example 3, below.

Figure 7 is for cell cycle assay, which shows that the HuFR anti-CD20 hits do not induce cell proliferation in human PBMC *in vitro*, as discussed in detail in Example 3, below.

Figure 8 is a bar graph of a CDC assay, as discussed in detail in Example 3, below.

Figure 9 is a bar graph of an ADCC assay, as discussed in detail in Example 3, below.

Figure 10 depicts the light chain (top) and heavy chain (bottom) nucleic acid sequences of DVSA-CD3, as discussed in detail in Example 4, below.

Figure 11 depicts the heavy chain (top) and light chain (middle) amino acid sequences of DVSA-CD3, as well as the light chain of DVSA-CD3 (bottom), as discussed in detail in Example 4, below.

Figure 12 provides an alignment of the heavy and light chains in the top 9 anti-CD3 hits.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides antibodies, such as chimeric human antibodies (chimeric antibodies with human components), recombinant antibodies, synthetic antibodies, the nucleic acids encoding them, and methods for making and using these immunoglobulins.

The invention provides a novel approach to designing antibodies, including chimeric antibodies and/or recombinant or synthetic antibodies. The approach is based, at least in part, upon generating consensus sequences for immunoglobulin variable region framework subregions, where the consensus sequence for each subregion is obtained independently of
5 the other subregions.

In one aspect, the consensus sequences are derived from (are compared to) germline sequences; thus, sequences that are most represented in the germline can be prioritized for antibody design. With a library of independently selected consensus sequences for each framework subregion, a combinatorial library of antibodies can be generated. For example,
10 keeping the CDR regions of a known antibody (that specifically binds to a known antigen), the CDR regions can be reassembled into different framework subregion combinations, thereby creating a large collection of antibodies having the same CDR regions, but different framework sequences. This collection of antibodies can then be tested to determine which framework sequences provide the least immunogenicity while maintaining sufficient binding
15 affinity and avidity toward the target antigen.

The invention provides compositions and libraries comprising heavy chain variable region polypeptides, including chimeric and/or recombinant, heavy chain variable region polypeptides, in addition to nucleic acids encoding them (e.g., that encode the chimeric heavy chain variable region polypeptides of the invention). The invention also provides
20 compositions and libraries of light chain variable region polypeptides, including chimeric and/or recombinant, light chain variable region polypeptides, and nucleic acids encoding them (e.g., that encode the chimeric heavy chain variable region polypeptides). The heavy chain variable region polypeptides of the invention, including the chimeric and/or recombinant heavy chain variable region polypeptides, can be associated with a light chain
25 variable region polypeptide (e.g., a light chain variable region polypeptide of this invention) in order to generate a bivalent immunoglobulin (Ig).

The invention also provides antibody compositions generated from the heavy chain and light chain variable regions comprising ICFs. In alternative embodiments, any CDR from any known antibody (for example, those exemplary antibodies shown in Tables 5–6)
30 can be combined or linked with ICFs, such as those of Tables 1–4. In addition, they can be further combined or linked to a constant domain (CD) (for example, those shown in Tables 7–8) to generate full-length heavy chain variable region polypeptides or full-length light chain variable region polypeptides. Upon combining the polypeptides to generate immunoglobulins, the Igs can serve as functional units for the following non-limiting

antibody examples: a single chain antibody, a bivalent antibody (such as a disulfide-linked antibody), a Fab fragment, and a single chain Fv.

Additionally, the present invention provides methods for generating a combinatorial library of nucleic acids that encode heavy chain and light chain variable regions that comprise ICFs. The present invention also provides methods for generating an antibody specific to an antigen and with a decreased immunogenicity.

In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) include and encompass (refer to), without limitation, monoclonal antibodies, multispecific antibodies, human antibodies, polyclonal antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, single domain antibodies, Fab fragments, F(ab) fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site. Antibody fragments can also include, but are not limited to, small “antibody mimetics” which are comprised of at least one CDR3 from either a heavy or light chain, at least one CDR1 or CDR2 from the immunoglobulin chain that did not provide the CDR3, and at least one framework region selected from either the heavy or light chain based on its ability to approximate the linkage of the CDRs in the parent molecule (the parent antibody). Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass.

In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) can comprise the equivalent of a native full-length antibody, e.g., comprising two heavy chains paired with two light chains. In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) can comprise a full-length heavy chain of about 50 kD in size (approximately 446 amino acids in length); which in one aspect can be encoded by a heavy chain variable region gene (about 116 amino acids) and a constant region gene. In alternative embodiments, different constant region genes encoding heavy chain constant region of different isotypes such as alpha, gamma (IgG₁, IgG₂, IgG₃, IgG₄), delta, epsilon, and mu sequences are used. In alternative embodiments, a full-length light chain of about 25 kD in size (approximately 214 amino acids in length), as is encoded by a light chain variable region gene and a constant region gene, is used. The variable regions of the light and/or heavy chain participate in

binding to an antigen, and the constant regions are generally responsible for the effector functions of the antibody.

In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) can comprise a “variable region” of a heavy and/or light antibody chain (which is an N-terminal mature domain of an antibody chain). All domains, CDRs, and residue numbers are assigned on the basis of sequence alignments and structural knowledge. In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) can comprise: V_H , which is the variable domain of an antibody heavy chain; or V_L , which is the variable domain of an antibody light chain, and alternatively can be of the kappa (κ) or of the lambda (λ) isotype.

In alternative embodiments, antibodies of the invention (e.g., the chimeric and/or recombinant antibodies of the invention) can comprise immunoglobulin light and/or heavy chain variable regions; which in one aspect can comprise or consist of a framework region (FR) that borders and encompasses three or four separate hypervariable regions, also called complementarity determining regions, or CDRs. In alternative embodiments, as in nature, the borderlines between the FR and the CDRs may not always be definite, and can depend on the particular antibody and its degree and location of variability relative to other, similar antibodies. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody—that is the combined framework regions of the constituent light and heavy chains—serves to position and align the CDRs. The CDRs are primarily responsible for binding to an epitope of an antigen.

In alternative embodiments, the Kabat system (a well known and widely used guide) is used to identify framework regions and CDRs of the invention - see Sequences of Proteins of Immunological Interest, E. Kabat et al., U.S. Department of Health and Human Services, (1987) and (1991). Identifying Kabat framework sequence is well known and thus is a routine protocol; see e.g., U.S. Patent No. 5,840,299; U.S. Pat. App. Pub. No. 20050261480. Kabat et al. list many amino acid sequences for antibodies for each subclass, and list the most commonly occurring amino acid for each residue position in that subclass. Kabat et al. use a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. Kabat et al.'s scheme is extendible to other antibodies not included in the compendium by aligning the antibody in question with one of the consensus sequences in Kabat et al. The use of the Kabat et al.

numbering system readily identifies amino acids at equivalent positions in different antibodies. For example, an amino acid at the L50 position of a human antibody occupies the equivalence position to an amino acid position L50 of a mouse antibody.

As used in the art, the term “CDR” refers to a complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3 for each of the variable regions. Because CDRs represent regions of increased variability (relative to the regions of similar sequences), the exact boundaries of these CDRs can be defined differently according to different systems. The widely used system described by Kabat (Kabat et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991)) provides a residue numbering system applicable to any variable region of an antibody, and provides residue boundaries defining the three CDRs. These CDRs may be referred to as “Kabat CDRs”. Chothia et al. (*Nature* (1989) 342:877-883; Chothia and Lesk, (1987) *J. Mol. Biol.* 196:901-917) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the “L” and the “H” designate the light chain and the heavy chains regions. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs.

The term “framework,” “framework region,” or “framework sequence” refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. In one embodiment, the positioning of the six CDRs (CDR1, 2, and 3 of light chain and CDR1, 2, and 3 of heavy chain) within the framework region effectively divides the framework region of each chain into four subregions, designated FR1, FR2, FR3, and FR4. CDR1 is positioned between FR1 and FR2; CDR2 between FR2 and FR3; and CDR3 between FR3 and FR4. Without specifying the particular subregions as FR1, FR2, FR3, or FR4, a framework region, as referred by others, represents the combined subregions FR1, FR2, FR3, and FR4, within the variable region of a single, naturally occurring immunoglobulin chain. In an alternative embodiment, a framework region (FR) of the invention comprises or consists of (represents) any portion of the entire framework sequence, including a sequence consisting of one of the four subregions. In an alternative embodiment, a framework region (FR) of the invention

comprises or consists of amino acids derived from a Kabat framework region (KF) domain, wherein the amino acid sequences are derived from germline immunoglobulin sequences.

In one embodiment, the term “germline sequence,” with respect to an immunoglobulin sequence, means a genomic sequence (containing immunoglobulin coding sequences) that has not undergone the maturation process that leads to genetic rearrangement and somatic hypermutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., (2002) *Crit. Rev. Immunol.* 22(3): 183-200; Marchalonis et al., (2001) *Adv. Exp. Med. Biol.* 484:13-30). A “germline” can include a lineage of cells that give rise to gametes and is continuous through many generations.

In one embodiment, the term “mature”, e.g., with respect to mature immunoglobulins, mature (Ab) sequences and/or mature (Ab) forms, and the like, can include any non-germline immunoglobulin sequence; for example, any rearranged or modified germline sequence of any isotype rearranged with any V region, including affinity-matured sequences (e.g., after the process of affinity maturation in vivo or in vitro).

In one embodiment, the term “consensus sequence” comprises, or consists of, (refers to) an amino acid sequence that comprises more frequently occurring amino acid residues at each location in a set of related proteins (for example, immunoglobulins of any particular subclass, e.g., e.g., light chain, such as a kappa or lambda, or isotype) or subunit structure). The consensus sequence may be based on immunoglobulins of a particular species or of many species. In an alternative embodiments, “more frequently” means at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% more frequently occurring amino acid residues at each residue location (position) in a set of related proteins, e.g., immunoglobulin sequences of any particular subclass (e.g., light chain, such as a kappa or lambda, or isotype) or subunit structure.

The term “derivative” refers to a molecule that can be formed from another molecule. In the context of a nucleotide (for example, a nucleic acid sequence) or a proteinaceous agent (such as, proteins, polypeptides, peptides and the like; for example, antibodies), a derivative can refer to the agent that comprises an original nucleic acid sequence (such as a germline sequence), or a proteinaceous agent that comprises an amino acid sequence, which has been obtained from an original source or altered by the introduction of amino acid residue

substitutions, deletions, and/or additions. A derivative of such an agent possesses a similar or identical sequence as the agent from which it was derived

As used herein, a “placeholder” is a nucleic acid sequence encoding an immunoglobulin variable region (for example, a light chain variable region) comprising Kabat framework regions 1, 2, and 3, and the CDRs 1, 2, and 3 of a known immunoglobulin. A placeholder is determined on the basis of a germline variable region nucleic acid sequence identity compared to that of a sequence of a processed, mature antibody (for example, those light chain variable region germline sequences that are most similar to the nucleic acid sequence of the mature antibody). The placeholder, once identified, can then be used as a temporary single chain molecule associated with, for example, a heavy chain variable region molecule of the invention, for the purpose of assessing the functional properties of the heavy chain while associated with a second chain. In one embodiment, the placeholder is a light chain variable region (for example, kappa chain or lambda chain).

As used herein, a “Kabat framework region” (KF) is a variable chain framework region (subregion) that corresponds to the standard Kabat scheme for numbering amino acid residues of immunoglobulins and assigning positions for FRs and CDRs (Kabat, et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition. NIH Publication No. 91-3242). For example, according to this scheme, the following Kabat numbers can be used to discern the variable heavy chain framework subregions: Kabat framework region 1 (KF1, which can correspond to FR1) comprises from residue 1 to about residue 29; Kabat framework region 2 (KF2, which can correspond to FR2) comprises from about residue 36 to about residue 49; Kabat framework region 3 (KF3, which can correspond to FR3) comprises from about residue 66 to about residue 94; and Kabat framework region 4 (KF4, which can correspond to FR4) comprises from about residue 103 to about residue 113.

In addition, according to this scheme, in alternative embodiments, the following Kabat numbers are used to discern the variable light chain framework regions: Kabat framework region 1 (KF1, which can correspond to FR1) comprises from residue 1 to about residue 23; Kabat framework region 2 (KF2, which can correspond to FR2) comprises from about residue 35 to about residue 49; Kabat framework region 3 comprises from about residue 57 to about residue 88 (KF3, which can correspond to FR3); and Kabat framework region 4 comprises from about residue 96 to about residue 109 (KF4, which can correspond to FR4).

As used herein, “Independently Consensused Framework” (ICF), means a framework region (for example, FR1, FR2, FR3, FR4, and therefore may correspond to KF1, KF2, KF3,

or KF4) having an amino acid or nucleic acid coding sequence that is a consensus sequence obtained, for example, from: (1) germline V or J genes, (2) rearranged VDJ genes, (3) rearranged VJ genes, and (4) amino acid sequences (and/or the nucleic acid sequences that encode identical or essentially identical amino acid sequences) of known immunoglobulins.

5 Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent
10 variations.” One of skill will recognize that each codon in a nucleic acid sequence (except AUG; which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques.

In one embodiment, each ICF comprises a consensus sequence that is selected independently from other ICFs in a variable region. In other words, in one aspect, an ICF
15 consensus sequence is obtained from analyzing a particular framework subregion independent from the other framework subregions (in contrast to a method that analyzes framework consensus sequences by examining entire framework regions and not by independently analyzing the separate framework subregions). Independent selection can entail aligning a pool of ICF germline nucleic acid sequences obtained from a plurality of
20 germline nucleic acid sequences encoding at least some portion of a variable chain framework region and subsequently clustering the sequences according to sequence similarity, wherein sequences from each framework cluster are then used to form a consensus sequence. Upon translation, the consensus sequence demonstrates the most frequent amino acid sequences occurring at each residue position. The domain can be ICF1, ICF2, ICF3, or
25 ICF4. The variable framework region amino acid residues can correspond to the standard Kabat numbering system. An ICF sequence can be identical to the original germline sequence used to determine the ICF domain. A consensus sequence also includes any wobble site changes in the nucleic acid consensus sequence wherein the nucleotide change will still encode the same amino acid sequence. For example, a consensus sequence can be
30 determined for a human based on human germline sequences. Consensus sequences also can be determined for the following non-limiting examples, such as canine, feline, ovine, equine, bovine, porcine, fowl, goat, salmon, and hybridoma cell line, utilizing the appropriate germline sequences.

Immunoglobulin structures

Immunoglobulins (Igs) are molecules that function as antibodies and are produced by plasma cells in response to an antigen (i.e., by way of an infection or immunization).

Immunoglobulins can bind specifically to one or a few closely related antigens. The primary
5 function of immunoglobulins is to bind to antigens, which mediates various effector functions that can ultimately result in protection of the animal. Igs are divided into five different classes, based on the differences in the amino acid sequences in the constant region of the heavy chains for example, gamma (γ) heavy chains (IgG), mu (μ) heavy chains (IgM), alpha (α) heavy chains (IgA), delta heavy chains (IgD), and epsilon (ϵ) heavy chains (IgE). All Igs
10 within a given class will have similar heavy chain constant regions. The Ig classes can be further divided into subclasses on the basis of small differences in the amino acid sequences in the constant region of the heavy chains. Igs within a subclass can have similar heavy chain constant region amino acid sequences, wherein differences are detected by serological means. For example, the IgG subclasses comprise IgG1, IgG2, IgG3, and IgG4, wherein the heavy
15 chain is classified as being a gamma 1 heavy chain, a gamma 2 heavy chain, and so on due to the amino acid differences. In another example, the IgA subclasses comprise IgA1 and IgA2, wherein the heavy chain is classified as being an alpha 1 heavy chain or an alpha 2 heavy chain due to the amino acid differences.

Immunoglobulins also comprise light chains, such as Kappa light chains or Lambda
20 light chains. The distinctions in the light chain types are based on differences in the amino acid sequence in the constant region of the light chain, which also can be detected by serological means. The light chains can also be divided into subtypes based on differences in the amino acid sequences in the constant region of the light chain. For example, the Lambda subtypes are classified as Lambda 1, Lambda 2, Lambda 3, and Lambda 4.

Immunoglobulins comprise a population of heterogeneous molecules because they are
25 composed of different classes and subclasses of heavy chains. Each heavy chain can subsequently associate with different types and subtypes of light chains. As a result, different immunoglobulin molecules can have different antigen binding properties due to the different V_H and V_L regions. Generally, immunoglobulins comprise a four-chain structure as their
30 basic unit. Full-length Igs comprise two heavy chains (~50-70 kD) covalently linked and two light chains (~23 kD each, such as lambda or kappa chains). Each light chain is also covalently linked to one of the heavy chains. For example, the two heavy chains and the heavy and light chains are held together by inter-chain disulfide bonds and by non-covalent

interactions. The number of inter-chain disulfide bonds can vary among different immunoglobulin molecules. Each chain has an N-terminal variable domain (V_H or V_L wherein each are ~110 amino acids in length) and one or more constant domains at the C-terminus. The constant domain of the light chain (C_L which is ~110 amino acids in length) is aligned with and disulfide bonded to the first constant domain of the heavy chain (C_H which is ~330-440 amino acids in length). The light chain variable domain is aligned with the variable domain of the heavy chain. The Ig heavy chain can comprise 2 or more additional C_H domains (such as, C_{H2} , C_{H3} and the like). For example, a hinge region can be identified between the C_{H1} and C_{H2} constant domains. This is the region where the arms of the antibody molecule form a Y-shape and allows for some flexibility in the molecule.

As discussed and defined above, the variable domains of the heavy and light chain include framework regions (FRs) and hypervariable regions called complementarity-determining regions (CDRs), and an intrachain disulfide bond. (See e.g. Chothia et al., (1985) *J. Mol. Biol.* 186:651-663; Novotny and Haber, (1985) *Proc. Natl. Acad. Sci. USA* 82:45924596; Padlar et al., (1986) *Mol. Immunol.*, 23(9): 951-960; and S. Miller, J. (1990) *Mol. Biol.*, 216:965-973).

The Ig heavy and light chain variable regions can be divided into groups and subgroups on the basis of their similarities and differences within the framework regions. The variability is the result of the products of the different variable region genes (such as the V, D, and J genes).

VDJ recombination, germline sequences, and immunoglobulin diversity

The heavy chain and light chain variable regions of an Ig molecule comprise a V segment (variable gene segment) and a J segment (joining gene segment). A V gene encodes the V-segment and the J-segment refers to a region encoded by a J gene. In addition, the heavy chain variable region comprises a D segment (diversity gene segment), which is encoded by the D gene. The V segments of heavy and light chain variable regions consist of FR1, CDR1, FR2, CDR2, FR3, and a few amino acids of CDR3. The J segment of a light chain variable region includes the remainder of CDR3 and FR4 in its entirety. In the heavy chain variable region, the J segment includes a portion of CDR3 and all of FR4 wherein the D segment comprises the remaining portion of CDR3. For example, to generate a light chain variable region, a J segment is added to the V segment as a consequence of rearrangement of the light chain variable region genes during B-cell differentiation. In the case of the heavy chain, a D segment in addition to a J segment is added to the V segment to generate the heavy chain variable region.

Immunoglobulin diversity is the result of various processes, such as combinatorial assembly (for example, V(D)J recombination), junctional assembly, light chain coupling (for example, different combinations of κ and λ light chains can be used but not all heavy chains pair equally well with a κ and λ), and somatic hypermutation.

5 Combinatorial assembly of multiple germline genes involves encoding variable regions and a variety of somatic events. V(D)J recombination assembles Ig genes from component V, D, and J gene segments in developing B cells. The somatic events include the random recombination of variable (V) gene segments with diversity (D) and joining (J) gene segments to make a complete V_H region – V(D)J domain of the heavy chain variable region. 10 Briefly, the first recombination event occurs between one D and one J gene segment of the heavy chain locus in the developing B cell, forming the DJ complex. DNA between these two genes is deleted. The D-J recombination event is then followed by the joining of one V gene, from a region upstream of the newly formed DJ complex, resulting in the formation of a rearranged VDJ gene. Other genes between the V and D segments of the new VDJ gene are 15 now deleted. The kappa (κ) and lambda (λ) chains of the immunoglobulin light chain loci recombine similarly to heavy chain variable regions, except the light chains lack a D segment wherein the events can also entail the random recombination of variable (V) and joining (J) gene segments to make a complete V_L region - VJ domain of the light chain variable region.

Junctional diversity also contributes to the Ig diversity achieved during the 20 recombination process. When the D gene segment is joined to the J gene segment, and the V gene segment is subsequently joined to the DJ region, the process in itself is imprecise, and can result in the loss or addition of nucleotides encoding various amino acids at the junctions of the V(D)J domain. These mechanisms involved in generating diversity occur in the developing B cell prior to antigen exposure.

25 After antigenic stimulation, the expressed Ig genes in B cells undergo somatic mutation or hypermutation (see Maizels (2005) *Ann. Rev. Genet.* 39:23-46), which further contributes to Ig variability. Mature B cells, following activation after encountering an antigen, have the capability to introduce point mutations into the variable regions of immunoglobulin genes (also referred to as affinity maturation); this occurs in specialized 30 lymphoid structures - the germinal centers. Some mutations can cause the Ig to have a higher affinity for the antigen. Antibodies that bind strongly to an antigen are selected for proliferation because they are stimulated more often than an antibody that weakly binds to its antigen.

In addition to the mechanisms described above to generate Ig diversity, a genetically diverse collection of nucleotides derived wholly or partially from sequences that encode expressed immunoglobulins can be used. For example, the sequences may be generated from a cell line by *in vitro* stimulation, in response to which the rearrangement occurs.

5 Alternatively, part or all of the sequences may be obtained by combining, e.g., unrearranged V segments with D and J segments, using nucleotide synthesis, randomized mutagenesis, and other methods, such as those disclosed in U.S. Pat. No. 5,565,332. Approximately 1.6×10^7 different antibodies can be produced based on the estimated number of germline gene segments (such as V, D, and J segments of the heavy and light chain variable regions), the
10 random recombination of these segments, and the random pairing of heavy and light chain variable regions (V_H - V_L) (*Fundamental Immunology* (3rd ed), ed. Paul, Raven Press, New York, N.Y., 1993; *Immunobiology: the immune system in health and disease*, 4th ed., Janeway et al., Elsevier Science/Garland Publishing, York, N.Y., 1999). When other processes that contribute to antibody diversity (such as somatic hypermutation) are taken into account,
15 approximately 10^{10} different Igs can be generated (*Immunoglobulin Genes*, 2nd ed., eds. Jonio et al., Academic Press, San Diego, Calif., 1995; *Immunology*, 3rd ed., Kuby, J., W.H. Freeman and Co., New York, NY., 1997).

Polypeptides

The present invention provides compositions of recombinant heavy chain variable
20 region polypeptides in addition to nucleic acids that encode the heavy chain variable region polypeptide. The invention also provides compositions of recombinant light chain variable region polypeptides as well as nucleic acids that encode the heavy chain variable region polypeptide. The recombinant heavy chain variable region polypeptide can be coupled to a light chain variable region polypeptide in order to generate an immunoglobulin. The nucleic
25 acid compositions in addition to the nucleic acid sequences that are useful in the methods of this invention, i.e., those that encode at least in part the individual light chain or heavy chain variable region peptides, polypeptides, or proteins, may be naturally occurring, synthetic or a combination thereof. They may be mRNA, DNA or cDNA. In some embodiments of the invention, the nucleic acids encode antibodies. In further embodiments, the nucleic acids
30 encode a single chain antibody, a bivalent antibody, a Fab fragment, or a single chain Fv.

In one embodiment, amino acid sequences that encode Independently Consensused Frameworks (ICFs) are provided to generate a recombinant heavy chain variable region (see Table 1). In another embodiment, nucleic acids that encode amino acid sequences corresponding to ICFs are provided to generate a recombinant heavy chain variable region

polypeptide (Table 2). In other embodiments, amino acid sequences that encode
Independently Consensused Frameworks are provided to generate a recombinant light chain
variable region (see Table 3). In yet further embodiments, nucleic acids that encode amino
acid sequences corresponding to ICFs are provided to generate a recombinant light chain
5 variable region polypeptide (Table 4). An ICF (for example, ICF1, ICF2, ICF3, or ICF4)
can be a Kabat framework (KF) region (i.e., KF1, KF2, KF3, or KF4) comprising an amino
acid or nucleic acid coding sequence that is a consensus sequence obtained, for example,
from: (1) germline V or J genes, (2) rearranged VDJ genes, (3) rearranged VJ genes, or (4)
amino acid sequences (and/or the nucleic acid sequences that encode identical or essentially
10 identical amino acid sequences) of known Igs.

**Table 1. Exemplary Amino Acid Sequences for Variable Heavy (V_H) Chain ICFs
Heavy Chain IFC1s:**

Identifier	Amino Acid sequence	SEQ ID NO:
GL1_8	EVQLVESGGGLVQPGGSLRLSCAAS	1
GL2	QVQLVESGGGVVQPGRSLRLSCAAS	2
GL3	QVQLQESGPGLVKPSETLSLTCAVS	3
GL4	QVTLKESGPALVKPTQTLTLTCTFS	4
GL5	QVQLQESGPGLVKPSQTLSLTCTVS	5
GL6	EVQLVQSGAEVKKPGESLKISCKGS	6
GL7	QVQLVQSGAEVKKPGASVKVSCKAS	7
GL1a	QVQLVQSGAEVKKPGSSVKVSCKAS	186
GL2a	QVTLRESGPALVKPTQTLTLTCTFS	187
GL4a	QVQLQESGPGLVKPSETLSLTCTVS	188
GL6a	QVQLQQSGPGLVKPSQTLSLTCAIS	189
GL7a	QVQLVESGAEVKKPGASVKVSCKAS	181
GL2b	QVQLVQSGGGVVQPGRSLRLSCAAS	199

Heavy Chain IFC2s:

		SEQ ID NO:
GL1_7_8	WVRQAPGKGLEWVS	8
GL2_3	WVRQAPGKGLEWVG	9
GL4	WVRQAPGQGLEWMG	10
GL5	WVRQAPGKGLEWMG	11

GL 6	WIRQPPGKGLEWIG	12
GL2a	WIRQPPGKALEWLG	190
GL5a	WVRQMPGKGLEWMG	191
GL6a	WIRQSPSRGLEWLG	192

Heavy Chain IFC3s:

		SEQ ID NO:
GL1	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	13
GL2	RFTISRDN SKNTLHLQMNSLRAEDTAVYYCKR	14
GL3	RFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR	15
GL4	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	16
GL5	RLTISKDTSKNQVVLTMTNMDPVD TATYYCAR	17
GL6	RFVFSLDTSVSTAYLQMSSLKAEDTAVYYCAR	18
GL7	RVTISADKSISTAYLQWSSLKASDTAMYYCAR	19
GL8	RVTITADKSTSTAYMELSSLRSED TAVYYCAR	20
GL1a	RFTISRDNAKNSLYLQMNSLRAEDTALYYCAKD	180
GL1b	RVTITADESTSTAYMELSSLRSED TAVYYCAR	193
GL1c	RVTMTRNTSISTAYMELSSLRSED TAVYYCAR	194
GL2a	RFTISRDN SKNTLHLQMNSLRAEDTAVYYCKK	182
GL3a	RFTISRDN SKNSLYLQMNSLR TEDTALYYCAKD	195
GL3b	RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR	196
GL5a	RLTISKDTSKNQVVLTMTNMDPVD TATYYCARI	183
GL6a	RFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR	197
GL6b	RITINPDTSKNQFSLQLNSVTPED TAVYYCAR	198
GL7a	HVTISADKSISTAYLQWSSLKASDTAMYYCAR	184
GL8a	RVTMTRDTSTSTAYMELSSLRSED TAVYYCAR	185

5

Heavy Chain IFC4s:

		SEQ ID NO:
GL1	VTVSSASTKGPS	21
	VTVSASTKGPS	206
	WGQGT VTVSASTKGPS	207

Table 2. Exemplary Nucleic Acid Sequences for Variable Heavy (V_H) Chain ICFs.

Heavy Chain IFC1s:

		SEQ ID NO :
GL1	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTG AGACTCTCCTGTGCAGCCTCT	22
GL2	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG AGACTCTCCTGTGCAGCCTCT	23
GL3	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTG TCCCTCACCTGCGCTGTCTCT	24
GL4	CAGGTCACCTTGAAGGAGTCTGGTCCTGCGCTGGTGAAACCCACACAGACCCTC ACACTGACCTGCACCTTCTCT	25
GL5	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTG TCCCTCACCTGCACTGTCTCT	26
GL6	GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTG AAGATCTCCTGTAAGGGTTCT	27
GL7	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTCGGTG AAGGTCTCCTGCAAGGCTTCT	28

5

Heavy Chain IFC2s:

		SEQ ID NO :
GL1_7_8	TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCA	29
GL2_3	TGGGTCCGCCAGGCTCCAGGCAAGGGGCTAGAGTGGGTGGCA	30
GL4	TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGC	31
GL5	TGGGTGCGACAGGCCCTTGACAAAGGGCTTGAGTGGATGGGA	32
GL6	TGGGTGCGACAGGCTCCTGGAAAAGGGCTTGAGTGGATGGGA	33

Heavy Chain IFC3s:

		SEQ ID NO :
GL1	CGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAAC AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGA	34
GL2	CGATTCACCATCTCCAGAGACAACAGCAAAAACCTCCCTGTATCTGCAAATGAAC	35

	AGTCTGAGAACTGAGGACACCGCCTTGTATTACTGTGCAAGA	
GL3	AGG TTCACCATCTCCAGAGATGATTCAAAGAACACGGCGTATCTGCAAATGAAC AGCCTGAAAACCGAGGACACGGCCGTGTATTACTGTACTAGA	36
GL4	CGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGAAGCTGAGC TCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGA	37
GL5	AGGCTCACCATCTCCAAGGACACCTCCAAAAACCAGGTGGTCCTTACAATGACC AACATGGACCCTGTGGACACAGCCACGTATTACTGTGCACGG	38
GL6	CGATTTGTCTTCTCCCTCGACACGTCTGTCAGCACGGCGTATCTTCAGATGTCT AGCCTAAAGGCTGAGGACACGGCCGTCTATTACTGTGCGCGA	39
GL7	CGCGTCACCATCTCAGCTGACAAGTCCATCAGCACTGCCTACCTGCAGTGGAGC AGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA	40
GL8	AGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGAGC AGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGA	41

Heavy Chain IFC4s:

		SEQ ID NO :
GL1	GTCACCGTCTCCTCCGCCTCCACCAAGGGCCCATCG	42

Table 3. Exemplary Amino Acid Sequences of Variable Light Chain (V_κ or V_λ) ICFs

		SEQ ID NO :
ICF1 kappa	Amino Acid sequence	
VK1_2	DIQMTQSPSSLSASVGDRVTITC	43
VK3	DIQMTQSPSTLSASVGDRVTITC	44
VK4	EIVMTQSPATLSVSPGERATLSC	45
VK5	EIVLTQSPATLSLSPGERATLSC	46
VK6	EIVLTQSPGTLSLSPGERATLSC	47
VK7	DIVMTQSPDSLAVSLGERATINC	48
VK8	DIVMTQSPLSLPVTPGEPASISC	49
VK1_2a	DIVMTQSPSSLSASVGDRVTITC	200
VK4a or VK5a	EIVMTQSPATLSLSPGERATLSC	201
VK7a	DIQMTQSPDFLAVSLGERATINC	202
VKa	EIVLTQSPSSLSASVGDRVTITC	203
	DIVMTQTPLSLPVTPGEPASISC	261
	DIVMTQTPLSLSVTPGQPASISC	262
	EIVLTQSPDFQSVTPKEKVTITC	263
	ETTLTQSPAFMSATPGDKVNISC	264

	AIRMTQSPFSLASVGDRVITTC	265
	AIQLTQSPSSLSASVGDRVITTC	266
	NIQMTQSPSAMSASVGDRVITTC	267
	DVVMTQSPPLSLPVTLGQPASISC	268
	DIVMTQTPLSSPVTLGQPASISC	269
	DVVMTQSPAFLSVTPGEKVTITC	270
	VIWMTQSPSLLSASTGDRVITISC	271
	AIRMTQSPSSFSASTGDRVITTC	272
ICF1 lambda	Amino Acid sequence	
VL1	QSVLTQPPSVSAAPGQKVTISC	50
VL2	QSVLTQPPSASGTPGQRTISC	51
VL3	QSALTQPASVSGSPGQSITISC	52
VL4	QSALTQPRSVSGSPGQSVTISC	53
VL5	SYVLTQPPSVSVAPGKTARITC	54
VL6	SSELTQDPAVSVALGQTVRITC	55
VL7	SYELTQPPSVSVSPGQTASITC	56
VL8	QLVLTQSPSASASLGASVKLTC	57

		SEQ ID NO:
ICF2 kappa	Amino Acid sequence	
VK1_2_3	WYQQKPGKAPKLLIY	58
VK4_5_6	WYQQKPGQAPRLLIY	59
VK7	WYQQKPGQPPKLLIY	60
VK8	WYLQKPGQSPQLLIY	61
VK4_5_6a	WYQQKPCQAPRLLIY	204
	WFQQKPGKAPKSLIY	273
	WYQQKPAKAPKLF IY	274
	WYLQKPGQPPQLLIY	275
	WYQQKPGKAPELLIY	276
	WYQQKPGKVPKLLIY	277
	WYQQKPEKAPKSLIY	278
	WFQQRPGQSPRRLIY	279
	WYQQKPDQSPKLLIK	280
	WFQQKPGKVPKHLLIY	281
	WYQQKPGKAPKRLIY	282
	WLQQRPGQPPRLLIY	283
	WYQQKPGEAAIFIIQ	284

		SEQ ID NO:
ICF 2 lambda	Amino Acid sequence	
VL1_2	WYQQLPGTAPKLLIY	62
VL3_4	WYQQHPGKAPKLMIY	63
VL5_6	WYQQKPGQAPVLVIY	64
VL7	WYQQKPGQSPVLVIY	65
VL8	WHQQQPEKGPRYLMY	66

		SEQ ID NO:
ICF3 kappa	Amino Acid sequence	
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	67
VK2	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	68
VK3	GVPSRFSGSGSGTEFTLTISSLQPDDEFATYYC	69
VK4	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	70
VK5	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC	71
VK6	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	72
VK7	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	73
VK8	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	74
	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC	253
	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	254
	GIPARFSGSGPGTDFTLTISSLQPEDFAVYYC	255
	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC	256
	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC	257
	GVPSRFSGSGSGTDFTFTISSLQAEEDAATYYC	258
	GIPPRFSGSGYGTDFTLTINNIESEDAAYYFC	259
	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	260

Table 4. Exemplary Nucleic Acid Sequences for Variable Light Chain ICFs

		SEQ ID NO:
ICF1 kappa	Nucleic Acid Sequence	
VK1_2	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGC	85
VK3	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGC	86
VK4	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGC	87

VK5	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGC	88
VK6	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGC	89
VK7	GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCG AGAGGGCCACCATCAACTGC	90
VK8	GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAG AGCCGGCCTCCATCTCCTGC	91

ICF1 lambda	Nucleic Acid Sequence	SEQ ID NO:
VL1	CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGA AGGTCACCATCTCCTGC	92
VL2	CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGA GGGTCACCATCTCTTGT	93
VL3	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGT CGATCACCATCTCCTGC	94
VL4	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTCCTGGACAGT CAGTCACCATCTCCTGC	95
VL5	TCCTATGTGCTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGA CGGCCAGGATTACCTGT	96
VL6	TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGA CAGTCAGGATCACATGC	97
VL7	TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGA CAGCCAGCATCACCTGC	98

ICF2 kappa	Nucleic Acid Sequence	SEQ ID NO:
VK1_2_3	TGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAGCTCCTGATCTAT	100
VK4_5_6	TGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT	101
VK7	TGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTAC	102
VK8	TGGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT	103

ICF2 lambda	Nucleic Acid Sequence	
VL1_2	TGGTACCAGCAGCTCCCAGGAACAGCCCCCAAACCTCCTCATCTAT	104
VL3_4	TGGTACCAACAGCACCCAGGCAAAGCCCCCAAACCTCATGATTTAT	105
VL5_6	TGGTACCAGCAGAAGCCAGGCCAGGCCCTGTGCTGGTCATCTAT	106

VL7	TGGTATCAGCAGAAGCCAGGCCAGTCCCCTGTGCTGGTCATCTAT	107
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		SEQ ID NO:
ICF3 kappa	Nucleic Acid Sequence	
VK1	GGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTC TCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGT	109
VK2	GGGGTCCCATCAAGGTTTCAGTGGGAAGTGGATCTGGGACAGATTTTACTT TCACCATCAGCAGCCTGCAGCCTGAAGATATTGCAACATATTACTGT	110
VK3	GGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCACTC TCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGC	111
VK4	GGCATCCCAGCCAGGTTTCAGTGGCAGTGGGTCTGGGACAGAGTTCACTC TCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAAGTTTATTACTGT	112
VK5	GGCATCCCAGCCAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTC TCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAAGTTTATTACTGT	113
VK6	GGCATCCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTC TCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAAGTTTATTACTGT	114
VK7	GGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGGGACAGATTTCACTC TCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGT	115
VK8	GGGGTCCCTGACAGGTTTCAGTGGCAGTGGATCAGGCACAGATTTTACAC TGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGT	116

		SEQ ID NO:
ICF3 lambda	Nucleic Acid Sequence	
VL1	GGGATTCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCC TGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATTATTACTGC	117
VL2	GGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCC TGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGT	118
VL3	GGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCC TGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGC	119
VL4	GGGGTCCCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCC TGACCATCTCTGGGCTCCAGGCTGAGGATGAGGCTGATTATTACTGC	120
VL5	GGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGGGAACACGGCCACCC TGACCATCAGCAGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGT	121
VL6	GGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCT TGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACTGT	122
VL7	GGGATCCCTGAGCGATTCTCTGGCTCCAAGTCTGGGAACACAGCCACTC TGACCATCAGCGGGACCCAGGCTATGGATGAGGCTGACTATTACTGT	123

		SEQ ID NO:
ICF4 kappa	Nucleic Acid Sequence	
VK1	TTCGGCCAAGGGACCAAGGTGGAAATCAAA	125

		SEQ ID NO:
ICF4 lambda	Nucleic Acid Sequence	
VL1	TTCGGCGGAGGGACCAAGCTGACCGTCCTA	126

5 In addition to the sequences listed in Tables 1 to 4, other ICFs that can be used in the invention are provided (colored or non-underlined subsequences) in Figures 24, 32, 33, 34, 38, 39, and in the assembled HuFR sequences disclosed in Examples 3 and 4.

For example, each ICF comprises a consensus sequence that is independently selected, wherein independent selection can involve aligning a pool of ICF germline nucleic acid sequences obtained from a plurality of germline nucleic acid sequences encoding at least some portion of a variable chain Kabat framework region and subsequently clustering the sequences according to sequence similarity. For example, the sequences from each framework cluster are then used to establish a consensus sequence for a heavy chain variable region. In a non-limiting example, the sequences of all germline human V_H exons can be compiled, and each of the exon sequences can be subsequently divided into the framework subregions, FR1, 2, 3, and 4 as prescribed by Kabat *et al.* (see above).

A set of FR subregion sequences (such as a pool of Kabat FR1 sequences), rather than a sequence of the entire framework that comprises framework subregions 1-4, are then aligned and clustered by sequence similarity. Sequences from each FR subregion cluster (for example, FR1, FR2, FR3, or FR4) can then be used to create a consensus sequence (for example ICF1, ICF2, ICF3, and ICF4), independently derived from the entire framework region, which comprises the most frequent amino acid occurring at each sequence position (see Tables 1 and 2). This consensus process can also be carried out for V_L exons in order to identify the consensus sequences within each framework subregion (Tables 3-4; see also Examples 1-2). For example, the ICF sequences used for assembling the heavy chain variable region resulted in a total of 336 heavy chain variable region combinations (i.e., 7 ICF1 sequences x 6 ICF2 sequences x 8 ICF3 sequences x 1 ICF4 sequence). In another

example using the ICF sequences to assemble a light chain variable region (for example a kappa light chain), a total of 224 light chain variable region combinations are possible (i.e., 7 IFC1 sequences x 4 ICF2 sequences x 8 ICF3 sequences x 1 ICF4 sequence). When both the heavy chain and light chain variable region combinations are associated with another, a total of 75,264 heavy chain-light chain complexes (for example, more human-like immunoglobulin molecules) can be generated.

In one embodiment, the heavy chain variable region ICF1 nucleic acid sequence comprises any of SEQ ID NO: 22, 23, 24, 25, 26, 27, or 28. In another embodiment, the heavy chain variable region ICF2 nucleic acid sequence comprises SEQ ID NO: 29, 30, 31, 32, or 33. In a further embodiment, the heavy chain variable region ICF3 nucleic acid sequence comprises SEQ ID NO: 34, 35, 36, 37, 38, 39, 40, or 41. In yet another embodiment of the invention, the heavy chain variable region ICF4 nucleic acid sequence is SEQ ID NO: 42.

In another example, the sequences from each framework cluster can also be used to establish a consensus sequence for a light chain variable region. In one embodiment, the light chain variable region ICF1 nucleic acid sequence comprises SEQ ID NO: 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, or 98. In another embodiment, the light chain variable region ICF2 nucleic acid sequence comprises SEQ ID NO: 100, 101, 102, 103, 104, 105, 106, or 107. In a further embodiment, the light chain variable region ICF3 nucleic acid sequence comprises SEQ ID NO: 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, or 123. In yet another embodiment of the invention, the light chain variable region ICF4 nucleic acid sequence is SEQ ID NO: 125 or 126.

Amino acid sequences from each framework cluster can also be used to establish a consensus sequence for a heavy chain variable region. In one embodiment, the heavy chain variable region ICF1 amino acid sequence comprises any of SEQ ID NO: 1-7, 186, 187, 188, 189, 181, 199. In another embodiment, the heavy chain variable region ICF2 amino acid sequence comprises any of SEQ ID NO: 8-12 or 190-192. In a further embodiment, the heavy chain variable region ICF3 amino acid sequence comprises SEQ ID NO: 13-20, 180, 182-185, or 193-198. In yet another embodiment of the invention, the heavy chain variable region ICF4 amino acid sequence is SEQ ID NO: 21, 206, or 207.

Additionally, amino acid sequences from each framework cluster can also be used to establish a consensus sequence for a light chain variable region. In one embodiment, the light chain variable region ICF1 amino acid sequence comprises any of SEQ ID NO: 43-57 or 200-204. In another embodiment, the light chain variable region ICF2 amino acid sequence

comprises SEQ ID NO: 58, 59, 60, 61, 62, 63, 64, 65, 66, or 204. In a further embodiment, the light chain variable region ICF3 amino acid sequence comprises SEQ ID NO: 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, or 82. In yet another embodiment of the invention, the light chain variable region ICF4 amino acid sequence is SEQ ID NO: 83 or 84.

5 Upon translation, the ICF1, ICF2, ICF3, or ICF4 consensus sequences demonstrate the most frequent amino acid sequences that occur at each residue position. An ICF sequence can be identical to the original germline sequence used to determine the ICF domain. In one embodiment, the ICF sequences comprising a heavy chain variable region are at least 80%, identical to a germline Kabat Framework Region (KFR). In another embodiment, the ICF
10 sequences are at least 85%, 90%, 93%, 95%, 99%, or 100% identical to a germline KFR.

 An ICF sequence of a light chain variable region polypeptides of the instant invention also can be identical to the original germline sequence used to determine the ICF domain. In another embodiments the ICF sequences comprising a light chain variable region are at least 70% identical to a germline KFR. In another embodiment, the ICF sequences are at least
15 50%, 60%, 70%, 80%, 85%, 90%, 93%, 95%, 99%, or 100% identical to a germline KFR.

 Upon translation, the ICF1, ICF2, ICF3, or ICF4 consensus sequences demonstrate the most frequent amino acid sequences that occur at each residue position. In one embodiment, the ICF sequences comprising a heavy chain variable region are at least 80%, 85%, 90%, 93%, 95%, 99%, or 100% identical to a mature antibody KFR.

20 An ICF sequence of a light chain variable region polypeptides of the instant invention also can be identical to the original mature antibody sequence used to determine the ICF domain. In one embodiment, the ICF sequences comprising a light chain variable region are at least 50% identical to a mature antibody KFR. In other embodiments, the ICF sequences comprising a light chain variable region are at least 60%, 70%, 80%, 85%, 90%, 93%, 95%,
25 99%, or 100% identical to a mature antibody KFR.

 The variable framework region amino acid residues can correspond to the standard Kabat numbering system as described above. The Kabat numbering system can correspond to the ICF amino acid sequences of the current invention. For example, ICF1 of the heavy chain variable region can comprise about 25 residues of a Kabat Framework (KF) 1. In one
30 embodiment, ICF1 of the heavy chain variable region comprises at least 20, 25, or at least 29 contiguous residues of a KF1.

 In one embodiment, ICF2 of the heavy chain variable region can comprise about 14 residues of a KF2. In one embodiment, ICF2 of the heavy chain variable region comprises at least 10, 12, or 14 contiguous residues of a KF2.

ICF3 of the heavy chain variable region can comprise about 32 residues of a KF3. In one embodiment, ICF3 of the heavy chain variable region comprises at least 25, 30, or 32 contiguous residues of a KF3.

ICF4 of the heavy chain variable region can comprise about 12 residues of a KF4. In one embodiment, ICF4 of the heavy chain variable region comprises at least 8, 10, 12 contiguous residues of a KF4.

The Kabat numbering system can also correspond to the ICF amino acid sequences of a light chain variable region polypeptide of the current invention. For example, ICF1 of a light chain (for example, V_K or V_L) variable region can comprise about 22 residues of a Kabat Framework (KF) 1. In one embodiment, ICF1 of a light chain variable region comprises at least 15, 20, or 23 contiguous residues of a KF1.

ICF2 of a light chain variable region can comprise about 15 residues of a KF2. In one embodiment, ICF2 of a light chain variable region comprises at least 10 contiguous residues of a KF2. In another embodiment, ICF2 comprises at least 12 contiguous residues of a KF2. In a further embodiment, ICF2 comprises at least 14 contiguous residues of a KF2.

ICF3 of a light chain variable region can comprise about 32 residues of a KF3. In one embodiment, ICF3 of a light chain variable region comprises at least 25, 30, or 32 contiguous residues of a KF3.

ICF4 of a light chain variable region can comprise about 10 residues of a KF4. In one embodiment, ICF4 of a light chain variable region comprises at least 8, 10, 13 contiguous residues of a KF4.

An ICF nucleic acid consensus sequence can include any wobble site changes in the nucleic acid consensus sequence wherein the nucleotide change will still encode the same amino acid sequence. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For example, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide.

In certain embodiments, a nucleic acid sequence encoding a heavy chain variable region ICF1 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 22, 23, 24, 25, 26, 27, or 28. In other embodiments, a nucleic acid sequence encoding a heavy chain variable region ICF2 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 29, 30, 31, 32, or 33. In some

embodiments, a nucleic acid sequence encoding a heavy chain variable region ICF3 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 34, 35, 36, 37, 38, 39, 40, or 41. In yet further embodiments, a nucleic acid sequence encoding a heavy chain variable region ICF4 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42.

In other embodiments, a nucleic acid sequence encoding a light chain variable region ICF1 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, or 98. In certain embodiments, a nucleic acid sequence encoding a light chain variable region ICF2 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 100, 101, 102, 103, 104, 105, 106, or 107. In some embodiments, a nucleic acid sequence encoding a light chain variable region ICF3 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, or 123. In yet other embodiments, a nucleic acid sequence encoding a light chain variable region ICF4 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 125 or 126.

Conservative amino acid changes refer to the interchangeability of amino acid residues having similar side chains changes. For example, a group of amino acids having basic side chains is lysine, arginine, and histidine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aromatic side chains is tyrosine, phenylalanine, and tryptophan; and a group of amino acids having sulfur-containing side chains is cysteine and methionine.

In certain embodiments, an amino acid sequence encoding a heavy chain variable region ICF1 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, 2, 3, 4, 5, 6, or 7. In other embodiments, an amino acid sequence encoding a heavy chain variable region ICF2 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8, 9, 10, 11, or 12. In some embodiments, an amino acid sequence encoding a heavy chain variable region ICF3 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, or 20. In yet further embodiments, an amino acid sequence encoding a heavy chain variable region ICF4 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 21.

In other embodiments, a amino acid sequence encoding a light chain variable region ICF1 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, or 57. In certain embodiments, a amino acid sequence encoding a light chain variable region ICF2 is at least 70%, 75%, 80%,
5 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 58, 59, 60, 61, 62, 63, 64, 65, or 66. In some embodiments, a amino acid sequence encoding a light chain variable region ICF3 is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, or 82. In yet other embodiments, an amino acid sequence encoding a light chain variable region ICF4 is at least
10 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 83 or 84.

An ICF nucleic acid or amino acid consensus sequence (for example, one that corresponds to ICF1, ICF2, ICF3, or ICF4) can be determined for a human based on human germline sequences or mature (i.e., rearranged) Antibody sequences. An ICF nucleic acid or
15 amino acid consensus sequence also can be determined for the following non-limiting examples, such as canine, feline, ovine, equine, bovine, porcine, fowl, goat, salmon, and hybridoma cell line, utilizing the appropriate germline sequences.

The present invention provides for antibody compositions generated from the heavy chain and light chain variable regions described above. According to the invention, any
20 CDRs from known antibodies (for example, those shown in Tables 5-6) can be combined with ICFs, such as those of Tables 1-4. In addition, they can be further combined to a constant domain (CD) (for example, those shown in Tables 7-8) to generate full-length heavy chain variable region polypeptides or a full-length light chain variable region polypeptide. These polypeptides can be combined subsequently to generate Igs, wherein the Igs can serve
25 as functional units for the following non-limiting antibody examples: a single chain antibody, a bivalent antibody (such as a disulfide-linked antibody), a Fab fragment, and a single chain Fv.

Immunoglobulin fragments can be generated by proteolytic digestion and have proven to be very useful in elucidating structure/function relationships in immunoglobulins. Ig
30 fragments can include combinations of heavy and light chain variable regions in order to form an antigen-binding site. Antibody fragments include, for example, Fab, Fab', F(ab')₂, Fv, scFv, Fd, and Fd' fragments.

For example, Fab fragments can be generated by digestion with papain, wherein the enzyme breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain

disulfide bond. This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain and additionally comprise the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. Fc fragments, for example can also be generated by digestion with
5 papain. The enzyme is able to produce a fragment that contains the remainder of the two heavy chains each containing a C_{H2} and C_{H3} domain.

Treatment of immunoglobulins with pepsin results in the cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites. This divalent fragment generated by pepsin digest is referred to as $F(ab')_2$. The
10 Fc region of the molecule is digested into small peptides by pepsin. The $F(ab')_2$ fragment can bind its antigen but does not generally mediate the effector functions of antibodies.

Compositions

Each of the compounds of this invention (e.g., compounds described herein) can be used as a composition (e.g., a pharmaceutical composition) when combined with an
15 acceptable carrier or excipient. These compositions (e.g., a pharmaceutical compositions) of the invention can be useful for *in vitro* or *in vivo* analysis or for administration to a subject (e.g., a human) *in vivo* or *ex vivo* for treating a subject.

Thus, pharmaceutical compositions of this invention can include, in addition to active ingredient(s), a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other
20 materials well known to those skilled in the art. In one aspect, these materials are non-toxic and do not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the formulation and route of administration.

Pharmaceutical formulations of this invention comprising a protein of the invention, e.g., an antibody or antigen binding fragment thereof of the invention (e.g., as identified by
25 the methods described herein), can be prepared for storage by, e.g., mixing the protein having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (see, e.g., Remington's Pharmaceutical Sciences latest edition, or the 16th edition, Osol, A. Ed. (1980)), e.g., in the form of lyophilized formulations or aqueous solutions. In alternative embodiments, acceptable carriers, excipients, or stabilizers are those that are non-
30 toxic to recipients (e.g., human patients) at the dosages and concentrations employed, and can include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol;

resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other
5 carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

In alternative embodiments, acceptable carriers are physiologically acceptable to the
10 administered individual (e.g., a human patient) and retain the therapeutic properties of the compounds with/in which it is administered. Acceptable carriers and their formulations are and generally described in, for example, Remington' pharmaceutical Sciences latest edition (see also the 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA 1990). In one aspect, an exemplary carrier is physiological saline.

15 "Pharmaceutically acceptable carriers" used to practice this invention can comprise a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject compounds from the administration site of one organ, or portion of the body, to another organ, or portion of the body, or in an *ex vivo* or *in vitro* assay system. In alternative
20 embodiments, each carrier is acceptable in the sense of being compatible with the other ingredients of the formulation and not injurious to a subject to whom it is administered. In alternative embodiments, acceptable carrier do not alter the specific activity of the subject compounds.

Pharmaceutical compositions or pharmaceutical formulations of this invention include
25 compositions suitable for pharmaceutical use in a subject. The pharmaceutical compositions and formulations of this invention can include an amount of a compound of this invention and a pharmaceutically or physiologically acceptable carrier.

Compositions (e.g., pharmaceutical compositions or pharmaceutical formulations) of this invention can be formulated to be compatible with a particular route of administration
30 (i.e., systemic or local). Thus, compositions of this invention can include carriers, diluents, or excipients suitable for administration by various routes.

In another embodiment, the compositions can further comprise, if needed, an acceptable additive in order to improve the stability of the compounds in composition and/or to control the release rate of the composition. Acceptable additives do not alter the specific

activity of the subject compounds. Exemplary acceptable additives include, but are not limited to, a sugar such as mannitol, sorbitol, glucose, xylitol, trehalose, sorbose, sucrose, galactose, dextran, dextrose, fructose, lactose and mixtures thereof. Acceptable additives can be combined with acceptable carriers and/or excipients such as dextrose. Alternatively,

5 exemplary acceptable additives include, but are not limited to, a surfactant such as polysorbate 20 or polysorbate 80 to increase stability of the peptide and decrease gelling of the solution. The surfactant can be added to the composition in an amount of 0.01% to 5% of the solution. Addition of such acceptable additives increases the stability and half-life of the composition in storage.

10 The pharmaceutical composition of this invention can be administered, for example, by injection. In alternative embodiments, compositions for injection include aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In alternative embodiments, for intravenous administration, suitable carriers include physiological saline, bacteriostatic water,

15 CREMOPHOR EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and

20 by the use of surfactants.

Antibacterial and antifungal agents can include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride may be included in the composition. The resulting solutions can be packaged for use as is, or lyophilized; the lyophilized preparation can later

25 be combined with a sterile solution prior to administration.

For intravenous, injection, or injection at the site of affliction, the active ingredient can be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Compositions of the invention can comprise (and those of relevant skill in the art are well able to prepare) suitable solutions using, for example,

30 isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included, as needed. Sterile injectable solutions can be prepared by incorporating an active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. In one aspect,

dispersions are prepared by incorporating the active ingredient into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, alternative methods of preparation are vacuum drying and freeze drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Compositions can be conventionally administered intravenously, such as by injection of a unit dose, for example. For injection, an active ingredient can be in the form of a parenterally acceptable aqueous solution which is substantially pyrogen-free and has suitable pH, isotonicity and stability. One can prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included, as required. Additionally, compositions can be administered via aerosolization. (Lahn et al., Aerosolized Anti-T-cell-Receptor Antibodies Are Effective against Airway Inflammation and Hyperreactivity, Int. Arch. Allergy Immuno., 134: 49-55 (2004)).

One embodiment contemplates the use of the compositions described herein to make a medicament for treating a condition, disease or disorder described herein. Medicaments can be formulated based on the physical characteristics of the patient/subject needing treatment, and can be formulated in single or multiple formulations based on the stage of the condition, disease or disorder. Medicaments can be packaged in a suitable package with appropriate labels for the distribution to hospitals and clinics wherein the label is for the indication of treating a subject having a disease described herein.

Treatment

In one aspect, polypeptides of the invention can specifically bind CD20, a transmembrane surface antigen on B-cell precursors and mature B-cells that is not internalized after binding nor shed from the cell surface. CD20 is also expressed a large percentage of B-cells involved in a wide variety of diseases. The antibodies or antigen binding fragments of this invention can be used to treat a subject with a tumorigenic disorder, e.g., a disorder characterized by the presence of tumor cells expressing CD20 including, for example, B cell lymphoma, e.g., NHL.

In alternative aspects, compositions of the invention, and methods of this invention, are used for "inhibition," "amelioration," "treatment" and/or "treating" a disease or condition, and these terms can be used interchangeably and can refer to, for example, stasis of symptoms, prolongation of survival, partial or full amelioration of symptoms, and partial or

full eradication of a condition, disease or disorder. The antibodies or antigen binding fragments of this invention can be used to treat a B-cell mediated disease. In one aspect, a “treatment” of the invention can include the suppression or abrogation of an immune response. The antibodies or antigen binding fragments of this invention can be used to suppress or abrogate a B-cell mediated immune response. The antibodies or antigen binding fragments of this invention can be used to in the treatment of cancers, including the stasis, partial or total elimination of a cancerous cells, growth, or tumor. In alternative aspects, treatment or partial elimination includes, for example, a fold reduction in cells, growth or tumor size and/or volume such as about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 20-fold, about 50-fold, or any fold reduction in between. In alternative aspects, treatment or partial elimination can include a percent reduction in cells, growth or tumor size and/or volume of about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or any percentage reduction in between.

Examples of tumorigenic diseases which can be treated and/or prevented using compositions or methods of this invention include B cell lymphoma, e.g., NHL, including precursor B cell lymphoblastic leukemia/lymphoma and mature B cell neoplasms, such as B cell chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, mantle cell lymphoma (MCL), follicular lymphoma (FL), including low-grade, intermediate-grade and high-grade FL, cutaneous follicle center lymphoma, marginal zone B cell lymphoma (MALT type, nodal and splenic type), hairy cell leukemia, diffuse large B cell lymphoma, Burkitt's lymphoma, plasmacytoma, plasma cell myeloma, post-transplant lymphoproliferative disorder, Waldenstrom's macroglobulinemia, and anaplastic large-cell lymphoma (ALCL).

Further examples of B cell non-Hodgkin's lymphomas which can be treated and/or prevented using compositions or methods of this invention are lymphomatoid granulomatosis, primary effusion lymphoma, intravascular large B cell lymphoma, mediastinal large B cell lymphoma, heavy chain diseases (including .gamma., .mu., and .alpha. disease), lymphomas induced by therapy with immunosuppressive agents, such as cyclosporine-induced lymphoma, and methotrexate-induced lymphoma.

Examples of immune disorders in which CD20 expressing B cells are involved which can be treated and/or prevented using compositions or methods of this invention include autoimmune disorders, such as psoriasis, psoriatic arthritis, dermatitis, systemic scleroderma and sclerosis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, respiratory distress syndrome, meningitis, encephalitis, uveitis, glomerulonephritis, eczema,

asthma, atherosclerosis, leukocyte adhesion deficiency, multiple sclerosis, Raynaud's syndrome, Sjogren's syndrome, juvenile onset diabetes, Reiter's disease, Behcet's disease, immune complex nephritis, IgA nephropathy, IgM polyneuropathies, immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpura and chronic idiopathic thrombocytopenic purpura, hemolytic anemia, myasthenia gravis, lupus nephritis, systemic lupus erythematosus, rheumatoid arthritis (RA), atopic dermatitis, pemphigus, Graves' disease, Hashimoto's thyroiditis, Wegener's granulomatosis, Omenn's syndrome, chronic renal failure, acute infectious mononucleosis, HIV, and herpes virus associated diseases. Further examples are severe acute respiratory distress syndrome and choreoretinitis.

In alternative aspects, other diseases and disorders that can be treated and/or prevented using compositions or methods of this invention include those caused by or mediated by infection of B-cells with virus, such as Epstein-Barr virus (EBV).

Table 5. Exemplary Nucleic Acid Sequences for CDR Fragments of the Variable Heavy Chain (V_H) and Variable Light Chain (V_K or V_L) of listed antibodies.

		SEQ ID NO:
VH CDR	Nucleic Acid Sequence	
CD20 IgG		
1	GGCTACACATTTACCAGTTACAATATGCAC	127
2	GCTATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGC	128
3	TCGCACTACGGTAGTAAGTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCTG	129
CD3 IgG		
1	GGCTACACCTTTACTAGGTACACGATGCAC	133
2	TACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGAC	134
3	TATTATGATGATCATTACTGCCTTGACTAC	135
LC CDR	Nucleic Acid Sequence	
CD20 IgG		
1	AGGGCCAGCTCAAGTTTAAGTTTCATGCAC	139
2	GCCACATCCAACCTGGCTTCT	140
3	CATCAGTGGAGTAGTAACCCGCTCACG	141
CD3 IgG		
1	AGTGCCAGCTCAAGTGTAAGTTACATGAAC	145
2	GACACATCCAACTGGCTTCT	146
3	CAGCAGTGGAGTAGTAACCCATTACG	147

Table 6. Exemplary Amino Acid Sequences for CDR Fragments of the Variable Heavy Chain (V_H) and Variable Light Chain (V_κ or V_λ) of listed antibodies.

		SEQ ID NO:
VH CDR	Amino Acid Sequence	
CD20 IgG		
1	GYTFTSYNMH	151
2	AIYPGNGDTSYNQKFKG	152
3	SHYGSNYVDYFDYWQGTL	153
CD3 IgG		
1	GYTFTRYTMH	157
2	YINPSRGYTNYNQKFKD	158
3	YYDDHYCLDY	159
LC CDR	Amino Acid Sequence	
CD20 IgG		
1	RASSSLSFMH	163
2	ATSNLAS	164
3	HQWSSNPLT	165
CD3 IgG		
1	SASSSVSYMN	169
2	DTSKLAS	170
3	QQWSSNPFT	171

5 Table 7. Amino Acid Sequences of Exemplary Constant Domains (CD) of an Ig Heavy Chain and κ Light Chain

		SEQ ID NO:
HC CD	Amino Acid Sequence	
anti- CD20	VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	175
	VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQ	208

	KSLSLSPGK	
κ CD	Amino Acid Sequence	
	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY	176
anti-CD20	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	209

Table 8. Nucleic Acid Sequences encoding Exemplary Constant Domains (CD) of an Ig Heavy Chain and κ Light Chain

		SEQ ID NO:
HC CD	Nucleic Acid Sequence	
	GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCA	178
	GGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA	
κ CD	Nucleic Acid Sequence	
	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC	179
	TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTAC	

In addition to the sequences listed in Tables 5 to 8, other CDRs (underlined) and constant region (double-underlined or yellow-highlighted) are provided in Figures 24, 32, 33, 34, 36, 38, 39, 42, and in the original and assembled HuFR sequences disclosed in Examples 3 to 6.

5

Exemplary Antibodies

In alternative embodiments, compositions of the invention, e.g., chimeric and/or recombinant antibodies of the invention, specifically bind to CD20, which is an unglycosylated phosphoprotein that is expressed on the surface of B cells and serves a B-cell marker. CD20 acts as a regulator of transmembrane calcium conductance and purportedly plays a role in B cell activation and proliferation. In one aspect of the invention, an antibody can be generated containing a more human-like variable region, that is directed at a surface protein of a eukaryotic cell (for example, B cells). In one embodiment, a recombinant heavy chain variable region polypeptide and a recombinant light chain variable region polypeptide of the invention containing the CDRs of an anti-CD20 antibody are used to form an antibody with variable regions more human in characterization. For example, the antibody binds to a CD20 antigen. In one embodiment, the antibody's light chain variable region comprises an ICF1 comprising an amino acid sequence of SEQ ID NOS:43, 44, 45, 46, 47, 38, or 49; a CDR1 comprising an amino acid sequence of SEQ ID NO:163; an ICF2 comprising an amino acid sequence of SEQ ID NOS:58, 59, 60, or 61; a CDR2 comprising an amino acid sequence of SEQ ID NO:164; an ICF3 comprising an amino acid sequence of SEQ ID NOS:67-71, 73, or 74; a CDR3 comprising an amino acid sequence of SEQ ID NO:165; and an ICF4 comprising an amino acid sequence of SEQ ID NO:83. In other embodiments, the antibody's heavy chain variable region comprises an ICF1 comprising an amino acid sequence of SEQ ID NOS: 6 or 7; a CDR1 comprising an amino acid sequence of SEQ ID NO:151; an ICF2 comprising an amino acid sequence of SEQ ID NOS:9, 10, or 11; a CDR2 comprising an amino acid sequence of SEQ ID NO:152; an ICF3 comprising an amino acid sequence of SEQ ID NOS:13, 17, 19, or 20; a CDR3 comprising an amino acid sequence of SEQ ID NO:153; and an ICF4 comprising an amino acid sequence of SEQ ID NO:21.

CD3 is a component of the T-cell receptor complex. It is a surface marker specific to T cells and, thus can be used to specifically identify T cells. According to the invention, an antibody can be generated containing a more human-like variable region, that is directed at a surface protein of a eukaryotic cell (for example, T cells). In one embodiment, a recombinant heavy chain variable region polypeptide and a recombinant light chain variable region

polypeptide of the invention containing the CDRs of an anti-CD3 antibody are used to form an antibody with variable regions more human in characterization. For example, the antibody binds to a CD3 antigen. In one embodiment, the antibody's light chain variable region comprises an ICF1 comprising an amino acid sequence of SEQ ID NOS:43–46, or 49; a

5 CDR1 comprising an amino acid sequence of SEQ ID NO:169; an ICF2 comprising an amino acid sequence of SEQ ID NOS:58, 59, or 60; a CDR2 comprising an amino acid sequence of SEQ ID NO:170; an ICF3 comprising an amino acid sequence of SEQ ID NOS:68–69, 72, 73, or 74; a CDR3 comprising an amino acid sequence of SEQ ID NO:171; and an ICF4 comprising an amino acid sequence of SEQ ID NO:83. In a further embodiment, the

10 antibody's heavy chain variable region comprises an ICF1 comprising an amino acid sequence of SEQ ID NOS:3, 7, or 181; a CDR1 comprising an amino acid sequence of SEQ ID NO:157; an ICF2 comprising an amino acid sequence of SEQ ID NOS:9 or 11; a CDR2 comprising an amino acid sequence of SEQ ID NO:158; an ICF3 comprising an amino acid sequence of SEQ ID NOS:15, 16, or 17; a CDR3 comprising an amino acid sequence of SEQ

15 ID NO:159; and an ICF4 comprising an amino acid sequence of SEQ ID NO:21.

Nucleic acids

In one embodiment, nucleic acid sequences of the invention that encode independently consensused heavy chain variable region domains ICF 1, 2, and 3 are provided

20 in addition to sequences encoding complementarity determining regions 1, 2, and 3 of a known immunoglobulin heavy chain variable region (such as that of anti-CD20, anti-CD3). In another embodiment, nucleic acid sequences of the invention that encode independently consensused light chain variable region domains ICF 1, 2, and 3 are provided in addition to sequences encoding complementarity determining regions 1, 2, and 3 of a known

25 immunoglobulin heavy chain variable region (such as that of anti-CD20, anti-CD3). In a further embodiment, nucleic acid sequences that encode independently consensused heavy chain and light chain variable region domain ICF4 are additionally provided. For example, heavy chain variable region ICF 1, 2, 3, and 4 domains and light chain variable region ICF 1, 2, 3, and 4 domains of the current invention have SEQ ID NOs listed in Tables 2 and 4.

30 Nucleic acid sequences corresponding to CDRs 1, 2, and 3 of a known immunoglobulin heavy chain variable region are found in Table 5.

The nucleic acids encoding the heavy chain or light chain variable region ICF domains and CDRs that are provided from above are fused in a 5'-to-3' orientation, forming nucleic acids that generate a heterogeneous population of single nucleic acid molecules. In

one embodiment, nucleic acids encoding the heavy chain variable region ICF domains and CDRs are fused in a 5'-to-3' orientation in the following order: a nucleic acid encoding ICF1; a nucleic acid encoding CDR1; a nucleic acid encoding ICF2; a nucleic acid encoding CDR2; a nucleic acid encoding ICF3; and a nucleic acid encoding CDR3. In another embodiment, nucleic acids encoding the light chain variable region ICF domains and CDRs are fused in a 5'-to-3' orientation in the following order: a nucleic acid encoding ICF1; a nucleic acid encoding CDR1; a nucleic acid encoding ICF2; a nucleic acid encoding CDR2; a nucleic acid encoding ICF3; and a nucleic acid encoding CDR3. In a further embodiment, nucleic acid sequences that encode heavy chain and light chain variable region domain ICF4 are fused in a 5'-to-3' orientation the C-terminus of heavy chain or light chain CDR3. For example, heavy chain variable region ICF 1, 2, 3, and 4 domains and light chain variable region ICF 1, 2, 3, and 4 domains of the current invention have SEQ ID NOS listed in Tables 2 and 4. Nucleic acid sequences corresponding to CDRs 1, 2, and 3 of a known immunoglobulin heavy chain variable region are found in Table 5.

An Ig chain obtained by HuFR can be further modified for desired properties using Gene Site Saturation Mutagenesis (GSSMTM) or Synthetic Ligation Reassembly (SLR or GeneReassemblyTM) evolution methods, as described in U.S. Patent No. 6,171,820, No. 6,537,776, No. 6,562,594, No. 6,605,449, and No. 6,764,835.

Vectors

Once the heavy chain or light chain variable region molecule is generated, it can then be cloned into a plasmid and transformed into cells so as to express the heavy chain or light chain variable region polypeptide. In one embodiment, plasmids carrying the heavy chain or light chain variable region polypeptide genes were amplified in *E. coli* and transfected into mammalian cells for production of full-length immunoglobulins.

The cells suitable for culturing can harbor introduced expression vectors (constructs), such as plasmids. The expression vector constructs can be introduced by transfection, lipofection, transformation, injection, electroporation, or infection. The expression vectors can contain coding sequences, or portions thereof, encoding the proteins for expression and production in the culturing process. Such expression vectors can include the required components for the transcription and translation of the inserted coding sequence. Expression vectors containing sequences encoding the produced proteins and polypeptides, as well as the appropriate transcriptional and translational control elements, can be generated using methods well known to and practiced by those skilled in the art. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination

which are described in J. Sambrook et al., (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y. and in F. M. Ausubel et al., 1989, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y. A more detailed description of the types of methods and tools that can be utilized is provided below.

5 Clones obtained by standard molecular biology protocols can be transfected into suitable host cells, such as mammalian cells, for expression of the desired product. Transfection techniques are carried out using standard techniques established in the art appropriate for the host cell being utilized. For example, mammalian cell transfection can be accomplished using lipofection, protoplast fusion, DEAE-dextran-mediated transfection,
 10 CaPO₄ co-precipitation, electroporation, direct microinjection, as well as other methods known in the art which can comprise: scraping, direct uptake, osmotic or sucrose shock, lysozyme fusion or erythrocyte fusion, indirect microinjection such as erythrocyte-mediated techniques, and/or by subjecting host cells to electric currents.

Expression

15 Expression of DNA encoding a protein of interest (for example, heavy chain or light chain variable region polypeptides, glycoproteins such as Igs) in eukaryotic host cells derived from multicellular organisms (for example, mammalian in origin) can be utilized in the context of this invention (*Tissue Cultures*, (1973) Academic Press, Cruz and Patterson, Eds.). Host cells derived from multicellular organisms have the ability to splice out introns and thus
 20 can be used directly to express genomic DNA fragments. Useful host cell lines capable of harboring, expressing, and secreting a protein of interest include Chinese hamster ovary cells (CHO), such as CHO-K1 (ATCC CCL-61), DG44 (Chasin et al., 1986, *Som. Cell Molec. Genet*, 12:555-556; Kolkekar et al., 1997, *Biochemistry*, 36:10901-10909; and WO 01/92337 A2), dihydrofolate reductase negative CHO cells (CHO/*dhfr*⁻, Urlaub and Chasin, 1980,
 25 *Proc. Natl. Acad. Sci. USA*, 77:4216), and dp12.CHO cells (U.S. Pat. No. 5,721,121); monkey kidney CV1 cells transformed by SV40 (COS cells, COS-7, ATCC CRL-1651); human embryonic kidney cells (e.g., 293 cells, or 293 cells subcloned for growth in suspension culture, Graham et al., 1977, *J. Gen. Virol.*, 36:59); baby hamster kidney cells (BHK, ATCC CCL-10); monkey kidney cells (CV1, ATCC CCL-70); African green monkey
 30 kidney cells (VERO-76, ATCC CRL-1587; VERO, ATCC CCL-81); mouse Sertoli cells (TM4, Mather, 1980, *Biol. Reprod.*, 23:243-251); human cervical carcinoma cells (HELA, ATCC CCL-2); canine kidney cells (MDCK, ATCC CCL-34); human lung cells (W138, ATCC CCL-75); human hepatoma cells (HEP-G2, HB 8065); mouse mammary tumor cells

(MMT 060562, ATCC CCL-51); buffalo rat liver cells (BRL 3A, ATCC CRL-1442); TRI cells (Mather, 1982, *Annals NY Acad. Sci.*, 383:44-68); MCR 5 cells; FS4 cells.

Expression vectors for eukaryotic cells, such as mammalian cells, can include promoters and control sequences compatible with mammalian cells that are well established in the art. Some regulatory elements can be, for example, a CMV promoter or the avian sarcoma virus (ASV) promoter found in various expression vectors. Other commonly used early and late promoters include those from Simian Virus 40 (SV 40) (Fiers, et al., (1973) *Nature* 273:113), or other viral promoters such as those derived from bovine papilloma, polyoma, and Adenovirus 2 virus. The regulatable promoter, hMTII (Karin, et al., 1982, *Nature* 299:797-802) can also be used, among others known in the art. For recombinant protein expression in cultured insect cells (for example, SF 9 cells), some baculovirus vectors available include the pVL series (Lucklow, V. A., and Summers, M. D., 1989, *Virology* 170:31-39) and the pAc series (Smith et al., 1983, *Mol. Cell Biol.* 3:2156-2165). A practitioner skilled in the art also understands that enhancer regions (those sequences found upstream or downstream of the promoter region in non-coding DNA regions) are also important in improving expression. Origins of replication can be employed, if needed, from viral sources, for example if utilizing a prokaryotic host for introduction of plasmid DNA.

Host cells

In alternative embodiments, in addition to mammalian host cells, other eukaryotic organisms also may be used as hosts to express a protein of interest (for example, a polypeptide of the invention, e.g., a heavy chain or light chain variable region polypeptide of the invention, including glycoproteins such as Igs). In alternative embodiments, laboratory strains of the budding yeast *Saccharomyces cerevisiae* can be used as well other yeast strains, such as the fission yeast *Schizosaccharomyces pombe*. Yeast vectors harboring DNA encoding a protein of interest (for example, a polypeptide of the invention) can utilize the 2 μ origin of replication (Broach et al., (1983) *Meth. Enz.* 101:307), or other origins of replications compatible with yeast (for example, Stinchcomb et al., 1979, *Nature* 282:39; Tschempe et al., 1980, *Gene* 10:157; and Clarke et al., 1983, *Meth. Enz.* 101:300). A regulatory element contained within yeast vectors can be a promoter for the synthesis of glycolytic enzymes (Hess et al., 1968, *J. Adv. Enzyme Reg.* 7:149; Holland et al., 1978, *Biochemistry* 17:4900). One skilled in the art can also utilize other promoters wherein growth conditions can regulate the transcription of a regulatable gene. Similar to mammalian expression systems, terminator sequences in yeast expression vectors are also desirable at the 3' end of the coding sequences and are found in the 3' untranslated region following the open

reading frame in yeast-derived genes. A recombinant protein of this invention, for example a heavy chain or light chain variable region polypeptide of this invention, glycoproteins such as Igs, can also be expressed in insect cells (for example, using a baculovirus vector).

Various culturing parameters can be used with respect to the host cell being cultured.

5 Appropriate culture conditions for mammalian cells are well known in the art (Cleveland et al., (1983) *J. Immunol. Methods*, 56: 221-234) or can be determined by the skilled artisan (see, for example, *Animal Cell Culture: A Practical Approach 2nd Ed.*, (1992) Rickwood, D. and Hames, B. D., eds. (Oxford University Press: New York,)), and vary according to the particular host cell selected. Commercially available media can be utilized and include, for
10 example, Minimal Essential Medium (MEM, Sigma, St. Louis, Mo.); Dulbecco's Modified Eagles Medium (DMEM, Sigma); Ham's F10 Medium (Sigma); HyClone cell culture medium (HyClone, Logan, Utah); RPMI-1640 Medium (Sigma); and chemically-defined (CD) media, which are formulated for particular cell types, e.g., CD-CHO Medium (Invitrogen, Carlsbad, Calif.). Any of these media can be supplemented as necessary with the
15 previously defined supplementary components or ingredients, including optional components, in appropriate concentrations or amounts, as necessary or desired.

A protein of interest (for example, a polypeptide of the invention), including a glycoprotein, an immunoglobulin, can be produced by growing cells expressing the desired protein product under a variety of cell culture conditions. A practitioner skilled in the art
20 understands that cell cultures and culturing runs for protein production can include three general types: continuous culture, batch culture, and fed-batch culture. In one aspect, a continuous culture process, a fresh culture medium supplement (for example, feeding medium) is supplied to cells during the culturing period while old culture medium is removed. The product produced during a continuous culture can also be harvested, for
25 example, on a daily basis or continuously. As long as the cells remain alive, and the environmental and culturing conditions are maintained, cells can remain in culture as long as is desired in a continuous culturing process.

The cells of the culture producing a protein of interest (for example, a polypeptide of the invention) can be propagated according to any scheme or routine that is most suitable for
30 the particular mammalian host cell and the particular production plan contemplated. Cell culture conditions can be developed to enhance expansion or growth of a population of mammalian host cells in the growth phase of the cell culture for a period of time that is maximized for such expansion and growth. Also, cell culture conditions can be developed to enhance protein production during the production phase of the cell culture for a period of

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time. Culture conditions, such as temperature, pH, dissolved oxygen (DO₂), that can be used are those used in culturing mammalian host cells that are understood by the individual skilled in the art. An appropriate temperature range for culturing mammalian host cells, such as CHO cells, is between 30 to 40°C, and in one embodiment about 37°C. The pH generally is
 5 adjusted to a level between about 6.5 and 7.5 using either an acid or base. A suitable DO₂ is between 5–90% of air saturation. These culture conditions can be used to facilitate the culturing of mammalian cells that produce a desired protein of interest.

Methods for making antibodies

The present invention also provides methods for generating an antibody specific to an
 10 antigen and with a decreased immunogenicity, wherein the antibody comprises heavy chain and light chain variable regions that comprise ICFs. The method for generating this collection comprises providing the combinatorial libraries of heavy chain and light chain nucleic acids (from above) expressed in a cell that produce heavy chain or light chain variable region polypeptides, wherein the variable regions comprising ICFs, and screening an
 15 antibody that binds to the antigen and has a reduced immunogenicity. In one embodiment, the combinatorial libraries of light chain and heavy chain variable region nucleic acids can be both transfected into cells according to methods established in the art, and thus have both collections being expressed by a cell. This would enable the light chains and heavy chains to recombine within the cells, generating antibodies that can be screened for binding affinities
 20 and/or reduced immunogenicity using methods known in the art.

In another embodiment, combinatorial libraries of heavy chains can be in a first population of cells and the combinatorial libraries of light chains can be in a second population of cells.

25

Combinatorial libraries

The present invention provides methods for generating a combinatorial library of nucleic acids that encode heavy chain and light chain variable regions that comprise ICFs. The method for generating this collection comprises providing nucleic acids that encode
 30 heavy chain and light chain variable regions comprising ICFs, joining the nucleic acids that encode heavy chain and light chain variable regions in a 5'-to-3' orientation, and expressing the nucleic acids in a cell.

In one embodiment, the method provides a combinatorial library of nucleic acids (or amino acid sequences encoded by them) of heavy chain variable regions. Table G shows an example of sets of ICF1, 2, 3 and 4 that can be used in the combinatorial library.

5 **Table G. Exemplary set of ICFs for making combinatorial heavy chain libraries**

ICF 1	ICF2	ICF3	ICF4
GL1_8	GL1_7_8	GL_1	GL_1
GL_2	GL2_3	GL_2	
GL_3	GL_4	GL_3	
GL_4	GL_5	GL_4	
GL_5	GL_6	GL_5	
GL_6		GL_6	
GL_7		GL_7	
		GL_8	

Using the 4 sets of ICFs in Table G can result in a total of 280 heavy chain combinations (7 ICF1s x 5 ICF2s x 8 ICF3s x 1 ICF4).

For a corresponding light chain library, the sets of ICFs in Table H are examples of

10 ICFs that can be used.

Table H. Exemplary sets of ICFs for making combinatorial light chain libraries

V _K . ICF 1	V _K . ICF2	V _K . ICF3	V _K . ICF4
VK1_2	VK1_2_3	VK1	VK1
VK3	VK4_5_6	VK2	
VK4	VK7	VK3	
VK5	VK8	VK4	
VK6		VK5	
VK7		VK6	
VK8		VK7	
		VK8	
V _λ . ICF 1	V _λ . ICF2	V _λ . ICF3	V _λ . ICF4
VL1	VL1_2	VL1	VL1
VL2	VL3_4	VL2	
VL3	VL5_6	VL3	
VL4	VL7	VL4	
VL5	VL8	VL5	
VL6		VL6	

VL7		VL7	
VL8		VL8	

Thus, combinatorial libraries of 224 kappa chains (7 x 4 x 8 x 1) or 320 (8 x 5 x 8 x 1) lambda chains can be obtained from these sets.

The combinatorial libraries of the invention can be assembled from other sets of ICFs.

5 For example, reduced libraries can be prepared, for example by combining ICF1, ICF2, and ICF3 having the same designation number: VK1_2 + VK1_2_3 + VK1 + VK1 or VK6_VK4_5_6 + VK6 + VK1, thereby obtaining a reduced, but representative set of 8 kappa chains. Other libraries can be prepared by replacing an ICF with another ICF, as provided in Tables 1 to 4. For example, when selecting a set of ICF1s for a heavy chain library, GL2 can
10 be (a) omitted, (b) replaced by GL2a, GL2b or other ICF1 listed in Table 1 or in the Examples, or (c) replaced by one or more sequences similar to GL2, GL2a, GL2b, or other ICF1, such as corresponding sequences in germline or mature antibodies.

Alternate exemplary embodiments

In another embodiment, HuFR can involve a two-step reassembly process involving
15 one or more placeholder nucleic acids. The placeholders can comprise a reduced set of light chain ICFs. A placeholder can also be determined on the basis of a known antibody or a germline variable region nucleic acid sequence identity compared to that of a sequence of a processed, mature antibody (for example, those light chain variable region germline sequences that are most similar to the nucleic acid sequence of the mature antibody). Once
20 identified, the placeholder nucleic acid sequence, after being transfected and expressed in a cell, can then be used as a temporary single light chain molecule that can be coupled to a heavy chain variable region molecule of the invention.

A heavy chain variable region polypeptides that have a desired property, such as binding to an antigen, the best heavy chains. In one embodiment, the nucleic acid sequences
25 encoding the polypeptides selected can be combined with the combinatorial library of light chain variable region nucleic acids expressed in a cell. In another embodiment, antibody clones can be screened, as described above. For example, antibody clones can be screened for enhanced binding affinities, for example the ability to induce apoptosis or to mediate cell death.

30 Light chain genes are synthesized to serve as placeholder light chains for HC screening purposes. A representative sequence of light chain frameworks from FR₁, FR₂, FR₃, and FR₄ was obtained that belongs to the same family (for example, derived from the

same original germline sequence) and was utilized as the placeholder light chain gene. 8 families of kappa framework regions and 8 families of lambda framework regions were selected, representing 8 potential kappa or lambda libraries that can be generated for screening purposes.

5 Once the heavy chain and placeholder light chain for each of the eight families is generated, each of the products can be associated into a library (e.g., the 245 heavy chains generated along with Family 1 of light chains; the 245 heavy chains along with Family 2 of the light chains; etc, until have 8 libraries total). From each of the 8 libraries (each library representing 1 germline family), a total of 1960 antibodies will be screened using binding
10 assays (such as ELISAs). Thus, after all 8 libraries are screened. A total of 15,680 HC candidates will have been examined. From that, the top 10 binding HCs will be further evaluated once the placeholder light chains are removed and replaced with the combinatorial library of light chain variable region nucleic acids that will be determined in the second phase of the HuFR process.

15 Following library synthesis and cloning, plasmids carrying the antibody genes can be amplified in *E. coli* and transfected into mammalian cells for the production of a full-length Ig. The resulting antibody supernatants can then be screened in the apoptosis assay. For example, in the case of a CD3 antibody, there were 326 hits selected from the primary screen, 52 confirmed hits, and the top 10 heavy chain hits were selected (see Example 4).

20 In the second round, the top 10 reassembled heavy chain genes identified by the apoptosis assay can subsequently be combined with a HuFR combinatorial light chain library. This library can be screened for identification of variants with identical or improved properties as compared to the control antibody. For example, in the case of a CD3 antibody, there were 268 hits from the primary screen, 37 confirmed hits, and the top 10 selected.
25 were successfully retransfected and assayed in confirmation assays (see Example 4).

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

EXAMPLES

30 Example 1: Framework Reassembly Fragments for Light Chain Libraries

The invention provides libraries of light and heavy chain framework region “fragments”, or working pieces, that can be used to build-construct chimeric antigen-binding polypeptides. The following example describes exemplary libraries of light chain framework

region “fragments”, or working pieces, that can be used to build-construct chimeric antigen-binding polypeptides, and an exemplary method for making them.

In one aspect, framework fragments are designed to represent the sequence diversity of human framework regions (FR), for example subregions FR1, FR2, and FR3. In this
5 example, fragment libraries were constructed based on the human germline immunoglobulin light chain variable domains (V_L).

Design of Light chain lambda (V_L) framework region fragments for a reassembly library

To identify sequences for lambda-chain framework regions, the Kabat database of antibody sequences was consulted to determine which human germline genes were used in
10 mature, functional antibodies. Sequence comparison software was used to identify the most similar germline gene for each mature V_L . Thus, genes can be compared by the percentage of mature antibodies that may have arisen from them. Based on functional full-length sequences (FIGS. 2-3), top full-length germline sequences were selected to obtain individual FR regions.

To obtain “consensus” sequences that are representative of human FRs, sequences of
15 all human V_k exons were compiled, and exon sequences were divided into FRs. The following steps were performed for each set of FR sequences. A set of FR sequences was aligned and clustered by sequence similarity. Sequences from each main FR cluster were used to create a consensus sequence, which consisted of the most frequent amino acid
20 occurring at each sequence position. The resulting sequences were 17 consensus FR_{1s} (also referred to as ICF1s), 16 consensus FR_{2s} (also referred to as ICF2s), and 15 consensus FR_{3s} (also referred to as ICF3s). Each of the consensus regions (for example ICF1, ICF2, ICF3) was at least 52% identical to a germline library FR fragment, and at least 65% identical to a mature FR fragment. The FR consensus sequences (for example, ICF1, ICF2, ICF3) were
25 converted to DNA sequences.

A subset of these ICFs can be selected, according to the desired coverage and screening capabilities. The subset fragments were chosen by first including the unique fragments from the ICF V_k library (in use at the time), and then supplementing this list with consensus fragments based primarily on their relative usage by mature antibodies and
30 secondarily on their coverage of any sequence space missed by the current library.

Example 2: Framework Reassembly Fragments for heavy chain libraries

The invention provides separate libraries for both light and heavy chain ICFs; these libraries made using exemplary methods of this invention. The following example describes exemplary libraries of heavy chain framework region “fragments”, or working pieces, that can be used to build-construct chimeric antigen-binding polypeptides, and an exemplary method for making them.

A separate library for heavy chain ICFs was constructed based on the human germline immunoglobulin heavy chain variable domains (V_H), and human V_H s that have been through the natural, immunological maturation process. Any V_H can be subdivided into complementarity-determining regions (CDRs) and FRs. For each FR, several fragments were designed to represent the diversity seen among natural V_H FRs.

The sequences of all human V_H exons were compiled, and exon sequences were divided into FRs. The following steps were performed for each set of FR sequences. A set of FR sequences was aligned and clustered by sequence similarity. Sequences from each main FR cluster were used to create a consensus sequence (for example, ICF1, ICF2, ICF3), which consisted of the most frequent amino acid occurring at each sequence position. Each FR family amino acid consensus sequence (for example, ICF1, ICF2, ICF3) was reverse-translated to codons in an unbiased manner. These preliminary nucleotide models were aligned with human V_H exons to determine “natural” codon usage. The exon regions that aligned with the primary nucleotide models were used to generate secondary nucleotide models. The secondary nucleotide models were translated for comparison to the original consensus primary structure (for example, ICF1, ICF2, ICF3). Codons in the secondary models, which resulted in a mutation from the consensus sequence (for example, ICF1, ICF2, ICF3), were replaced with human codons that code for the residue seen in the consensus sequence. FR₃ had twelve representative fragment sequences; in one experimental library, eight fragments were used. These were selected in order to minimize the difference in sequence diversity between the set of twelve and the set of eight.

Example 3: Anti-CD20 Antibody

The invention provides a chimeric polypeptide and a chimeric bivalent antibody that specifically binds to the polypeptide CD20, e.g., in one embodiment, human CD20.

A mouse antibody that specifically binds to human CD20 was identified, having biological properties similar to a reference antibody. The mouse hybridoma was cultured,

and binding of the mouse antibody to a human CD20+ B cell line (Daudi) was confirmed by Fluorescent-Activated Cell Sorting (FACS) analysis.

Prior to further characterization and assay development, the selected parental mouse antibody was converted to a chimeric anti-CD20 antibody. The chimeric antibody was required for performing comparative biological studies of the selected antibody versus reference antibody (mouse-human chimera). Furthermore, the chimeric antibody was prepared to serve as the appropriate control for the screening assays used in the modification. The parental chimera was prepared so that the sequences encoding the variable regions from the immunoglobulin heavy chain (HC) and light chain (LC) genes were isolated and cloned into a mammalian expression vector containing a human IgG1 constant domain. The resulting chimeric anti-CD20 antibody is referred to as DVSA-CD20.

Assay Development

A cell-based ELISA was established as a simple, rapid, primary screen for the identification of HuFR variants with CD20-binding properties similar to or better than DVSA-CD20. This assay was developed using CD20+ B cell lines in suspension as well as with a stable, adherent HEK-293 cell line expressing the human CD20 protein.

CDC (Complement-Dependent Cytotoxicity) Assay. A fluorescence-based, 96-well plate assay was developed for evaluating the ability of anti-CD20 variants to bind to CD20+ lymphoma cells. Complement activation was assessed by measurement of cell viability. For this assay, the reference antibody and DVSA-CD20 served as the positive controls. The negative controls included untreated cells, cells treated with complement only, cells treated with an unrelated human IgG and complement, and cells treated with vector control supernatants and complement.

ADCC (antibody-dependent, cell-mediated cytotoxicity assay). The ability of anti-CD20 antibodies to induce ADCC was assayed. The anti-CD20 antibody variants were tested for similar or improved binding to DVSA-CD20 in the CD20 cellular ELISA and that have similar or improved activity compared to DVSA-CD20 in the CDC assay. To confirm that the anti-CD20 variants retained this effector function, a 96-well ADCC assay was established. Cell death was measured using LDH release. Positive controls for the assay included the reference antibody and the DVSA-CD20 antibody.

Apoptosis Assay. A FACS-based assay, which measured the loss of plasma membrane integrity, was developed for assessing the ability of anti-CD20 variants to induce

apoptosis. For this assay, human CD20 positive B cell lymphoma cells were treated with anti-CD20, stained with Annexin V and propidium iodide, followed by FACS analysis.

Cell Cycle Assay. The fully murine, parental antibody of DVSA-CD20 has been reported to induce cell proliferation in human PBMCs *in vitro* in the presence of cross-linking antibodies. The reference antibody did not demonstrate this undesirable activity. To ensure that the anti-CD20 variants did not induce cell proliferation in human B cells, an anti-CD3 cell cycle assay was adapted for this screen. The murine CD20 (muCD20) as reported in the literature induces a modest level of cell proliferation when incubated in the presence of cross-linking antibodies.

Construction of the HuFR Library

Human Framework Reassembly was performed in two rounds. For the first round, a heavy chain library was prepared. The following table shows the ICFs used for CD20 heavy chain assembly (see Tables 1 and 2 for sequences of ICFs) with mouse CDRs, as described schematically in Figure 1.

heavy chain ID	ICF1	ICF2	ICF3	ICF4
BD20332	GL7	GL2_3	GL7	GL1
BD20333	GL7	GL2_3	GL8	GL1
BD20335	GL7	GL5	GL1	GL1
BD20336	GL7	GL2_3	GL1	GL1
BD20337	GL7	GL4	GL7	GL1
BD20338	GL6	GL5	GL7	GL1
BD20339	GL7	GL5	GL8	GL1
BD20341	GL7	GL4	GL8	GL1

The complete nucleotide and amino acid sequences for the heavy chain variable regions are provided as follows:

Nucleotide sequences of heavy chain variable regions:

```
>BD20332 (SEQ ID NO:99)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
TGCAGTGGGTCCGCCAGGCTCCAGGCAAGGGGCTAGAGTGGGTGGGTGCT
ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
AGTCACCATCTCAGCTGACAAGTCCATCAGCACTGCCTACCTGCAGTGGA
GCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGATCGCAC
TACGGTAGTAACTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
GGTCACCGTCTCCTCC
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>BD20333 (SEQ ID NO:108)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
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GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 AGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGA
 5 GCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20335 (SEQ ID NO:124)

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
 GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTGCGACAGGCTCCTGGAAAAGGGCTTGAGTGGATGGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 ATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGA
 15 ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20336 (SEQ ID NO:130)

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
 GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 ATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGA
 25 ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20337 (SEQ ID NO:131)

30 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
 GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 AGTCACCATCTCAGCTGACAAGTCCATCAGCACTGCCTACCTGCAGTGGA
 35 GCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20338 (SEQ ID NO:132)

40 GAGGTGCAGCTGGTGCAGTCTGGGGCAGAGGTGAAAAGCCCGGGGAGTC
 TCTGAAGATCTCCTGTAAGGGTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTGCGACAGGCTCCTGGAAAAGGGCTTGAGTGGATGGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 AGTCACCATCTCAGCTGACAAGTCCATCAGCACTGCCTACCTGCAGTGGA
 45 GCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20339 (SEQ ID NO:136)

50 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
 GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA
 TGCAGTGGGTGCGACAGGCTCCTGGAAAAGGGCTTGAGTGGATGGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAAGTTCAAAGGCAG
 AGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGA
 55 GCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACCTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

>BD20341 (SEQ ID NO:137)

60 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTC
 GGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATA

5 TGCACCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGTGCT
 ATTTATCCAGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAG
 AGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGA
 GCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGATCGCAC
 TACGGTAGTAACTACGTAGACTACTTTGACTACTGGGGCCAGGGCACCCCT
 GGTCACCGTCTCCTCC

Amino acid sequences of heavy chain variable regions:

10 >BD20332 (SEQ ID NO:138)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGKGLEWVGA
 IYPGNGDTSYNQKFKGRVTISADKSISTAYLQWSSLKASDTAMYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

15 >BD20333 (SEQ ID NO:142)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGKGLEWVGA
 IYPGNGDTSYNQKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

20 >BD20335 (SEQ ID NO:143)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGKGLEWMGA
 IYPGNGDTSYNQKFKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

25 >BD20336 (SEQ ID NO:144)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGKGLEWVGA
 IYPGNGDTSYNQKFKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

30 >BD20337 (SEQ ID NO:148)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGQGLEWMGA
 IYPGNGDTSYNQKFKGRVTISADKSISTAYLQWSSLKASDTAMYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

35 >BD20338 (SEQ ID NO:149)
 EVQLVQSGAEVKKPGESLKISCKGSGYTFSTSYNMHWVRQAPGKGLEWMGA
 IYPGNGDTSYNQKFKGRVTISADKSISTAYLQWSSLKASDTAMYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

40 >BD20339 (SEQ ID NO:150)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGKGLEWMGA
 IYPGNGDTSYNQKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

45 >BD20341 (SEQ ID NO:154)
 QVQLVQSGAEVKKPGASVKVSCKASGYTFSTSYNMHWVRQAPGQGLEWMGA
 IYPGNGDTSYNQKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARSH
 YGSNYVDYFDYWGQGTLVTVSS

50

The signal sequence and constant regions associated with the heavy chains are as follows:

55 >HC signal (SEQ ID NO:155)
 ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGT

>HC signal (SEQ ID NO:156)
 MEFGLSWLFLVAILKGVQC

>HC constant (SEQ ID NO:160)

GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
 GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTG
 CACACCTTCCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC
 5 TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
 AAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
 TTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC
 CACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG
 GAGGAGCAGTACAACAGCACGTACCGTGTGGTTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAG
 10 GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
 CCCCAGAAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGC
 CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAG
 ACCACGCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGG
 CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC
 15 CTGTCTCCGGGTAAATGA

>HC constant (SEQ ID NO:161)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
 LGTQTYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
 20 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNV FSCSVMHEALHNHYTQKSLSLSPGK

The heavy chain library was then associated with eight placeholder light chains. Each
 25 light chain consisted of a fixed set of human frameworks and the mouse CDRs. Plasmids
 carrying the antibody genes were amplified in *E. coli* and transfected into mammalian cells
 for production of full-length IgG-containing supernatants for screening in the cellular ELISA.
 The best reassembled heavy chain genes identified by the cellular ELISA were then
 combined with a reassembled light chain library as follows:

light chain ID	ICF1	ICF2	ICF3	ICF4
BD22084	VK8	VK7	VK5	VK1
BD22107	VK8	VK8	VK5	VK1
BD22086	VK8	VK4_5_6	VK7	VK1
BD22103	VK8	VK1_2_3	VK7	VK1
BD22088	VK8	VK7	VK2	VK1
BD22108	VK8	VK4_5_6	VK2	VK1
BD22094	VK8	VK4_5_6	VK3	VK1
BD22085	VK7	VK4_5_6	VK1	VK1
BD22109	VK7	VK7	VK5	VK1
BD22090	VK8	VK8	VK8	VK1
BD22092	VK1_2	VK8	VK7	VK1
BD22100	VK3	VK4_5_6	VK2	VK1
BD22105	VK6	VK8	VK7	VK1
BD22111	VK7	VK1_2_3	VK3	VK1
BD22104	VK4	VK8	VK1	VK1
BD22087	VK6	VK1_2_3	VK3	VK1

BD22096	VK5	VK1_2_3	VK3	VK1
BD22091	VK5	VK7	VK4	VK1
BD22089	VK5	VK7	VK2	VK1
BD22095	VK4	VK7	VK2	VK1
BD22106	VK6	VK4_5_6	VK2	VK1
BD22097	VK6	VK7	VK1	VK1
BD22101	VK5	VK7	VK1	VK1
BD22102	VK4	VK7	VK1	VK1

The complete nucleotide and amino acid sequences for the light chain variable regions are provided as follows:

5 Nucleotide sequences of light chain variable regions:

>BD22084 (SEQ ID NO:162)

10 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGATCCCAGCCAGGTTTCAGTGGCAGTGGGTCTGG
GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAG
TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
15 ACCAAGGTGGAAATCAAA

>BD22107 (SEQ ID NO:166)

20 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
GGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATGCCACA
TCCAACCTGGCTTCTGGGATCCCAGCCAGGTTTCAGTGGCAGTGGGTCTGG
GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAG
TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

25 >BD22086 (SEQ ID NO:167)

30 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGG
GACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAG
TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

35 >BD22103 (SEQ ID NO:168)

40 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGG
GACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAG
TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

45 >BD22088 (SEQ ID NO:172)

GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA

GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAGTGGAAGTGGATCTGG
 GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
 5 CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

>BD22108 (SEQ ID NO:173)

10 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
 GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAGTGGAAGTGGATCTGG
 GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
 15 CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

>BD22094 (SEQ ID NO:174)

20 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
 GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGG
 GACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAA
 CTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 25 ACCAAGGTGGAAATCAAA

>BD22085 (SEQ ID NO:177)

30 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGA
 GAGGGCCACCATCAACTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTGG
 GACAGATTTTACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA
 CTTACTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 35 ACCAAGGTGGAAATCAAA

>BD22109 (SEQ ID NO:205)

40 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGA
 GAGGGCCACCATCAACTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
 TCCAACCTGGCTTCTGGGATCCCAGCCAGGTTTCAGTGGCAGTGGGTCTGG
 GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCA
 TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 45 ACCAAGGTGGAAATCAAA

>BD22090 (SEQ ID NO:210)

50 GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGA
 GCCGGCCTCCATCTCCTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCTGACAGGTTTCAGTGGCAGTGGATCAGG
 CACAGATTTTACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGG
 TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 55 ACCAAGGTGGAAATCAAA

>BD22092 (SEQ ID NO:211)

60 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA
 CAGAGTCACCATCACTTGCAGGGCCAGCTCAAGTTTAAGTTTCATGCACT
 GGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGG
 GACAGATTTTACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAG
 TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 65 ACCAAGGTGGAAATCAAA

>BD22100 (SEQ ID NO:212)
 GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGA
 CAGAGTCACCATCACTTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
 5 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAAGTGGATCTGG
 GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
 CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

10 >BD22105 (SEQ ID NO:213)
 GAAATTGTGTTGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGA
 AAGAGCCACCCTCTCCTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTGG
 15 GACAGATTTTACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAG
 TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

20 >BD22111 (SEQ ID NO:214)
 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGA
 GAGGGCCACCATCAACTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGGGAAAGCCCCCTAAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAAGCGGCAGTGGATCTGG
 GACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAA
 25 CTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

30 >BD22104 (SEQ ID NO:215)
 GAAATAGTGATGACCCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGA
 AAGAGCCACCCTCTCCTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAAGTGGCAGTGGATCTGG
 GACAGATTTTACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA
 CTTACTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 35 ACCAAGGTGGAAATCAAA

40 >BD22087 (SEQ ID NO:216)
 GAAATTGTGTTGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGA
 AAGAGCCACCCTCTCCTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGGGAAAGCCCCCTAAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAAGCGGCAGTGGATCTGG
 GACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAA
 CTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 45 ACCAAGGTGGAAATCAAA

50 >BD22096 (SEQ ID NO:217)
 GAAATTGTGTTGACCCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA
 AAGAGCCACCCTCTCCTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGGGAAAGCCCCCTAAGCTCCTGATCTATGCCACA
 TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTTCAAGCGGCAGTGGATCTGG
 GACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAA
 CTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
 ACCAAGGTGGAAATCAAA

55 >BD22091 (SEQ ID NO:218)
 GAAATTGTGTTGACCCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA
 AAGAGCCACCCTCTCCTGCAGGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
 GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
 TCCAACCTGGCTTCTGGGATCCAGCCAGGTTTCAAGTGGCAGTGGGTCTGG
 60 GACAGAGTTCACTCTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCA
 TTTATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT

ACCAAGGTGGAAATCAAA

>BD22089 (SEQ ID NO:219)

5 GAAATTGTGTTGACCCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGAAGTGGATCTGG
10 GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

>BD22095 (SEQ ID NO:220)

15 GAAATAGTGATGACCCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGAAGTGGATCTGG
GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
20 ACCAAGGTGGAAATCAAA

>BD22106 (SEQ ID NO:221)

25 GAAATTGTGTTGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGAAGTGGATCTGG
GACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATATTGCAA
CATATTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

>BD22097 (SEQ ID NO:222)

30 GAAATTGTGTTGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGG
35 GACAGATTTTACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA
CTTACTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

>BD22101 (SEQ ID NO:223)

40 GAAATTGTGTTGACCCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGG
GACAGATTTTACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA
45 CTTACTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
ACCAAGGTGGAAATCAAA

>BD22102 (SEQ ID NO:224)

50 GAAATAGTGATGACCCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGA
AAGAGCCACCCTCTCCTGCAGGGCCAGCTCAAGTTTAAAGTTTCATGCACT
GGTATCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTATGCCACA
TCCAACCTGGCTTCTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGG
GACAGATTTTACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAA
CTTACTACTGTCATCAGTGGAGTAGTAACCCGCTCACGTTTCGGCCAAGGT
55 ACCAAGGTGGAAATCAAA

Amino acid sequences of light chain variable regions:

60 >BD22084 (SEQ ID NO:225)

DIVMTQSPVLPVTPGEPASISCRASSSLSEFMHWYQQKPGQPPKLLIYAT

SNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCHQWSSNPLTFGQG
TKVEIK

- 5 >BD22107 (SEQ ID NO:226)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYLQKPGQSPQLLIYAT
SNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCHQWSSNPLTFGQG
TKVEIK
- 10 >BD22086 (SEQ ID NO:227)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQWSSNPLTFGQG
TKVEIK
- 15 >BD22103 (SEQ ID NO:228)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYQQKPGKAPKLLIYAT
SNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQWSSNPLTFGQG
TKVEIK
- 20 >BD22088 (SEQ ID NO:229)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK
- 25 >BD22108 (SEQ ID NO:230)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK
- 30 >BD22094 (SEQ ID NO:231)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCHQWSSNPLTFGQG
TKVEIK
- 35 >BD22085 (SEQ ID NO:232)
DIVMTQSPDSLAVSLGERATINCRASSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQWSSNPLTFGQG
TKVEIK
- 40 >BD22109 (SEQ ID NO:233)
DIVMTQSPDSLAVSLGERATINCRASSLSFMHWYQQKPGQPPKLLIYAT
SNLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCHQWSSNPLTFGQG
TKVEIK
- 45 >BD22090 (SEQ ID NO:234)
DIVMTQSPSLPVTGPGEPAISCRASSLSFMHWYLQKPGQSPQLLIYAT
SNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGYYCHQWSSNPLTFGQG
TKVEIK
- 50 >BD22092 (SEQ ID NO:235)
DIQMTQSPSSLSASVGDRVTITCRASSLSFMHWYLQKPGQSPQLLIYAT
SNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQWSSNPLTFGQG
TKVEIK
- 55 >BD22100 (SEQ ID NO:236)
DIQMTQSPSTLSASVGDRVTITCRASSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK
- 60 >BD22105 (SEQ ID NO:237)
EIVLTQSPGTLSPGERATLSCRASSLSFMHWYLQKPGQSPQLLIYAT
SNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQWSSNPLTFGQG

TKVEIK

>BD22111 (SEQ ID NO:238)

5 DIVMTQSPDSLAVSLGERATINCRASSSLSFMHWYQQKPGKAPKLLIYAT
SNLASGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22104 (SEQ ID NO:239)

10 EIVMTQSPATLSVSPGERATLSCRASSSLSFMHWYLQKPGQSPQLLIYAT
SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22087 (SEQ ID NO:240)

15 EIVLTQSPGTLSSLSPGERATLSCRASSSLSFMHWYQQKPGKAPKLLIYAT
SNLASGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22096 (SEQ ID NO:241)

20 EIVLTQSPATLSLSPGERATLSCRASSSLSFMHWYQQKPGKAPKLLIYAT
SNLASGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22091 (SEQ ID NO:242)

25 EIVLTQSPATLSLSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGIPARFSGSGSGTEFTLTISLQSEDFAVYYCHQWSSNPLTFGQG
TKVEIK

>BD22089 (SEQ ID NO:243)

30 EIVLTQSPATLSLSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK

>BD22095 (SEQ ID NO:244)

35 EIVMTQSPATLSVSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK

>BD22106 (SEQ ID NO:245)

40 EIVLTQSPGTLSSLSPGERATLSCRASSSLSFMHWYQQKPGQAPRLLIYAT
SNLASGVPSRFSGSGSGTDFTFITISLQPEDATYYCHQWSSNPLTFGQG
TKVEIK

>BD22097 (SEQ ID NO:246)

45 EIVLTQSPGTLSSLSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22101 (SEQ ID NO:247)

50 EIVLTQSPATLSLSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWSSNPLTFGQG
TKVEIK

>BD22102 (SEQ ID NO:248)

55 EIVMTQSPATLSVSPGERATLSCRASSSLSFMHWYQQKPGQPPKLLIYAT
SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWSSNPLTFGQG
TKVEIK

The signal sequence and constant regions associated with the light chains are as follows:

```

5  >LC signal (SEQ ID NO:249)
   ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAAATGT

   >LC signal (SEQ ID NO:250)
   MDMRVPAQLLGLLLLWLPGAKC

10  >LC constant (SEQ ID NO:251)
   CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTT
   GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGT
   AACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGC
   AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG
15  AGCTTCAACAGGGGAGAGTGTTAA

   >LC constant (SEQ ID NO:252)
   RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS
   KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
20

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These were transfected via plasmid Ig chain expression vectors into HEK-293 suspension cells, and the resulting cell culture supernatants were screened.

HuFR Library Screening Results. The primary high-throughput screening data for the final HuFR library is shown in Fig. 26, which illustrates a graph of data from an anti-CD20 ELISA assay demonstrating the specific activity of the anti-CD20 HuFR clones in the anti-CD20 cellular ELISA, using adherent CD20+ HEK-293 cells. The specific activity of DVSA-CD20 was set at 1.0. Clones with an activity greater than or equal to 1.0 were tested in the CDC assay. The signal:noise was 4.2 for the quantitative ELISA (CV 8.8%) and 3.6 for the anti-CD20 cellular ELISA (CV 5.7%). The specific activities of the HuFR clones were determined by normalizing the anti-CD20 cellular ELISA binding activity by antibody expression levels (as determined by a quantitative IgG ELISA). The specific activity of DVSA-CD20 was set at 1.0 and arranged by highest specific activity. The best putative hits (> 80) were chosen for further analysis. The top putative hits identified from the cellular ELISA were profiled in the CDC assay. A comparison of the top hits in the CD20 cellular ELISA and the CDC assay illustrated that many of the cellular ELISA hits retained cytotoxicity activities similar to DVSA-CD20, as illustrated in Figure 5. Figure 5 is a bar graph comparing the specific activity of the top anti-CD20 HuFR clones in the anti-CD20 ELISA (purple, left bars) with the top clone activity in the CDC assay (aqua, right bars). The activities of the DVSA-CD20 positive control (cDVSA) and negative control (unrelated human IgG) are indicated.

Based on the results of the cellular ELISA and the CDC assay, the top HuFR variants were selected for confirmation and further analysis in the panel of secondary assays. The HuFR heavy and light chains associated in these variants are as follows:

LC & HC combination	Light Chain	Heavy chain
1	BD22084	BD20332
2	BD22085	BD20335
3	BD22086	BD20335
4	BD22088	BD20337
5	BD22087	BD20335
6	BD22089	BD20335
7	BD22090	BD20337
8	BD22095	BD20337
9	BD22091	BD20337
10	BD22108	BD20337
11	BD22092	BD20338
12	BD22094	BD20337
13	BD22096	BD20337
14	BD22092	BD20337
15	BD22102	BD20337
16	BD22097	BD20335
17	BD22104	BD20337
18	BD22085	BD20339
19	BD22107	BD20339
20	BD22100	BD20335
21	BD22103	BD20337
22	BD22105	BD20337
23*	BD22108	BD20337
24	BD22101	BD20335
25	BD22106	BD20333
26	BD22108	BD20338
27	BD22109	BD20341

28	BD22111	BD20336
29*	BD22104	BD20337

* Note that LC & HC combination number 23 has the same combination of light chain and heavy chain as number 10 (BD22108 and BD20337). Number 29 has the same heavy/light chain combination as number 17 (BD22104 and BD20337). Nevertheless, combination numbers 23 and 29 have been maintained for consistent presentation of the results below.

The top variants were transfected into HEK-293 suspension cells and the resulting (unpurified) cell culture supernatants were tested in a panel of secondary assays: apoptosis, see Figure 6; cell cycle, see Figure 7; CDC, see Figure 8; and ADCC, see Figure 9.

Figure 6 is a bar graph of an apoptosis assay, which demonstrates that several of the top HuFR hits have activities equal to or better than reference antibody and DVSA-CD20. Positive controls were staurosporine, the reference antibody, and DVSA-CD20. Negative controls were untreated cells (media no stain), untreated cells cross-linking antibody only (media GAH only), and cells treated with an unrelated human IgG (human). Figure 7 is for cell cycle assay, which shows that the HuFR anti-CD20 hits do not induce cell proliferation in human PBMC *in vitro*. DVSA-CD3 was the positive control (lane 1). Negative controls included untreated cells with cross-linking antibody and cells treated with an unrelated human IgG. Figure 8 is a bar graph of a CDC assay. Several anti-CD20 HuFR hits induce CDC as well as, or better than the reference antibody and DVSA-CD20 (lanes 3 and 4). Negative controls for this assay (100% viability) were untreated cells and cell treated with an unrelated human IgG (lanes 1 and 2).

Figure 9 is a bar graph of an ADCC assay, as discussed in detail in Example 4, below. Preliminary ADCC data with a subset of the top anti-CD20 HuFR hits suggest that several of these hits have activity similar to the reference antibody and DVSA-CD20 at a concentration of 1 µg/ml. The negative control for this assay was CD20+ target cells incubated with an irrelevant human IgG (Human) anti-CD3.

A summary of the assay data is shown in Table A. The variants were ranked in order from 1 to 29, starting with best binding activity in the cellular ELISA. A performance of ++ was equivalent to reference antibody. The top 12 variants overall are starred.

Table A. Summary of anti-CD20 variants in panel of secondary cell-based assays

Variant	CDC	Apoptosis	Cell Cycle	ADCC
1	–	–	++	+
2	+	+	+	++
3	+	+	++	++
4*	+	+	++	+++
5	–	++	++	+++
6*	+	++	++	++
7	+	+	++	++
8*	+	+++	++	++
9*	++	++	++	++
10*	++	+	++	++
11	++	+	–	+
12	–	+	++	+++
13	–	–	++	+++
14*	+	+++	++	++++
15*	+	+	++	+++
16	–	+	++	+++
17*	++	+	++	++
18*	+++	++	+	+++
19*	++	+	+	+++
20	–	+	++	+++
21	++	+	+++	+
22*	++	+++	++	+
23	++	+	++	+
24	++	+	++	+
25	–	++	++	+++
26	–	+	+++	+
27	+	++	++	+
28*	++	+++	++	++
29	++	+	+	+

Example 4: Anti-CD3 antibody

The invention provides a chimeric polypeptide and a chimeric bivalent antibody that specifically binds to the polypeptide CD3, e.g., in one embodiment, human CD3. In one aspect, a polypeptide of the invention, e.g., a chimeric polypeptide or a chimeric bivalent antibody of the invention, are used to suppress or abrogate an immune response, e.g., to treat (ameliorate) acute allograft rejection in renal transplant patients and steroid-resistant acute

allograft rejection in cardiac and hepatic transplant patients, and to treat (ameliorate) autoimmune diseases, serious graft-versus-host disease, to treat (ameliorate) psoriasis and ulcerative colitis, and to ameliorate Type-I diabetes, e.g., by maintaining or improving insulin production in diabetes patients, including recently diagnosed Type-I diabetes patients.

5 In alternative embodiments, the anti-CD3 antibodies of the invention are useful to treat acute allograft rejection in renal transplant patients and steroid-resistant acute allograft rejection in cardiac and hepatic transplant patients. In alternative embodiments, these antibodies of the invention also are useful to treat autoimmune diseases, including psoriasis and ulcerative colitis, and serious graft-versus-host disease, and to maintain or improve
10 insulin production in recently diagnosed Type-I diabetes patients. Modified anti-CD3s are being evaluated in Phase 2 studies for psoriasis and ulcerative colitis studies.

A reference mouse anti-CD3 antibody was converted to a chimeric, anti-CD3 antibody of this invention. A single amino acid change (T299V) was inserted into the Fc region of the antibody to reduce undesirable cytokine side effects associated with the
15 reference antibody (the Fc region having this T299V mutation is referred to as "Fc null"). Fc null served as an additional control.

The chimeric antibody was also prepared to serve as the appropriate control for establishing the screening assays used in the modification. The parental chimera was prepared so that the reference sequences encoding the variable regions were cloned into a
20 mammalian expression vector containing a human IgG1 constant domain.

Figure 9 is a bar graph of an ADCC assay, as discussed in detail in Example 4, below. Preliminary ADCC data with a subset of the top anti-CD20 HuFR hits suggest that several of these hits have activity similar to the reference antibody and DVSA-CD20 at a concentration of 1 µg/ml. The negative control for this assay was CD20+ target cells incubated with an
25 irrelevant human IgG (Human) anti-CD3.

The resulting chimeric, anti-CD3 antibody is referred to DVSA-CD3, shown in Figure 10 and Figure 11. Figure 10 depicts the light chain (top) and heavy chain (bottom) nucleic acid sequences of DVSA-CD3. The yellow highlighted text denotes the CDRs. Figure 11 depicts the heavy chain (top) and light chain (middle) amino acid sequences of DVSA-CD3,
30 as well as the light chain of DVSA-CD3 (bottom). The yellow highlighted text denotes constant regions.

Apoptosis Assay

Jurkat T cells (ATCC Cat. TIB-152), cultured in cell medium (RPMI-1640 (ATCC Cat. 30-2001)/10% FBS (Invitrogen Cat. 10082-147)/0.05 mM 2-mercaptoethanol (Sigma M-7522)), were plated 2 days after the last subculturing at a density of about 2.5×10^4 cells.

5 Cells were then centrifuged for 5 minutes at 200g, room temperature. The spent cell culture medium subsequently was aspirated and cells were gently resuspended in fresh medium. The cell number was adjusted to 4.0×10^5 cells/ml with fresh cell culture medium after cells were counted. Cells were then plated ($\sim 50 \mu\text{l}$ /well) in a 96-well plate. An antibody solution (100 ng/ml, 50 ng/ml, 25 ng/ml or 12.5 ng/ml IgG) made up in cell culture medium was added to
10 the cells and incubated for 24 hours, at 37°C , 5% CO_2 . The antibodies to be tested (20 ng/ml) were those identified in the screening process. Irrelevant human IgG1 (EMD Biosciences Cat. 400120), DVSA-CD3, DVSA-CD3 (Fc null) served as control antibodies.

The APO-ONE APOPTOSIS ASSAY™ (Promega Cat. G7791) was used. The assay readout was based on the cleavage of fluorescently labeled tetrapeptide substrates in a 96-
15 well format (APO-ONE™ HOMOGENEOUS CASPASE-3/7 Assay, Promega Cat. G7790, G7791). $100 \mu\text{l}$ /well of the APO-ONE™ reagent/substrate (100:1 dilution) was added to each well and was incubated at room temperature in the dark for 24 hours. The *in vitro* apoptosis assay established measures the induction of caspase activity in human CD3+ T cells following anti-CD3 antibody treatment. Plates were then read using a fluorescent
20 microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Construction of the HuFR anti-CD3 Libraries

HuFR was performed as in Example 3. In the first round, the antibody supernatants (heavy chain HuFR library associated with kappa placeholder chains) were screened in a
25 high-throughput assay that measured the ability of the antibody variants to induce T-cell signal transduction and subsequent apoptosis. In one experimental run, there were 326 hits selected from the primary screen, 52 hits were confirmed, and the top 10 heavy chain hits were selected. Tables C and D show the top heavy and light chain sequences (see Tables 1 to 4).

30 In the second round, the top 10 reassembled heavy chain genes identified by the apoptosis assay were then combined with the HuFR light chain library. This library was screened for identification of variants with identical or improved properties as compared to the control DVSA-CD3 (Fc-null). In one experiment, there were 268 hits from the primary

screen, 37 confirmed hits, and the top 10 selected. 9 candidate clones were successfully retransfected and assayed in confirmation assays (**Table B**). The ICFs appearing in the top heavy and light chains are shown in **Table C** and **D**.

Table B. Heavy and light chains in the top anti-CD3 HuFR antibodies

HuFR antibody	Heavy chain ID	Light chain ID
1	BD20610	BD21130
2	BD20613	BD21131
3	BD20611	BD21132
4	BD20611	BD21133
5	BD20611	BD21134
6	BD20611	BD21135
7	BD20611	BD21136
8	BD20611	BD21137
9	BD20613	BD21138

5

Table C. ICFs used in the top heavy chains for anti-CD3

Heavy chain ID	ICF1	ICF2	ICF3	ICF4
BD20610	GL_7a	GL_5	GL_4	GL1
BD20611	GL_7a	GL_5	GL_5	GL1
BD20613	GL_3	GL2_3	GL_3	GL1

Table D. ICFs used in the top kappa light chains for anti-CD3

Light chain ID	ICF1	ICF2	ICF3	ICF4
BD21130	VK1_2	VK7	VK8	VK1
BD21131	VK3	VK4_5_6	VK8	VK1
BD21132	VK5	VK1_2_3	VK3	VK1
BD21133	VK8	VK7	VK3	VK1
BD21134	VK4	VK7	VK3	VK1
BD21135	VK4	VK4_5_6	VK7	VK1
BD21136	VK3	VK1_2_3	VK6	VK1
BD21137	VK3	VK7	VK2	VK1
BD21138	VK3	VK1_2_3	VK8	VK1

10 Figure 12 provides an alignment of the heavy and light chains in the top 9 anti-CD3 hits.

HuFR Library Screening Results

The top nine (9) CD3 antibody variant heavy chain and light chain candidates were transfected into HEK-293 suspension cells and the resulting cell culture supernatants were tested for apoptosis activity and thermostability. All 9 variants obtained through the human framework reassembly reaction displayed apoptosis activities that were the same or better than the DVSA-CD3 (Fc-null) *in vitro* (Table E). Negative controls were untreated cells (media) and an irrelevant human IgG (huIgG).

Table E. Apo-One Apoptosis Assay of HuFR antibodies

	12.5 ng/ml	25 ng/ml	50 ng/ml	12.5 ng/ml	25 ng/ml	50 ng/ml
media	380	380	384			
huIgG	250	259	234			
Fc null	494	729	1191	1.0	1.0	1.0
variant 1	797	1217	1753	1.6	1.7	1.5
variant 2	854	1435	2156	1.7	2.0	1.8
variant 3	649	854	1132	1.3	1.2	1.0
variant 4	1390	2348	3303	2.8	3.2	2.8
variant 5	1163	1663	2165	2.4	2.3	1.8
variant 6	1277	2224	3498	2.6	3.1	2.9
variant 7	2268	3477	4744	4.6	4.8	4.0
variant 8	969	1632	2559	2.0	2.2	2.1
variant 9	885	1383	2041	1.8	1.9	1.7

10 Thermostability

The 9 HuFR variants were also assayed for thermostability assay to ensure that the structural integrity of the antibody had not been compromised by any of the amino acid changes. The 9 variants obtained through the human framework reassembly reaction have higher melting temperatures than the DVSA-CD3 (Fc-null) antibody.

15 Table F. The thermostability of the 9 variants was not affected by HuFR

The invention provides chimeric polypeptides, described below as variant 1 through variant 9, capable of binding antigen that have thermostable binding activity:

Antibody	T _m (°C)
DVSA-CD3 (Fc-null)	59.6
variant 1	66.5
variant 2	70.5
variant 3	64.7
variant 4	65.8
variant 5	67.7

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variant 6	65.2
variant 7	65.5
variant 8	70.7
variant 9	63.5

What is claimed is:

1. An antibody or antigen-binding fragment thereof comprising at least one variable region having a combination of:

- (1) light chain BD22088 (SEQ ID NO:229) and heavy chain BD20337 (SEQ ID NO:148);
- (2) light chain BD22090 (SEQ ID NO:234) and heavy chain BD20337 (SEQ ID NO:148);
- (3) light chain BD22095 (SEQ ID NO:244) and heavy chain BD20337 (SEQ ID NO:148);
- (4) light chain BD2209I (SEQ ID NO:242) and heavy chain BD20337 (SEQ ID NO:148);
- (5) light chain BD22108 (SEQ ID NO:230) and heavy chain BD20337 (SEQ ID NO:148);
- (6) light chain BD22094 (SEQ ID NO:231) and heavy chain BD20337 (SEQ ID NO:148);
- (7) light chain BD22096 (SEQ ID NO:241) and heavy chain BD20337 (SEQ ID NO:148);
- (8) light chain BD22092 (SEQ ID NO:235) and heavy chain BD20337 (SEQ ID NO:148);
- (9) light chain BD22102 (SEQ ID NO:248) and heavy chain BD20337 (SEQ ID NO:148);
- (10) light chain BD22104 (SEQ ID NO:239) and heavy chain BD20337 (SEQ ID NO: 148);
- (11) light chain BD22103 (SEQ ID NO:228) and heavy chain BD20337 (SEQ ID NO:148); or
- (12) light chain BD22105 (SEQ ID NO:237) and heavy chain BD20337 (SEQ ID NO:148).

2. An antibody or antigen-binding fragment thereof comprising at least a portion of a heavy chain variable region and at least a portion of a light chain variable region, wherein the light chain portion and the heavy chain portion is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or complete sequence identity to the full length sequence of the respective light and heavy chains of at least one of the following combinations:

- (1) light chain BD22088 (SEQ ID NO:229) and heavy chain BD20337 (SEQ ID NO:148);
- (2) light chain BD22090 (SEQ ID NO:234) and heavy chain BD20337 (SEQ ID NO:148);
- (3) light chain BD22095 (SEQ ID NO:244) and heavy chain BD20337 (SEQ ID NO:148);
- (4) light chain BD22091 (SEQ ID NO:242) and heavy chain BD20337 (SEQ ID NO:148);
- (5) light chain BD22108 (SEQ ID NO:230) and heavy chain BD20337 (SEQ ID NO:148);
- (6) light chain BD22094 (SEQ ID NO:231) and heavy chain BD20337 (SEQ ID NO:148);
- (7) light chain BD22096 (SEQ ID NO:241) and heavy chain BD20337 (SEQ ID NO:148);
- (8) light chain BD22092 (SEQ ID NO:235) and heavy chain BD20337 (SEQ ID NO: 148);
- (9) light chain BD22102 (SEQ ID NO:248) and heavy chain BD20337 (SEQ ID NO: 148);
- (10) light chain BD22104 (SEQ ID NO:239) and heavy chain BD20337 (SEQ ID NO:148);
- (11) light chain BD22103 (SEQ ID NO:228) and heavy chain BD20337 (SEQ ID NO:148); or

- (12) light chain BD22105 (SEQ ID NO:237) and
heavy chain BD20337 (SEQ ID NO:148);

and wherein the light chain, the heavy chain, or both are derived from sequences made by the method comprising:

- (1) providing an Independently Consensused Framework I (ICFI) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from a Kabat framework region I (KFI) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;
- (2) providing a complementarity determining region 1 (CDR1) derived from the variable region of a 1F5 antibody;
- (3) providing an Independently Consensused Framework 2 (ICF2) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from a Kabat framework region 2 (KF2) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;
- (4) providing a complementarity determining region 2 (CDR2) derived from the variable region of a 1F5 antibody;
- (5) providing an Independently Consensused Framework 3 (ICF3) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from a Kabat framework region 3 (KF3) domain, wherein the plurality of amino acid sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;
- (6) providing a complementary determining region 3 (CDR3) derived from the variable region of a 1F5 antibody; and
- (7) providing an Independently Consensused Framework 4 (ICF4) domain, comprising an amino acid consensus sequence derived from a plurality of amino acid sequences each comprising amino acids derived from a Kabat framework region 4 (KF4) domain, wherein the plurality of amino acid

sequences are translated from a germline sequence of an immunoglobulin variable region gene or obtained from a mature immunoglobulin;

wherein at least one ICF is derived from a genomic nucleic acid sequence,

(8) joining, in a 5'-to-3' orientation, nucleic acids encoding the ICFI-CDR1-ICF2-CDR2-ICF3-CDR3 and ICF4 domains

wherein the sequences of the CDR1, CDR2, and CDR3 of said antibody or antigen-binding fragment thereof confers specificity for binding CD20, and wherein said method does not require computer modeling for the selection of the various domains.

3. The antigen binding antibody fragment of claim 2, wherein the antibody fragment is an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, a single-chain antibody, an Fv fragment, an scFv fragment, an antibody mimetic, an Fd fragment, or an Fd' fragment.

4. The antigen binding antibody fragment of claim 3, wherein the antibody fragment is fused to an Fc.

5. A recombinant, synthetic or isolated antibody having a structure comprising at least one variable region combination of claim 2.

6. A chimeric antibody or antigen binding fragment thereof comprising at least one variable region combination of claim 2.

7. A chimeric antigen binding antibody fragment of claim 6, wherein the chimeric antibody fragment is a chimeric Fab, a chimeric Fab', a chimeric F(ab')₂, a chimeric single-chain antibody, a chimeric Fv, a chimeric scFv, an antibody mimetic, a chimeric Fd, or a chimeric Fd'.

8. A pharmaceutical composition or formulation comprising: an antibody or antigen binding fragment thereof of claim 2 and an excipient.

9. The pharmaceutical composition or formulation of claim 8, further comprising a pharmaceutically acceptable carrier.

10. The use of an antibody or antigen binding fragment thereof of claim 2 to suppress or abrogate a B-cell mediated immune response.
11. The use of an antibody or antigen binding fragment thereof of claim 2 to treat B-cell lymphoma.
12. The use of an antibody or antigen binding fragment thereof of claim 2, for the manufacture of a pharmaceutical composition for treating a subject having a B-cell mediated disease.
13. The use as claimed in claim 13 wherein the disease is B-cell lymphoma.

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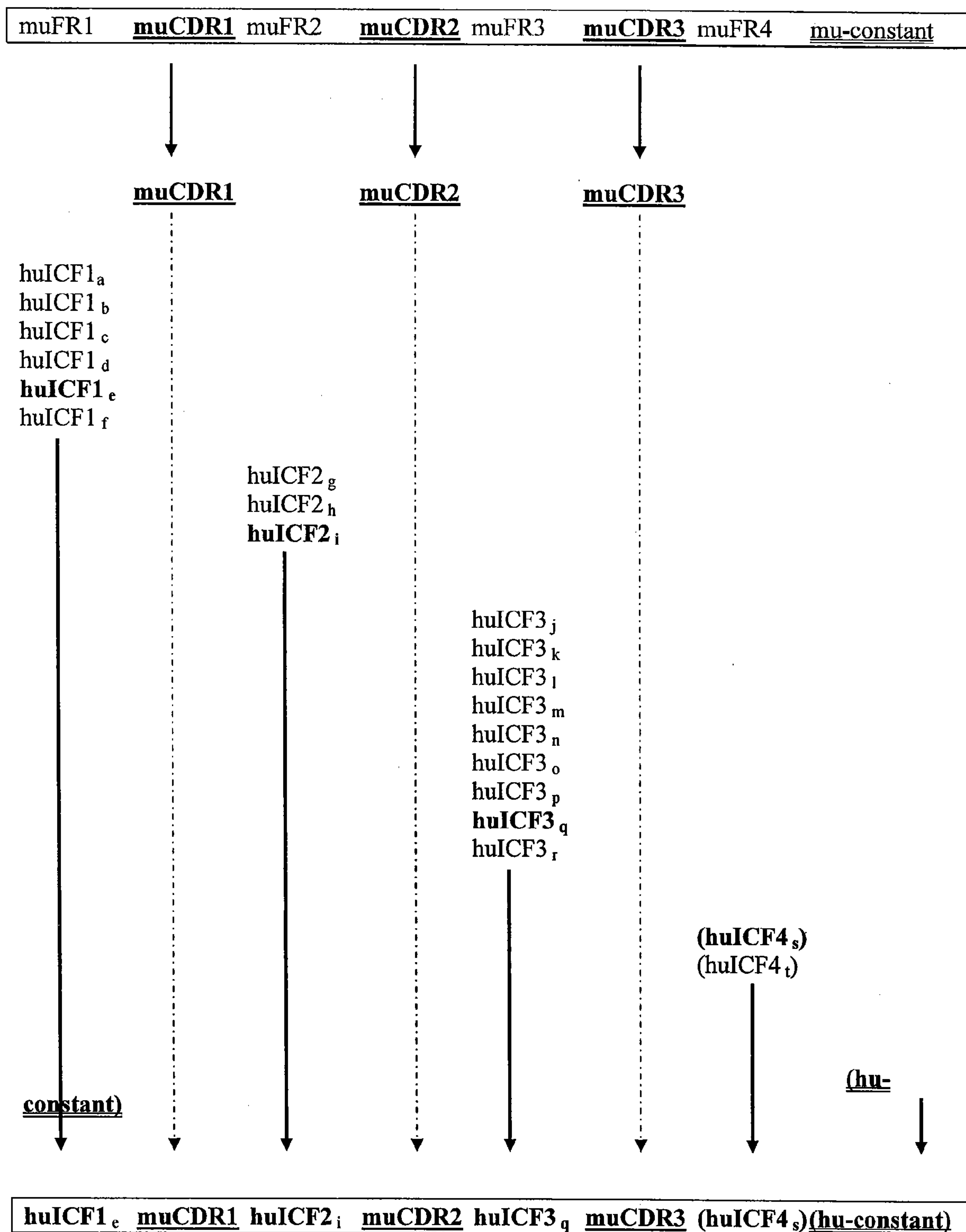


FIG. 1: Schematic of Human Framework Reassembly

(SEQ ID NO : 285) DIQMTQSPSSLSASVGDVRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP
(SEQ ID NO : 286) DIQMTQSPSSLSASVGDVRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP
(SEQ ID NO : 287) DIQMTQSPSSLSASVGDVRVTITCQASQDISYLNWYQQKPGKAPKLLIYDASNLETGVPSPRFSGSGSGTDFTFTISSLQPEDFATYYCQQYDNLP
(SEQ ID NO : 288) DIQMTQSPSSLSASVGDVRVTITCQASQDISYLNWYQQKPGKAPKLLIYDASNLETGVPSPRFSGSGSGTDFTFTISSLQPEDFATYYCQQYDNLP
(SEQ ID NO : 289) DIQMTQSPSSLSASVGDVRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQKYNLAP
(SEQ ID NO : 290) DIQMTQSPSSLSASVGDVRVTITCRASQGIKNDLWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCCLQHNLYP
(SEQ ID NO : 291) NIQMTQSPSAMSASVGDVRVTITCRARQGISNYLAWFQQKPGKVPKHLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCCLQHNLYP
(SEQ ID NO : 292) DIQMTQSPSSLSASVGDVRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYP
(SEQ ID NO : 293) DIQMTQSPSSLSASVGDVRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYP
(SEQ ID NO : 294) AIQLTQSPSSLSASVGDVRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYP
(SEQ ID NO : 295) AIQLTQSPSSLSASVGDVRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYP
(SEQ ID NO : 296) DIQMTQSPSSLSASVGDVRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFP
(SEQ ID NO : 297) DIQMTQSPSSLSASVGDVRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFP
(SEQ ID NO : 298) DIQLTQSPSSLSASVGDVRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNLYP
(SEQ ID NO : 299) AIRMTQSPFSLASVGDVRVTITCWASQGISSYLAWYQQKPAKAPKLIYDASSLQSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYYSTP
(SEQ ID NO : 300) AIRMTQSPSFSASTGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSFP
(SEQ ID NO : 301) VIWMTQSPSLLSASTGDRVTISCRMSQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISCLQSEDFATYYCQQYYSFP
(SEQ ID NO : 302) AIQMTQSPSSLSASVGDVRVTITCRASQGIKNDLWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCCLQDYNLYP
(SEQ ID NO : 303) DIQMTQSPSTLSASVGDVRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYNSYS
(SEQ ID NO : 304) DIVMTQTPLSLPVTTPGEPASISCRSSQSLDSDGNTYLDWYLOKPGQSPQLLIYTLSYRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQRIEFP
(SEQ ID NO : 305) DIVMTQTPLSLPVTTPGEPASISCRSSQSLDSDGNTYLDWYLOKPGQSPQLLIYTLSYRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQRIEFP
(SEQ ID NO : 306) DVVMTQSPSLPVTTLGQPASISCRSSQSLVSDGNTYLNWFQQRPQSPRLLIYKVSNRDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQGTHWP
(SEQ ID NO : 307) DVVMTQSPSLPVTTLGQPASISCRSSQSLVSDGNTYLNWFQQRPQSPRLLIYKVSNRDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQGTHWP
(SEQ ID NO : 308) DIVMTQTPLSLVTPGQPASISCRSSQSLVSDGNTYLNWFQKTPQSPQLLIYEVSSRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQGIHLP
(SEQ ID NO : 309) DIVMTQTPLSLVTPGQPASISCRSSQSLVSDGNTYLNWFQKTPQSPQLLIYEVSSRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQSIQLP
(SEQ ID NO : 310) DIVMTQSPSLPVTTPGQPASISCRSSQSLVSDGNTYLNWFQKTPQSPQLLIYKVSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQALQTP
(SEQ ID NO : 311) DIVMTQSPSLPVTTPGEPASISCRSSQSLVSDGNTYLNWFQKTPQSPQLLIYKVSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQALQTP
(SEQ ID NO : 312) DIVMTQTPLSPVTTPGEPASISCRSSQSLVSDGNTYLNWFQKTPQSPQLLIYKVSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYCMQATQFP
(SEQ ID NO : 313) EIVLTQSPGTLSLSPGERATLSASVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFATYYCQQYGSSE
(SEQ ID NO : 314) EIVLTQSPATLSLSPGERATLSASVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFATYYCQQYGSSE
(SEQ ID NO : 315) EIVMTQSPATLSVSPGERATLSASVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISLQSEDFATYYCQQYNNWP
(SEQ ID NO : 316) EIVMTQSPATLSVSPGERATLSASVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISLQSEDFATYYCQQYNNWP
(SEQ ID NO : 317) EIVLTQSPATLSLSPGERATLSASVSSSYLAWYQQKPGQAPRLLIYDASNRRATGIPARFSGSGSGTDFTLTISLQSEDFATYYCQQRSNWP
(SEQ ID NO : 318) EIVLTQSPATLSLSPGERATLSASVSSSYLAWYQQKPGQAPRLLIYDASNRRATGIPARFSGSGSGTDFTLTISLQSEDFATYYCQQRSNWP
(SEQ ID NO : 319) EIVMTQSPATLSLSPGERATLSASVSSSYLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTDFTLTISLQSEDFATYYCQQDYNLP
(SEQ ID NO : 320) DIVMTQSPDLSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQAPRLLIYDASNRRATGIPARFSGSGSGTDFTLTISLQSEDFATYYCQQYYSSTP
(SEQ ID NO : 321) ETTLTQSPAFMSATPGDKVNISCKASQDIDDDMNWYQQKPEAAIFIIQEAATLVPGIPPRFSGSGYGTFTLTINNIESEDAAYYFCLQHDNFP
(SEQ ID NO : 322) EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSHLWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAATYYCHQSSSLP
(SEQ ID NO : 323) EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSHLWYQQKPDQSPKLLIKYASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAATYYCHQSSSLP
(SEQ ID NO : 324) DVVMTQSPAFLSVTPGEPKVTITCQASEGIGNLYWYQQKPDQAPKLLIKYASQSIGVPSRFSGSGSGTDFTFTISLQSEDAEDAATYYCQQGNKHP

FIG. 2: Exemplary sequences derived from germline kappa genes

(SEQ ID NO : 325) QSVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVWYQQLPGKAPKLLIYYDDLLPSGVSDRFGSKSGTASLAISGLQSEDEADYYCAAWDDSLNG
(SEQ ID NO : 326) QSVLTQPPSVSGAPGQRVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFGSKSGTASLAITGLQAEDEADYYCQSYDSSLG
(SEQ ID NO : 327) QSVLTQPPSASGTPGQRVITISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSCVPDRFGSKSGTASLAISGLQSEDEADYYCAAWDDSLNG
(SEQ ID NO : 328) QSVLTQPPSASGTPGQRVITISCSGSSSNIGSNVYVWYQQLPGTAPKLLIYRNNQRPSCVPDRFGSKSGTASLAISGLQSEDEADYYCAAWDDSLG
(SEQ ID NO : 329) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNVYSWYQQLPGTAPKLLIYDNNKRPSCVPDRFGSKSGTASLTGITGLQGTGDEADYYCGTWDSSLSA
(SEQ ID NO : 330) QSALTQPPSASGSPGQSVTISCTGTSSDVGGYVSWYQQHPGKAPKLMIEVSKRPSGVPDRFGSKSGTASLTVSGLQAEDEADYYCSSYAGSNNF
(SEQ ID NO : 331) QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYVSWYQQHPGKAPKLMIDVSKRPSGVPDRFGSKSGTASLTVSGLQAEDEADYYCCSYAGSYTF
(SEQ ID NO : 332) QSALTQPASVSGSPGQSITISCTGTSSDVGGYVSWYQQHPGKAPKLMIEVSNRPSGVNRFGSKSGTASLTVSGLQAEDEADYYCSTSSSTL
(SEQ ID NO : 333) QSALTQPPSVSGSPGQSVTISCTGTSSDVGSYNRVSWYQQPGTAPKLMIEVSNRPSGVPDRFGSKSGTASLTVSGLQAEDEADYYCSLYTSSSTF
(SEQ ID NO : 334) QSALTQPASVSGSPGQSITISCTGTSSDVGSYNLVSWYQQHPGKAPKLMIEVSKRPSGVNRFGSKSGTASLTVSGLQAEDEADYYCCSYAGSSTF
(SEQ ID NO : 335) SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQOKPGQSPVLVIYQDSKRPSGIPERFGSGNSGNTATLTISGTQAMDEADYYCQAWDSSTA
(SEQ ID NO : 336) SYELTQPLSVSVALGQTARITCGGNNIGSKNVHWYQOKPGQAPVLVIYRDSNRPSGIPERFGSGNSGNTATLTISRAQAGDEADYYCQVWDSSTA
(SEQ ID NO : 337) SYELTQPPSVSVSPGQTARITCSGDALPKKYAWYQOKSGQAPVLVIYEDSKRPSGIPERFGSSSGTMA TLTVISGAQVEDEADYYCYSTDSSGNH
(SEQ ID NO : 338) SYELTQPPSVSVSLGQMARITCSGEALPKKYAWYQOKPGQFPVLVIYKDSERPSPGIPERFGSSSGTIVTLTVISGVQAEDEADYYCLADSSGTY
(SEQ ID NO : 339) SSELTDQFAVSVALGQTVRITCQGDSLRYSYASWYQOKPGQAPVLVIYGNRNPSPGIPDRFGSSSGTASLTVISGAQAEDEADYYCNSRDSGNNH
(SEQ ID NO : 340) SYVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQOKPGQAPVLVIYVSDRPSGIPERFGSGNSGNTATLTISRVEAGDEADYYCQVWDSSTDH
(SEQ ID NO : 341) SYELTQLPSVSVSPGQTARITCSGDVLGENYADWYQOKPGQAPVLVIYEDSERYPGIPERFGSGTSGNTTTLTVISRVLTEDEADYYCLSGDEDN
(SEQ ID NO : 342) SYEIMQPPSVSVSPGQTARITCSGDALPKQYAWYQOKPGQAPVLVIYKDSERPSPGIPERFGSSSGTTVTLTVISGVQAEDEADYYCQSADSSGTY
(SEQ ID NO : 343) SYELTQPSSVSVSPGQTARITCSGDVLAKKYARWFQOKPGQAPVLVIYKDSERPSPGIPERFGSSSGTIVTLTVISGAQVEDEADYYCYSAADNN
(SEQ ID NO : 344) LPVLTQPPSASALLGASIKLTCTLSSEHSTYTIIEWYQORPGRSPQYIMKVSDGSHSKGDGIPDRFMGSSSGADRYLTVSNLQSDDEAFYHCGESHVIDGQVG
(SEQ ID NO : 345) QPVLTOSSSASASLGSVVKLTCTLSSGHSSYIIAHHQQQPGKAPRYIMKLEGSYKNGSGVDPDRFGSSSGADRYLTVISNQLQLEDEADYYCETWDSNT
(SEQ ID NO : 346) QLVLTQSPSASASLGASVKLTCTLSSGHSSYAIAMHQQQPEKGPRLMKLNSDGSKSGDIPDRFGSSSGAERYLTVISLQSEDEADYYCMIWPSNAS
(SEQ ID NO : 347) QPVLTPPPSSASPGEASRLTCTLPSDINVGSYNIYWYQOKPGSPPYLLIYYSDSKGQSGVPSRFGSGKDASANTGILLISGLQSEDEADYYCMIWPSNAS
(SEQ ID NO : 348) QAVLTQPASLSASPGASASLTCTLRSGINVTYRIYWYQOKPGSPPYLLRYKSDSDKQQSGVPSRFGSGKDASANAGILLISGLQSEDEADYYCMIWHSSAS
(SEQ ID NO : 349) QPVLTPQSSHASGASVRLTCTMLSSGFSVGDFWIRWYQOKPGNPPRYLLIYHSDSNKGGSGVPSRFGSGNDASANAGILRISGLQPEDEADYYCGTWHNSKST
(SEQ ID NO : 350) NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQORPGSSPTTVIYEDNQRPSCVPDRFGSIDSSSNSASLTVISGLKTEDEADYYCQSYDSSN
(SEQ ID NO : 351) QTVVTOEPSLTVSPGGTVTLTCASSTGAVTSGYYPNWFQOKPGQAPRALIYSTSNKHSWTPARFGSLLGGKAALTVLSGVQPEDEAFYCYCLLYYGGAAQ
(SEQ ID NO : 352) QAVVTQEPSLTVSPGGTVTLTCSSSTGAVTSGHYYPWFQOKPGQAPRTLIIYDTSNKHSTWTPARFGSLLGGKAALTVLSGAQPEDEAFYCYCLLYSYSGAR
(SEQ ID NO : 353) QTVVTOEPSFVSVPGGTVTLTCGLSSGSVSTSYPSWYQQTGQAPRTLIIYSTNTRSSGVPDRFGSILGNKAALTVITGAQADDESDDYCYVLYMGSGI
(SEQ ID NO : 354) QPVLTPPPSASASLGASVTLTCTLSSGSYNYKVDWYQORPGKPRFVVRVGTGGIVGSKGDGIPDRFVLSGLNRYLTVIKNTQEEDESDYHCGADHSGSGSNFV
(SEQ ID NO : 355) QAGLTQPPSVSKGLRQTATLTCTGNSNNVGNQAAWLQQHQGHPPPKLLSYRNNNRPSGISERLSASRSGNTASLTVITGLQPEDEADYYCSAWDSSLSA

FIG. 3: Exemplary sequences derived from germline lambda genes

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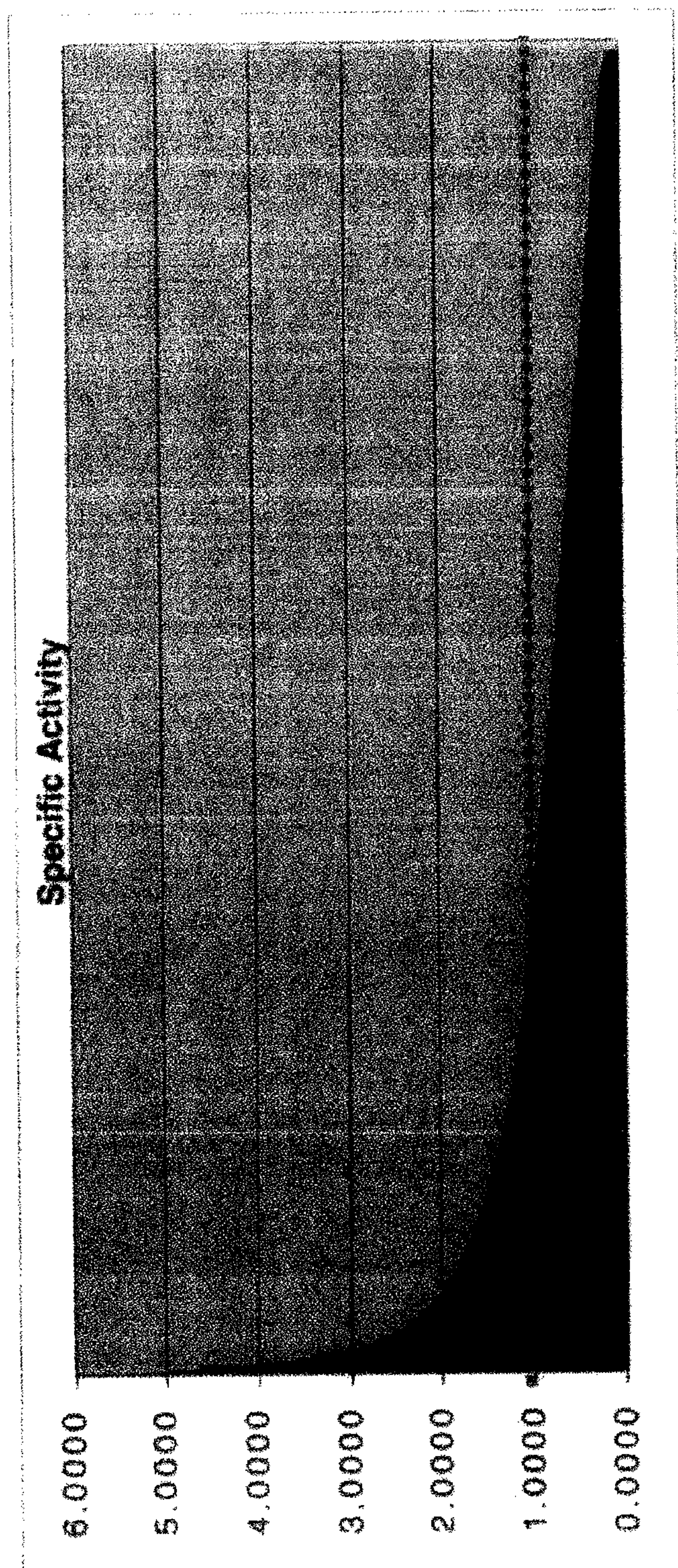


FIG. 4: Primary ELISA screen of HuFR antibodies for specific activity, Relative to DVSA-CD20 (=1.0 dotted baseline)

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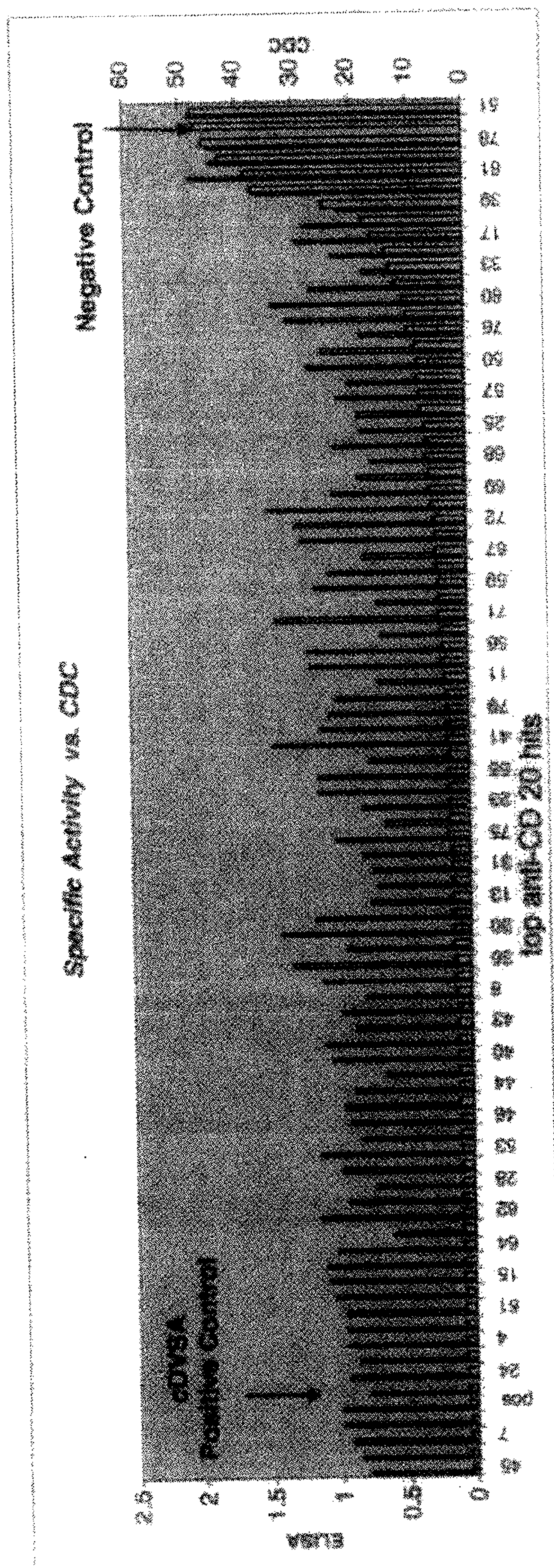


FIG. 5

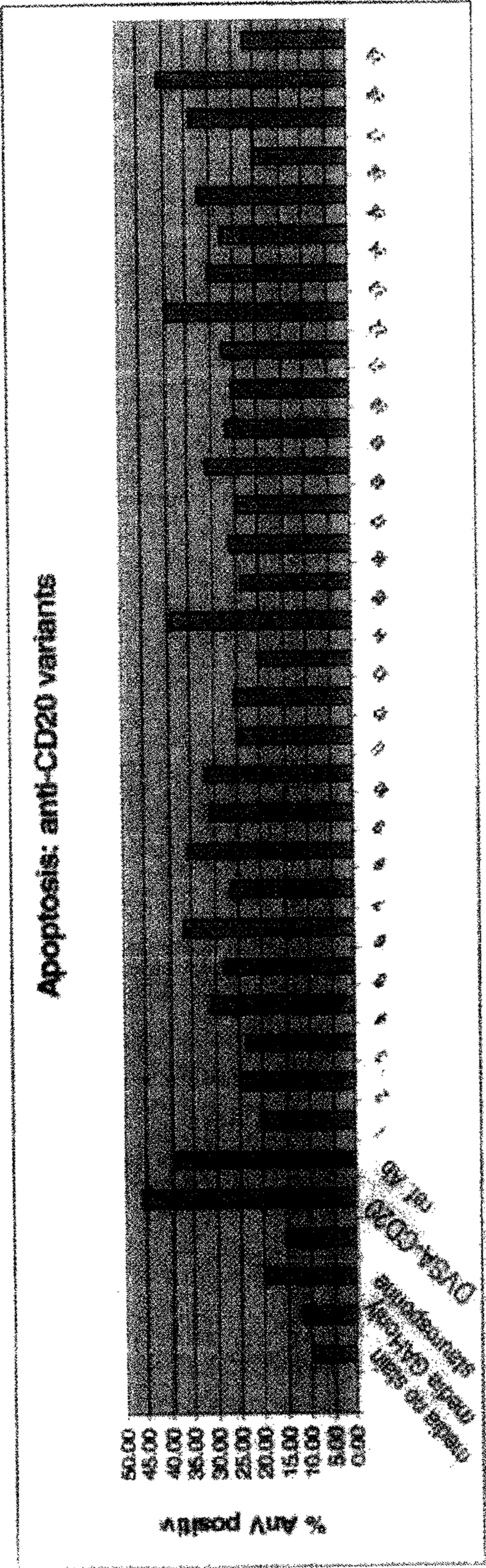


FIG. 6

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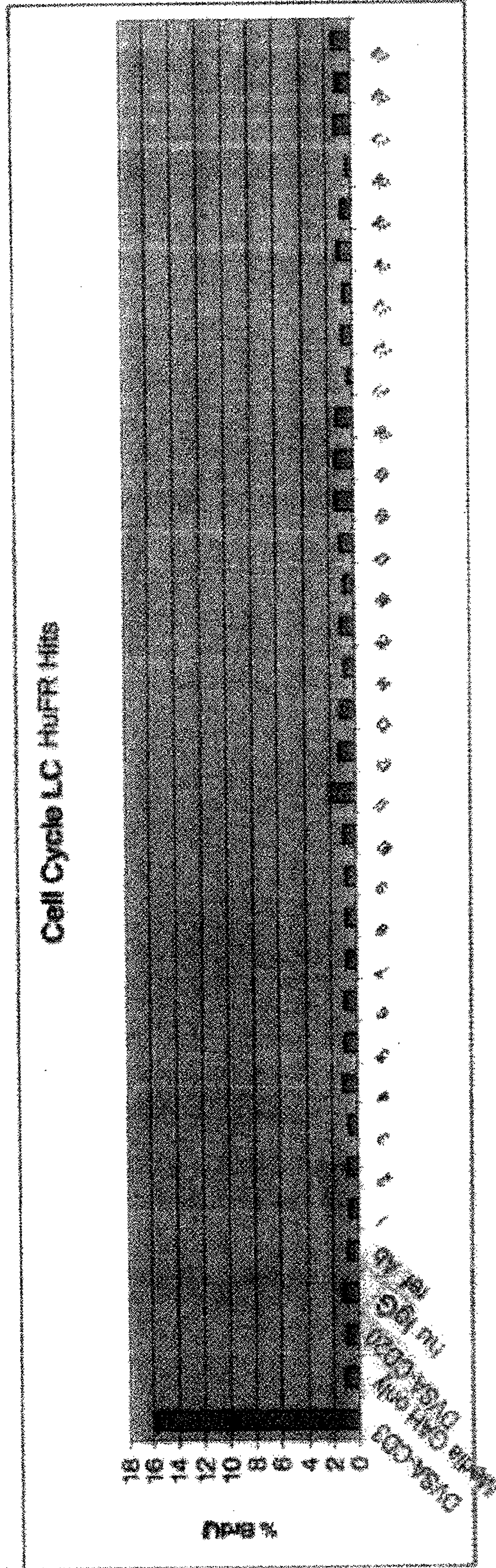


FIG. 7

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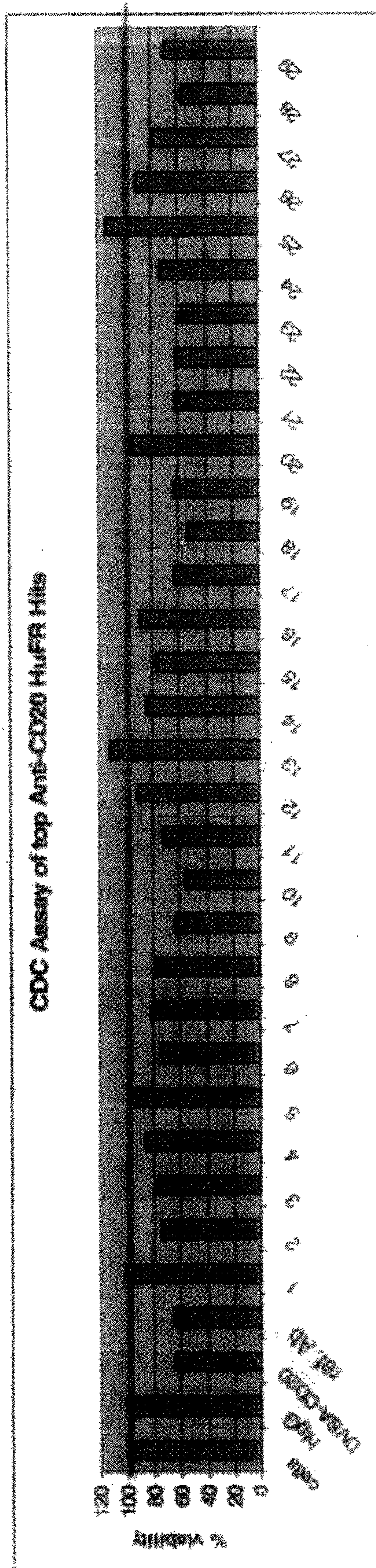


FIG. 8

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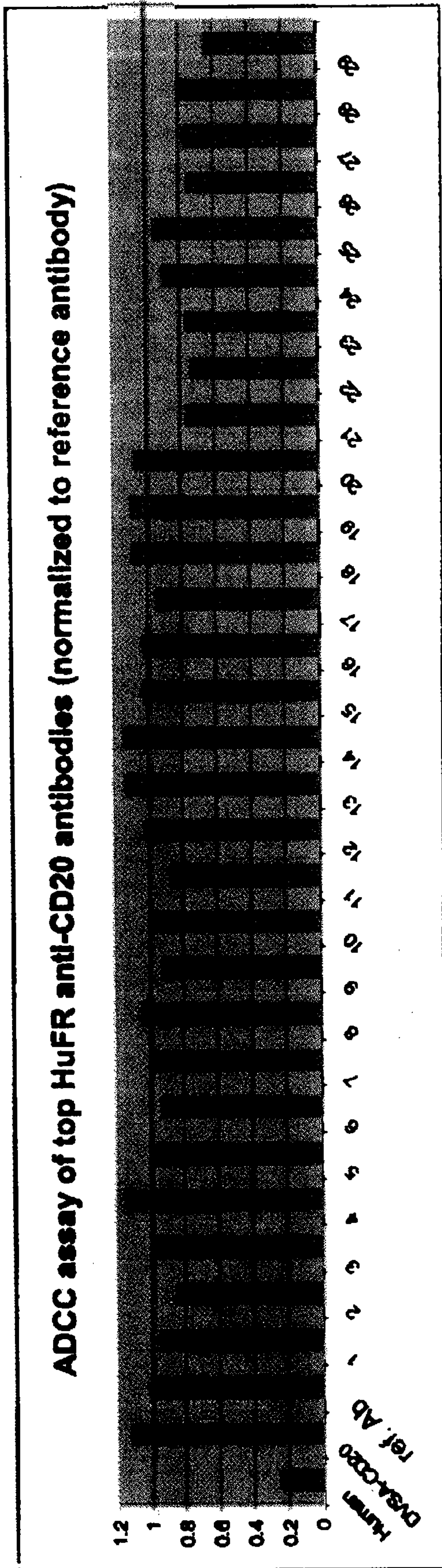


FIG. 9

heavy chain construct: (SEQ ID NO :356)

ATGGAGTTTGGGCTGAGCTGGCTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGTCCAGTCCAGCTGCAGC
 AGTCTGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTTTAC
 TAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGC
 CGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAATCCTCCAGCACAG
 CCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCA
 TTAAGTGCCTTGAAGTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTC
 TTTCCCTTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
 TCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCT
 ACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
 ATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAA
 CTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACC
 CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT
 GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
 ACAACAGCACGTACCGTGTGGTCAAGCTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
 GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
 GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGG
 TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACATAAGAC
 CACGCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
 CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT
 CCCTGTCTCCGGGTAAATGA

light chain construct: (SEQ ID NO :357)

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCCAAATGTCAAATTG
 TTCTCACCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTACCATGACCTGCAGTGCCAGCTC
 AAGTGTAAGTTACATGAACTGGTACCAGCAGAAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCC
 AAAGTGGCTTCTGGAGTCCCTGCTCACTTCAGGGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCG
 GCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAGTAGTAACCCATTACAGTTTCGGCTCGGG
 GACAAAGTTGGAAATAAACCGGGCTGATCGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT
 GAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
 AGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG
 CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAA
 GTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTAA

**FIG. 10: Light (top) and heavy (bottom) sequences
 for a HuFR anti-CD3 antibody**

HC DVSA-CD3 (Fc Null): (SEQ ID NO :358)

MEFGLSWLFLVAILKGVQCQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRG
 YTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSASTKGPSVFPLA
 PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
 KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSVYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPOVYTLPPS
 RDELTKNOVSLTCLVKGFYPSDIAVEWESNGOPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQOQGNVFSCSVMH
 EALHNHYTQKSLSLSPGK

HC DVSA-CD3: (SEQ ID NO :359)

MEFGLSWLFLVAILKGVQCQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRG
 YTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSASTKGPSVFPLA
 PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
 KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPOVYTLPPS
 RDELTKNOVSLTCLVKGFYPSDIAVEWESNGOPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQOQGNVFSCSVMH
 EALHNHYTQKSLSLSPGK

LC DVSA-CD3: (SEQ ID NO :360)

MDMRVPAQLLGLLLLWLPGAKCQIVLTQSPAISASPGKVTMTCSASSSVSYMNYQQKSGTSPKRWIYDTSKL
 ASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRADRTVAAPSVFIFPPSDEOLK
 SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHOGL
 SSPVTKSFNRGEC

**FIG 11: Light (top) and heavy (bottom) chains
 for HuFR anti-CD3 antibodies**

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Top Heavy chains (SEQ ID NOS :361-363)

	1	50
BD20610	QVQLVESGAIEVKKPGASVKVSC	KASGYTFTRYTMHWVRQAPGKGLEWMGY
BD20611	QVQLVESGAIEVKKPGASVKVSC	KASGYTFTRYTMHWVRQAPGKGLEWMGY
BD20613	QVQLQESGPGLVKPSETLSLTCAV	SGYTFTRYTMHWVRQAPGKGLEWVG
	51	100
BD20610	INPSRGYTNYNQKFKDRVTISVD	TSKNQFSLKLSSVTAADTAVYYCARY
BD20611	INPSRGYTNYNQKFKDRLTISKD	TSKNQVVLTMNMDPVDATYYCARY
BD20613	INPSRGYTNYNQKFKDRFTISR	DDSKNTAYLQMNSLKTEDTAVYYCTRY
	101	136
BD20610	DDHYCLDYWGQGT	LTVSSASTKGPSVFPLAPSSKS
BD20611	DDHYCLDYWGQGT	LTVSSASTKGPSVFPLAPSSKS
BD20613	DDHYCLDYWGQGT	LTVSSASTKGPSVFPLAPSSKS

Top Light (kappa) chains (SEQ ID NOS :364-372)

	1	55
BD21130	DIQMTQSPSSLSASVGDRVTITCS	ASSSVSYMNWYQQKPGQPPKLLIYDTSKLAS
BD21131	DIQMTQSPSTLSASVGDRVTITCS	ASSSVSYMNWYQQKPGQAPRLLIYDTSKLAS
BD21132	EIVLTQSPATLSLSPGERATLSC	SASSSVSYMNWYQQKPGKAPKLLIYDTSKLAS
BD21133	DIVMTQSPSLSLPVTIPGEPASIS	CSASSSVSYMNWYQQKPGQPPKLLIYDTSKLAS
BD21134	EIVMTQSPATLSVSPGERATLSC	SASSSVSYMNWYQQKPGQPPKLLIYDTSKLAS
BD21135	EIVMTQSPATLSVSPGERATLSC	SASSSVSYMNWYQQKPGQAPRLLIYDTSKLAS
BD21136	DIQMTQSPSTLSASVGDRVTITCS	ASSSVSYMNWYQQKPGKAPKLLIYDTSKLAS
BD21137	DIQMTQSPSTLSASVGDRVTITCS	ASSSVSYMNWYQQKPGQPPKLLIYDTSKLAS
BD21138	DIQMTQSPSTLSASVGDRVTITCS	ASSSVSYMNWYQQKPGKAPKLLIYDTSKLAS
	56	106
BD21130	GVPDRFSGSGSGTDFTLKISR	VEAEDVGVYYCQQWSSNPFTFGQGTKVEIK
BD21131	GVPDRFSGSGSGTDFTLKISR	VEAEDVGVYYCQQWSSNPFTFGQGTKVEIK
BD21132	GVPSRFSGSGSGTEFTLTIS	SSLQPDFFATYYCQQWSSNPFTFGQGTKVEIK
BD21133	GVPSRFSGSGSGTEFTLTIS	SSLQPDFFATYYCQQWSSNPFTFGQGTKVEIK
BD21134	GVPSRFSGSGSGTEFTLTIS	SSLQPDFFATYYCQQWSSNPFTFGQGTKVEIK
BD21135	GVPDRFSGSGSGTDFTLTIS	SLQAEDVAVYYCQQWSSNPFTFGQGTKVEIK
BD21136	GIPDRFSGSGSGTDFTLTIS	RLEPEDFAVYYCQQWSSNPFTFGQGTKVEIK
BD21137	GVPSRFSGSGSGTDFTFTIS	SSLQPEDATYYCQQWSSNPFTFGQGTKVEIK
BD21138	GVPDRFSGSGSGTDFTLKISR	VEAEDVGVYYCQQWSSNPFTFGQGTKVEIK

FIG. 12: HuFR HC and LC variable regions for anti-CD3 antibody