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(54) Title: HYBRID ANTIBODIES

(57) Abstract: Hybrid antibodies and/or hybrid antibody fragments and methods of making them are provided. In one embodiment the hybrid antibodies and/or hybrid antibody fragments contain heavy and/or light variable regions that contain two or more frame-
work regions derived from at least two antibodies. In another embodiment, at least two of the framework regions are classified in the same germline gene family. In one embodiment, at least two framework regions are classified in the same germline gene family member. The hybrid antibodies or hybrid antibody fragments may contain human framework regions and nonhuman CDRs.



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HYBRID ANTIBODIES

BACKGROUND

5 RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 10/453,698 filed June 3, 2003 which is a continuation-in-part of U.S. Application Serial No. 10/308,817 filed December 3, 2002 which claims priority to U.S. Provisional Application No. 60/336,591 filed on December 3, 2001.

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TECHNICAL FIELD

The present description relates to hybrid antibodies and hybrid antibody fragments derived from one species which preferentially bind a target object and which have reduced immunogenicity in a different species.

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BACKGROUND OF RELATED ART

Antibodies are proteins produced by lymphocytes known as B cells in vertebrates in response to stimulation by antigens. The basic structural unit of an antibody (a.k.a. immunoglobulin (Ig)) molecule consists of four polypeptide chains which come together in the shape of a capital letter "Y". Two of the four chains are identical light (L) chains and two are identical heavy (H) chains. There are five different kinds (isotypes) of heavy chains which divide antibodies into five classes, namely, IgA, IgD, IgE, IgG and IgM. In addition, there are two different isotypes of light chains designated κ and λ . Each class of heavy chains can combine with either of the light chains. The heavy and light chains each contain a variable region (VH and VL, respectively) that is involved in antigen binding and a constant (C) region. The antigen binding site is composed of six hypervariable regions (a.k.a. complementarity determining regions (CDRs)). Three CDRs from the heavy chain and three CDRs from the light chain are respectively positioned between four relatively conserved anti-parallel β -sheets which are called framework regions (FR1, FR2, FR3 and FR4), on each chain. By convention,

numbering systems have been utilized to designate the location of the component parts of VH and VL chains. The Kabat definition is based on sequence variability and the Chothia definition is based on the location of structural loop regions.

For each type of Ig chain synthesized by B cells, there is a separate pool of gene segments, known as germline genes, from which a single polypeptide chain is synthesized. Each pool is located on a different chromosome and typically contains a relatively large number of gene segments encoding the V region and a lesser number of gene segments encoding the C region. Each light chain V region is encoded by a nucleic acid sequence assembled from two kinds of germline gene segments, i.e., a long V gene segment, a short joining (J) gene segment, and a C segment. The heavy chain is encoded by four kinds of germline gene segments, three for the variable region and one for the constant region. The three germline gene segments that encode the heavy chain variable region are a V segment, a J segment and a diversity (D) segment. Human germline V, D and J gene sequences have been characterized. The human germline VH gene segments (such "segments" are also referred to herein as family members) are classified into seven families (VH1-VH7) based on sequence homology of at least 80%. See, e.g., Matsuda, et al. J. Exp. Med. (1998) 188:2151-2162. There are approximately fifty-one VH segments (family members). The first two CDRs and three framework regions of the heavy chain variable region are encoded by VH. CDR3 is encoded by a few nucleotides of VH, all of DH and part of JH, while FR4 is encoded by the remainder of the JH gene segment. With regard to light chains, V Kappa (Vk) or V lambda (Vλ) gene segments (family members) encode the first two CDR and three framework regions of the V region along with a few residues of CDR3. J Kappa (Jk) and J Lambda (Jλ) segments encode the remainder of the CDR3 region in a Vk or Vλ region, respectively. DNA encoding the κ chain includes approximately forty Vk segments (family members) that are classified into six families (Vk I-Vk VI) based on sequence homology. DNA encoding the λ chain includes approximately thirty-one Vλ segments (family members) that are classified into ten families. See Figs. 1, 2, 3 and 6.

Antibodies and antibody fragments have become promising therapeutic agents in connection with various human diseases in both acute and chronic settings. There are several methods being utilized to generate antibodies including hybridoma technology, bacterial display, ribosome display, yeast display, and recombinant expression of human antibody fragments on the surface of replicative bacteriophage. Monoclonal antibodies (mAbs), which may be produced by hybridomas, have been applied successfully as diagnostics for many years, but their use as therapeutic agents is just emerging. The vast majority of mAbs are of non-human (largely rodent) origin, posing the problem of immunogenicity in humans. When antibodies of rodent origin are administered to humans, anti-rodent antibodies are generated which result in enhanced clearance of the rodent antibody from the serum, blocking of its therapeutic effect and hypersensitivity reactions. These limitations have prompted the development of engineering technologies known as "humanization".

The first humanization strategies were based on the knowledge that heavy and light chain variable domains are responsible for binding to antigen, and the constant domains for effector function. Chimeric antibodies were created, for example, by transplanting the variable domains of a rodent mAb to the constant domains of human antibodies (e.g. Neuberger MS, et al., *Nature* 314, 268-70, 1985 and Takeda, et al., *Nature* 314, 452-4, 1985). Although these chimeric antibodies induce better effector functions in humans and exhibit reduced immunogenicity, the rodent variable region still poses the risk of inducing an immune response. When it was recognized that the variable domains consist of a beta sheet framework surmounted by antigen-binding loops (complementarity determining regions or CDR's), humanized antibodies were designed to contain the rodent CDR's grafted onto a human framework. Several different antigen-binding sites were successfully transferred to a single human framework, often using an antibody where the entire human framework regions have the closest homology to the rodent sequence (e.g., Jones PT, et al., *Nature* 321, 522-5, 1986; Riechmann L. et al., *Nature* 332, 323-327, 1988; and Sato K. et al., *Mol. Immunol.* 31, 371-8, 1994). Alternatively, consensus human frameworks were built based on

several human heavy chains (e.g., Carter P. et al., Proc. Nat. Acad. Sci. USA 89, 487-99, 1992). However, simple CDR grafting often resulted in loss of antigen affinity. Other possible interactions between the β -sheet framework and the loops had to be considered to recreate the antigen binding site (Chothia C, et al., Mol. Biol. 196, 901-917, 1987).

Comparison of the essential framework residues required in humanization of several antibodies, as well as computer modeling based on antibody crystal structures revealed a set of framework residues termed as "Vernier zone residues" (Foote J., et al., Mol Biol 224, 487-99, 1992) that most likely contributes to the integrity of the binding site. In addition, several residues in the VH-VL interface zone might be important in maintaining affinity for the antigen (Santos AD, et al., Prog. Nucleic Acid Res Mol Biol 60, 169-94 1998). Initially, framework residues were stepwise mutated back to the rodent sequence (Kettleborough CA, et al. Protein Engin. 4, 773-783, 1991). However, this mutation approach is very time-consuming and cannot cover every important residue.

For any particular antibody a small set of changes may suffice to optimize binding, yet it is difficult to select from the set of Vernier and VH/VL residues. Combinatorial library approaches combined with selection technologies (such as phage display) revolutionized humanization technologies by creating a library of humanized molecules that represents alternatives between rodent and human sequence in all important framework residues and allows for simultaneous determination of binding activity of all humanized forms (e.g. Rosok MJ, J Biol Chem, 271, 22611-8, 1996 and Baca M, et al. J Biol Chem 272, 10678-84, 1997).

The above approaches utilize entire framework regions from a single antibody variable heavy or variable light chain to receive the CDRs. It is advantageous to provide highly homologous engineered antibodies based on antibodies from an originating species which exhibit reduced immunogenicity while maintaining an optimum binding profile that can be administered to a target species for therapeutic and diagnostic purposes.

SUMMARY

In one aspect, a method for producing a hybrid antibody or hybrid antibody
5 fragment is provided which includes providing an initial antibody having specificity for a
target; determining the sequence of at least a portion of a variable region of the initial
antibody; and (i) selecting a first component of the variable region selected from the
group consisting of FR1, FR2, FR3 and FR4; comparing the sequence of the first
10 selected component to sequences contained in a reference database of antibody
sequences or antibody fragment sequences from a target species; and selecting a
sequence from an antibody in the database which demonstrates a high degree of
homology to the first component; (ii) selecting a second component of the variable
region which is different than the first component, the second component selected from
the group consisting of FR1, FR2, FR3 and FR4; comparing the sequence of the
15 second component to sequences contained in a reference database of antibody
sequences or antibody fragment sequences from the target species; selecting a
sequence from the database which demonstrates a high degree of homology to the
second component and which is from a different antibody than the antibody selected in
step (i); and (iii) operatively linking the selected framework sequences to one or more
20 CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.
The method described above may be continued with respect to the remaining
components of the variable region until an entire variable region is synthesized. The
remaining components may be from the same or different antibodies than those
selected from the database in steps (i) and (ii) above. The first, second and/or
25 remaining components above may include one or more CDRs. It should be understood
that combinations of the framework regions within the first, second and/or remaining
components can be used for comparison in the steps set forth above. The variable
region of the initial antibody may be a variable light chain or a variable heavy chain. The
sequences referred to above may be amino acid sequences or nucleic acid sequences.

The antibody may be any known antibody form known to those skilled in the art, e.g., whole antibodies, chimeric antibodies, bivalent antibodies and the like. The antibody fragment referred to above may be selected from the group consisting of scFv, Fab, Fab', F(ab')₂, Fd, , antibody light chains and antibody heavy chains. The target species
5 may be human.

In one embodiment, the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. In another embodiment, the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree
10 of homology. In another embodiment, the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. In another embodiment, the FR4 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. The reference database may contain germline or rearranged
15 antibody sequences of the target species.

In another aspect, a method for producing a hybrid antibody or hybrid antibody fragment is provided which includes providing an initial antibody having specificity for a target; determining the sequence of at least a portion of a variable framework region of the initial antibody; and (i) selecting a first component of the variable region selected
20 from the group consisting of FR1, FR2 and FR3; comparing the sequence of the first component of the variable region to sequences contained in a reference database of antibody sequences or antibody fragment sequences from a target species; selecting a sequence from the database which demonstrates a high degree of homology to the first component; and determining the germline gene family from which the sequence was
25 derived; (ii) selecting a second component of the variable region which is different than the first component, the second component selected from the group consisting of FR1, FR2 and FR3; comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species; selecting a sequence from the database which

demonstrates a high degree of homology to the second component and which corresponds to the same germline gene family as the first sequence selected from the database in step (i) of this paragraph; and (iii) operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment. The method described in this aspect may be continued with respect to the third component of the framework region. In one embodiment, FR4 is added and operatively linked to the product of step (iii) of this paragraph and an entire variable region is synthesized. The method can be extended until an entire hybrid antibody is produced. The variable framework region of the initial antibody may be a light chain or a heavy chain. The first, second and/or third components in this paragraph may include one or more CDRs. It should be understood that combinations of the framework regions within the first, second and/or third components can be used for comparison in the steps set forth in this paragraph.

In one embodiment, two or more of the sequences selected from the reference database are from different antibodies. The sequences referred to above may be amino acid sequences or nucleic acid sequences. The antibody may be any known antibody form known to those skilled in the art, e.g., whole antibodies, chimeric antibodies, bivalent antibodies and the like. The antibody fragment referred to above may be selected from the group consisting of scFv, Fab, Fab', F(ab')₂, Fd, antibody light chains and antibody heavy chains. The target species may be human.

In one embodiment, the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment, the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment, the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high

degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment, the FR4 region sequence from the initial antibody is used individually to search the reference database for FR4 germline sequences which are chosen from those which have the highest homology of human JH, JK, or JA family members. The reference database may contain germline or rearranged sequences of the target species. In one embodiment, at least two of the selected sequences correspond to the same family member in the germline gene family.

In another aspect, a hybrid antibody or hybrid antibody fragment includes a first heavy chain framework region from a first antibody, and a second heavy chain framework region from a second antibody. In one embodiment, the hybrid antibody or hybrid antibody fragment includes a third heavy chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. In another embodiment, the hybrid antibody or hybrid antibody fragment includes a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, and a second light chain framework region from a second antibody. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first heavy chain framework region from a first antibody, the first heavy chain framework region corresponding to a particular VH family, and a second heavy chain framework region from a second antibody, the second heavy chain framework region corresponding to the same VH family as the first heavy chain framework region. In one embodiment, the hybrid antibody includes a third heavy chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same VH family as the first heavy chain framework region. In another embodiment, the hybrid antibody includes a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second heavy chain framework region and the third heavy chain framework region correspond to the same member of the VH family as the first heavy chain framework region. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin. Germline FR4 is selected from those of the highest homology of corresponding J region.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular Vk family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same Vk family as the first light chain framework region. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same Vk family as the first light chain framework region. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third

antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the V κ family as the first light chain framework region. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular V λ family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same V λ family as the first light chain framework region. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same V λ family as the first light chain framework region. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the V λ family as the first light chain framework region. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a library of antibodies or antibody fragments is provided which includes hybrid antibodies and/or hybrid antibody fragments according to the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart depicting germline genes of the V κ gene locus. V κ exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are

according to the structural criteria defined by Chothia. CDRs are according to Kabat, et al.

FIG. 2 is a chart depicting germline genes of the VH gene locus. VH exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are according to the structural criteria defined by Chothia. CDRs are according to Kabat, et al.

FIG. 3 is a chart depicting germline genes of the Vλ gene locus. Vλ exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are according to the structural criteria defined by Chothia. CDRs are according to Kabat, et al.

FIG. 4A depicts the amino acid sequence (Seq. Id. No. 123) of a murine antibody variable light chain directed to human mannose binding lectin (i.e., the light chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 4B depicts the amino acid sequence (Seq. Id. No. 124) of human antibody variable light chain sequence gene identification (GI) number 3747016, separating the sequence into framework and CDR component parts.

FIG. 4C depicts the amino acid sequence (Seq. Id. No. 125) of human antibody variable light chain sequence gene identification (GI) number 5833827, separating the sequence into framework and CDR component parts.

FIG. 4D depicts the amino acid sequence (Seq. Id. No. 126) of human antibody variable light chain sequence gene identification (GI) number 722614, separating the sequence into framework and CDR component parts.

FIG. 4E depicts the amino acid sequence (Seq. Id. No. 127) of human antibody variable light chain sequence gene identification (GI) number 1785870, separating the sequence into framework and CDR component parts.

FIG. 4F depicts the amino acid sequence of a hybrid humanized antibody light chain (Seq. Id. No. 128), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody light chain of FIG. 4A is provided.

FIG. 4G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain (see FIG. 4F) and the initial murine monoclonal antibody light chain (see FIG. 4A) in terms of framework regions alone, CDRs alone and whole Vk chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain and the most similar human germline sequence VkVI (A10/A26). Also shown is the degree of homology between the most similar human rearranged CDR grafted variable light chain obtained by prior art methods and the initial murine monoclonal antibody light chain. Also shown is the most similar human rearranged CDR grafted VL versus the most similar human germline sequence VkVI (A14).

FIG. 4H depicts an amino acid sequence (Seq. Id. No. 129) resulting from a BLAST query in Genbank using the entire variable light chain of the initial murine monoclonal antibody depicted in FIG. 4a.

FIG. 4I depicts an amino acid sequence (Seq. Id. No. 130) resulting from a BLAST query in Genbank using only the combined framework regions of the variable light chain of the initial murine monoclonal antibody depicted in FIG. 4a.

FIG. 5A depicts the amino acid sequence (Seq. Id. No. 131) of a murine antibody variable heavy chain directed to human mannose binding lectin (i.e., the heavy chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 5B depicts the amino acid sequence (Seq. Id. No. 132) of human antibody variable heavy chain sequence gene identification (GI) number 563649, separating the sequence into framework and CDR component parts.

FIG. 5C depicts the amino acid sequence (Seq. Id. No. 133) of human antibody variable heavy chain sequence gene identification (GI) number 951263, separating the sequence into framework and CDR component parts.

FIG. 5D depicts the amino acid sequence (Seq. Id. No. 134) of human antibody variable heavy chain sequence gene identification (GI) number 484852, separating the sequence into framework and CDR component parts.

FIG. 5E depicts the amino acid sequence (Seq. Id. No. 135) of human antibody variable heavy chain sequence gene identification (GI) number 2367531, separating the sequence into framework and CDR component parts.

FIG. 5F depicts the amino acid sequence of a hybrid humanized antibody heavy chain (Seq. Id. No. 136), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody heavy chain of FIG. 5a is provided.

FIG. 5G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain (see FIG. 5F) and the initial murine monoclonal antibody heavy chain (see FIG. 5A) in terms of framework regions alone, CDRs alone and whole VH chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain and the most similar human germline sequence VH4-31. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable heavy chain obtained by prior art methods and the initial murine monoclonal antibody heavy chain. Also shown is the degree of homology between the most similar human rearranged CDR grafted VH versus the most similar germline sequence VH4-31.

FIG. 5H depicts an amino acid sequence (Seq. Id. No. 137) resulting from a BLAST query in Genbank using the entire variable heavy chain of the murine antibody depicted in FIG. 5A.

FIG. 5I depicts an amino acid sequence (Seq. Id. No. 138) resulting from a BLAST query in Genbank using only the combined framework regions of the variable heavy chain of the murine monoclonal antibody depicted in FIG. 5A.

FIG. 6 is a chart depicting translated germline genes of the JH, JK and JL gene loci in terms of amino acid sequence alignment.

FIG. 7 depicts the nucleic acid (Seq. Id. No. 154) and amino acid (Seq. Id. No. 155) sequences of the hybrid humanized variable light chain and of the nucleic acid sequence (Seq. Id. No. 156) and amino acid sequence (Seq. Id. No. 157) of the hybrid humanized variable heavy chain and indicates the positions of particular nucleotides

and amino acids that were altered as compared to the initial murine antibody sequences. Framework regions are underlined and altered nucleotides and amino acids are boldface.

FIG. 8 depicts the nucleotide sequences of oligonucleotide chains that were
5 utilized for site directed mutagenesis of the initial murine antibody variable light and variable heavy chains. The chains are designated as follows: for VL: Oligo 1 (Seq. Id. No. 158), Oligo 2 (Seq. Id. No. 159), Oligo 3 (Seq. Id. No. 160), Oligo 4 (Seq. Id. No. 161), Oligo 5 (Seq. Id. No. 162), Oligo 6 (Seq. Id. No. 163), Oligo 7 (Seq. Id. No. 164); for VH: Oligo 8 (Seq. Id. No. 165), Oligo 9 (Seq. Id. No. 166), Oligo 10 (Seq. Id. No.
10 167), Oligo 11 (Seq. Id. No. 168), Oligo 12 (Seq. Id. No. 169), Oligo 13 (Seq. Id. No. 170), Oligo 14 (Seq. Id. No. 171).

FIG. 9A depicts the amino acid sequence (Seq. Id. No. 172) of a murine antibody variable light chain directed to h-DC-SIGN (i.e., the light chain of the initial antibody), separating the sequence into framework and CDR components.

15 FIG. 9B depicts the amino acid sequences (Seq. Id. Nos. 173 and 174) of human antibody variable light chain sequence gene identification (GI) numbers 441333 and 5578780, separating the sequence into framework and CDR component parts.

FIG. 9C depicts the amino acid sequences (Seq. Id. Nos. 175 and 176) of human antibody variable light chain sequence gene identification (GI) number 4324018 and
20 18041766, separating the sequence into framework and CDR component parts.

FIG. 9D depicts the amino acid sequence (Seq. Id. No. 177) of human antibody variable light chain sequence gene identification (GI) numbers 553476 and 33251, separating the sequence into framework and CDR component parts.

FIG. 9E depicts the amino acid sequence (Seq. Id. No. 178) of human antibody
25 variable light chain sequence gene identification (GI) number 446245, separating the sequence into framework and CDR component parts.

FIG. 9F depicts the amino acid sequences of hybrid humanized antibody light chain (Seq. Id. Nos. 179, 180 and 181), separating the sequence into framework and

CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody light chain of FIG. 9A is provided.

FIG. 9G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain (see FIG. 9F) and the initial murine monoclonal antibody light chain (see FIG. 9A) in terms of framework regions alone, CDRs alone and whole Vk chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain and the most similar human germline sequence. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable light chain obtained by prior art methods and the initial murine monoclonal antibody light chain. Also shown is the most similar human rearranged CDR grafted VL versus the most similar human germline sequence.

FIG. 9H depicts an amino acid sequence (Seq. Id. No. 182) resulting from a BLAST query in Genbank using the entire variable light chain of the initial murine monoclonal antibody (excluding CDRs) depicted in FIG. 9A.

FIG. 10A depicts the amino acid sequence (Seq. Id. No. 183) of a murine antibody variable heavy chain directed to h-DC-SIGN (i.e., the heavy chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 10B depicts the amino acid sequences (Seq. Id. Nos. 184 and 185) of human antibody variable heavy chain sequence gene identification (GI) numbers 18698373 and 392677, separating the sequence into framework and CDR component parts.

FIG. 10C depicts the amino acid sequences (Seq. Id. Nos. 186 and 187) of human antibody variable heavy chain sequence gene identification (GI) numbers 886288 and 999106, separating the sequence into framework and CDR component parts.

FIG. 10D depicts the amino acid sequence (Seq. Id. No. 188) of human antibody variable heavy chain sequence gene identification (GI) number 5542538, separating the sequence into framework and CDR component parts.

FIG. 10E depicts the amino acid sequences (Seq. Id. Nos. 189, 190 and 191) of human antibody variable heavy chain sequence gene identification (GI) numbers 4530559, 5834122 and 106709, separating the sequence into framework and CDR component parts.

5 FIG. 10F depicts the amino acid sequences of a hybrid humanized antibody heavy chain (Seq. Id. Nos. 192 and 193), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody heavy chain of FIG. 10A is provided.

10 FIG. 10G depicts an amino acid sequences (Seq. Id. Nos. 194 and 195) resulting from a BLAST query in Genbank using the entire variable heavy chain of the murine antibody depicted in FIG. 10A.

15 FIG. 10H is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain (see FIG. 10F) and the initial murine monoclonal antibody heavy chain (see FIG. 10A) in terms of framework regions alone, CDRs alone and whole VH chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain and the most similar human germline sequence. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable heavy chain obtained by prior art methods and the initial murine monoclonal antibody heavy chain. Also shown is the degree of homology between the most similar human
20 rearranged CDR grafted VH versus the most similar germline sequence.

FIG. 11 shows the results of competition ELISA experiments involving an antibody in accordance with the present disclosure and comparative antibodies.

25 FIG. 12 shows the results of binding affinity testing on the initial antibody and a hybrid antibody directed to mannan-binding lectin (MBL).

FIG 13.shows the results of binding affinity testing on the initial antibody and hybrid antibodies directed to h-DC-SIGN.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The techniques described herein provide hybrid antibodies or hybrid antibody fragments (collectively referred to herein as "hybrids") which are active against a target object and which reduce the risk of immunogenicity when administered to a target species. The present disclosure provides techniques which maximize homology between framework regions of antibodies or antibody fragments obtained from an originating species and those of a target species. Hybrids that have been constructed by incorporation of highly homologous framework regions from two or more antibodies of a target species and which have been manipulated in accordance with the present disclosure maintain a high degree of affinity to the target object while reducing the risk of an adverse immune response when administered to the target species. In addition, hybrids that have been constructed by incorporation of highly homologous framework regions from one or more antibodies of a target species which correspond to the same family of germline gene sequences and which have been manipulated in accordance with the present disclosure also maintain a high degree of affinity to the target object while reducing the risk of an adverse immune response when administered to the target species. In one embodiment, the target species is human and the engineered antibody is humanized.

Technical and scientific terms used herein have the meanings commonly understood by one of ordinary skill in the art to which the present teachings pertain, unless otherwise defined herein. Reference is made herein to various methodologies known to those of skill in the art. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full. Practice of the methods described herein will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such conventional techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch, and Maniatis, *Molecular Cloning; Laboratory Manual* 2nd ed. (1989); *DNA Cloning, Volumes I and II* (D.N Glover ed. 1985); *Oligonucleotide*

Synthesis (M.J. Gait ed, 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); the series, Methods in Enzymology (Academic Press, Inc.), particularly Vol. 154 and Vol. 155 (Wu and Grossman, eds.); PCR-A Practical Approach (McPherson, Quirke, and Taylor, eds., 1991); Immunology, 2d Edition, 1989, Roitt et al., C.V. Mosby Company, and New York; Advanced Immunology, 2d Edition, 1991, Male et al., Grower Medical Publishing, New York.; DNA Cloning: A Practical Approach, Volumes I and II, 1985 (D.N. Glover ed.); Oligonucleotide Synthesis, 1984, (M.L. Gait ed); Transcription and Translation, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R.I. Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; and Gene Transfer Vectors for Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); WO97/08320; US. Patent Nos. 5,427,908; 5,885,793; 5,969,108; 5,565,332; 5,837,500; 5,223,409; 5,403,484; 5,643,756; 5,723,287; 5,952,474; Knappik et al., 2000, J. Mol. Biol. 296:57-86; Barbas et al., 1991, Proc. Natl. Acad. Sci. USA 88:7978-7982; Schaffitzel et al. 1999, J. Immunol. Meth. 10:119-135; Kitamura, 1998, Int. J. Hematol., 67:351-359; Georgiou et al., 1997, Nat. Biotechnol. 15:29-34; Little, et al., 1995, J. Biotech. 41:187-195; Chauthaiwale et al., 1992, Microbiol. Rev., 56:577-591; Aruffo, 1991, Curr. Opin. Biotechnol. 2:735-741; McCafferty (Editor) et al., 1996, Antibody Engineering: A Practical Approach, the contents of which are incorporated herein by reference.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the methods described herein; however, preferred materials and/or methods are described. Materials, reagents and the like to which reference is made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The hybrid antibodies and hybrid antibody fragments include complete antibody molecules having full length heavy and light chains, or any fragment thereof, such as Fab, Fab', F(ab')₂, Fd, scFv, , antibody light chains and antibody heavy chains. Chimeric antibodies which have variable regions as described herein and constant regions from various species are also suitable.

Initially, a predetermined target object is chosen to which an antibody may be raised. Techniques for generating monoclonal antibodies directed to target objects are well known to those skilled in the art. Examples of such techniques include, but are not limited to, those involving display libraries, xeno or humab mice, hybridomas, etc.

- 5 Target objects include any substance which is capable of exhibiting antigenicity and are usually proteins or protein polysaccharides. Examples include receptors, enzymes, hormones, growth factors, peptides and the like. It should be understood that not only are naturally occurring antibodies suitable for use in accordance with the present disclosure, but engineered antibodies and antibody fragments which are directed to a
10 predetermined object are also suitable.

- Antibodies (Abs) that can be subjected to the techniques set forth herein include monoclonal and polyclonal Abs, and antibody fragments such as Fab, Fab', F(ab')₂, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments derived from phage or phagemid display technologies. To begin with, an initial antibody
15 is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target antigen is needed. The originating species is any species which was used to generate the antibodies or antibody libraries, e.g., rat, mice, rabbit, chicken, monkey, human, etc. Techniques for
20 generating and cloning monoclonal antibodies are well known to those skilled in the art. After a desired antibody is obtained, the variable regions (VH and VL) are separated into component parts (i.e, frameworks (FRs) and CDRs) using any possible definition of CDRs (e.g., Kabat alone, Chothia alone, Kabat and Chothia combined, and any others known to those skilled in the art). Once that has been obtained, the selection of
25 appropriate target species frameworks is necessary. One embodiment involves alignment of each individual framework region from the originating species antibody sequence with variable amino acid sequences or gene sequences from the target species. Programs for searching for alignments are well known in the art, e.g., BLAST and the like. For example, if the target species is human, a source of such amino acid

sequences or gene sequences (germline or rearranged antibody sequences) may be found in any suitable reference database such as Genbank, the NCBI protein databank (<http://ncbi.nlm.nih.gov/BLAST/>), VBASE, a database of human antibody genes (<http://www.mrc-cpe.cam.ac.uk/imt-doc>), and the Kabat database of immunoglobulins (<http://www.immuno.bme.nwu.edu>) or translated products thereof. If the alignments are done based on the nucleotide sequences, then the selected genes should be analyzed to determine which genes of that subset have the closest amino acid homology to the originating species antibody. It is contemplated that amino acid sequences or gene sequences which approach a higher degree homology as compared to other sequences in the database can be utilized and manipulated in accordance with the procedures described herein. Moreover, amino acid sequences or genes which have lesser homology can be utilized when they encode products which, when manipulated and selected in accordance with the procedures described herein, exhibit specificity for the predetermined target antigen. In certain embodiments, an acceptable range of homology is greater than about 50%. It should be understood that target species may be other than human.

In one aspect, after determining the degree of homology of an individual framework region from an originating species, i.e., FR1, FR2, FR3 or FR4, with the most similar matches from two or more different antibodies in the reference database of the target species, a set of homologous sequences is selected which can include, e.g., the top 100 hits. This is done with each individual framework region while looking for matches in the database with the closest homology to the antibody from the originating species. It is contemplated that at least two of the selected sequences may be obtained from different antibodies in the database. For example, FR1 may come from antibody one, FR2 may come from antibody two, FR3 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR4 may come from either antibody one, antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three, with the caveat that at least two FRs are from different antibodies. As another example, FR1 may come from

antibody one, FR3 may come from antibody two, FR2 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR4 may come from either antibody one, antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three, with the caveat
5 that at least two FRs are from different antibodies. As another example, FR1 may come from antibody one, FR4 may come from antibody two, FR2 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR3 may come from either antibody one, antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three,
10 with the caveat that at least two FRs are from different antibodies. After selecting suitable framework region candidates, either or both the heavy and light chains variable regions are produced as further discussed below by grafting the CDRs from the originating species into the hybrid framework regions.

In another aspect, after determining the degree of homology of an individual
15 framework region from an originating species, i.e., FR1, FR2, FR3 or FR4, with the most similar matches of germline or rearranged antibody sequences, a set of homologous sequences is selected which can include, e.g, the top 100 hits. At that point, with respect to FR1, FR2, and FR3, the members of the set are categorized into original germline families, i.e., VH1, VH2, VH3, etc., Vkl, VklI, VklII, etc., and Vλ1, Vλ2, Vλ3, etc.,
20 and further, into family members where possible. See Figs. 1, 2 and 3 for a more complete listing of families and family members. Although not always the case, the most similar sequence matches for each individual framework region will typically come from different antibodies or antibody fragments. In one embodiment, two or more framework regions come from antibodies in the same variable family. In another embodiment, two
25 or more framework regions come from a different antibody from the same family member. In another embodiment, up to three framework regions can be from the same antibody. It is contemplated that even though there may be framework sequences in the database from a different family with a higher degree of homology, the more preferable candidate sequence may actually have lower homology but be from the

same family as the other selected frameworks. Similarly, there may be framework sequences in the database from the same family with high homology, but from different members of the same family; the more preferable candidates may be from the same family member as the other selected frameworks. An optional selection criteria involves
5 checking to see which framework sequences most closely resemble the somatic mutations contained in the originating species antibody. Somatic mutations cause the sequences of antibodies to be different even if they come from the same family member. In certain embodiments it is preferable to make a selection that is closer to the somatic mutations occurring in the originating species sequence.

10 FR4 regions are not matched between families and family members of FR1, FR2 and FR3. Indeed, FR4 is encoded by J segments (See Fig.6) and a choice of suitable FR4 sequences can be determined based on homology between the initial antibody FR4 sequences and the most similar FR4 sequences in a reference database. In one embodiment, the FR4 is chosen based on the degree of maximum homology between
15 the initial antibody and those found in rearranged antibody sequence reference databases. In certain embodiments, 100% homology is preferred between the FR4 from the initial antibody and the FR4 selected from the reference database of the target species. Choices based on the germline sequence databases, while not necessarily completely homologous to the initial antibody may also be appropriate. An optional
20 selection criteria involves checking to see which framework sequences most closely resemble the somatic mutations contained in the originating species antibody. Somatic mutations cause the sequences of antibodies to be different even if they come from the same family member. In certain embodiments it is preferable to make a selection that is closer to the somatic mutations occurring in the originating species sequence.

25 After selecting suitable frame work region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard to either of the above aspects can be

accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (i.e., frameworks based on the target species and CDRs from the originating species) may be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions may also be isolated from the originating species antibodies using suitable restriction enzymes and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of the originating species antibody may be changed by site-directed mutagenesis.

Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

Assembly of a physical antibody or antibody fragment library is preferably accomplished using synthetic oligonucleotides. In one example, oligonucleotides are designed to have overlapping regions so that they could anneal and be filled in by a polymerase, such as with polymerase chain reaction (PCR). Multiple steps of overlap extension are performed in order to generate the VL and VH gene inserts. Those fragments are designed with regions of overlap with human constant domains so that they could be fused by overlap extension to produce full length light chains and Fd heavy chain fragments. The light and heavy Fd chain regions may be linked together by overlap extension to create a single Fab library insert to be cloned into a display vector. Alternative methods for the assembly of the humanized library genes can also be used. For example, the library may be assembled from overlapping oligonucleotides using a Ligase Chain Reaction (LCR) approach. See, e.g., Chalmers and Curnow, *Biotechniques* (2001) 30-2, p249-252.

Various forms of antibody fragments may be generated and cloned into an appropriate vector to create a hybrid antibody library or hybrid antibody fragment library.

For example variable genes can be cloned into a vector that contains, *in-frame*, the remaining portion of the necessary constant domain. Examples of additional fragments that can be cloned include whole light chains, the Fd portion of heavy chains, or fragments that contain both light chain and heavy chain Fd coding sequence.

- 5 Alternatively, the antibody fragments used for humanization may be single chain antibodies (scFv).

Any selection display system may be used in conjunction with a library according to the present disclosure. Selection protocols for isolating desired members of large libraries are known in the art, as typified by phage display techniques. Such systems, in
10 which diverse peptide sequences are displayed on the surface of filamentous bacteriophage (Scott and Smith (1990) *Science*, 249: 386), have proven useful for creating libraries of antibody fragments (and the nucleotide sequences that encode them) for the *in vitro* selection and amplification of specific antibody fragments that bind a target antigen. The nucleotide sequences encoding the VH and VL regions are linked
15 to gene fragments which encode leader signals that direct them to the periplasmic space of *E. coli* and as a result the resultant antibody fragments are displayed on the surface of the bacteriophage, typically as fusions to bacteriophage coat proteins (e.g., pIII or pVIII). Alternatively, antibody fragments are displayed externally on lambda phage or T7 capsids (phagebodies). An advantage of phage-based display systems is
20 that, because they are biological systems, selected library members can be amplified simply by growing the phage containing the selected library member in bacterial cells. Furthermore, since the nucleotide sequence that encode the polypeptide library member is contained on a phage or phagemid vector, sequencing, expression and subsequent genetic manipulation is relatively straightforward. Methods for the
25 construction of bacteriophage antibody display libraries and lambda phage expression libraries are well known in the art (see, e.g., McCafferty *et al.* (1990) *Nature*, 348: 552; Kang *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.*, 88: 4363).

One display approach has been the use of scFv phage-libraries (see, e.g., Huston *et al.*, 1988, Proc. Natl. Acad. Sci U.S.A., 85: 5879-5883; Chaudhary *et al.* (1990) Proc. Natl. Acad. Sci U.S.A., 87: 1066-1070. Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described. Refinements of phage display approaches are also known, for example as described in WO96/06213 and WO92/01047 (Medical Research Council *et al.*) and WO97/08320 (Morphosys), which are incorporated herein by reference. The display of Fab libraries is also known, for instance as described in WO92/01047 (CAT/MRC) and WO91/17271 (Affymax).

Hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintained good binding activity because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. See for example Barbas III, *et al.* (2001) Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the contents of which are incorporated herein by reference. For example, in the case of Fab fragments, the light chain and heavy chain Fd products are under the control of a lac promoter, and each chain has a leader signal fused to it in order to be directed to the periplasmic space of the bacterial host. It is in this space that the antibody fragments will be able to properly assemble. The heavy chain fragments are expressed as a fusion with a phage coat protein domain which allows the assembled antibody fragment to be incorporated into the coat of a newly made phage or phagemid particle. Generation of new phagemid particles requires the addition of helper phage which contain all the necessary phage genes. Once a library of antibody fragments is presented on the phage or phagemid surface, a process termed panning follows. This is a method whereby) the antibodies displayed on the surface of phage or phagemid particles are bound to the desired antigen, ii) non-binders are washed away, iii) bound particles are eluted from the antigen, and iv) eluted particles are exposed to fresh bacterial hosts in order to amplify the enriched pool for an

additional round of selection. Typically three or four rounds of panning are performed prior to screening antibody clones for specific binding. In this way phage/phagemid particles allow the linkage of binding phenotype (antibody) with the genotype (DNA) making the use of antibody display technology very successful. However, other vector formats could be used for this humanization process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

After selection of desired hybrid antibodies and/or hybrid antibody fragments, it is contemplated that they can be produced in large volume by any technique known to those skilled in the art, e.g., prokaryotic or eukaryotic cell expression and the like. For example, hybrid antibodies or fragments may be produced by using conventional techniques to construct an expression vector that encodes an antibody heavy chain in which the CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity (as engineered according to the techniques described herein) are derived from the originating species antibody and the remainder of the antibody is derived from a target species immunoglobulin which may be manipulated as described herein, thereby producing a vector for the expression of a hybrid antibody heavy chain.

Additionally, an expression vector can be constructed that encodes an antibody light chain in which one or more CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity which may be manipulated as provided herein are derived from the originating species antibody, and the remainder of the antibody is derived from a target species immunoglobulin which can be manipulated as provided herein, thereby producing a vector for the expression of hybrid antibody light chain.

The expression vectors may then be transferred to a suitable host cell by conventional techniques to produce a transfected host cell for expression of optimized

engineered antibodies or antibody fragments. The transfected or transformed host cell is then cultured using any suitable technique known to those skilled in the art to produce hybrid antibodies or hybrid antibody fragments.

In certain embodiments, host cells may be cotransfected with two expression
5 vectors, the first vector encoding a heavy chain derived polypeptide and the second
encoding a light chain derived polypeptide. The two vectors may contain different
selectable markers but, with the exception of the heavy and light chain coding
sequences, are preferably identical. This procedure provides for equal expression of
heavy and light chain polypeptides. Alternatively, a single vector may be used which
10 encodes both heavy and light chain polypeptides. The coding sequences for the heavy
and light chains may comprise cDNA or genomic DNA or both.

In certain embodiments, the host cell used to express hybrid antibodies or hybrid
antibody fragments may be either a bacterial cell such as *Escherichia coli*, or preferably
a eukaryotic cell. Preferably a mammalian cell such as a chinese hamster ovary cell or
15 NSO cells, may be used. The choice of expression vector is dependent upon the
choice of host cell, and may be selected so as to have the desired expression and
regulatory characteristics in the selected host cell.

Once produced, the hybrid antibodies or hybrid antibody fragments may be
purified by standard procedures of the art, including cross-flow filtration, ammonium
20 sulphate precipitation, affinity column chromatography (e.g., protein A), gel
electrophoresis and the like.

The hybrid antibodies or hybrid antibody fragments may be used in conjunction
with, or attached to other proteins (or parts thereof) such as human or humanized
monoclonal antibodies. These other proteins may be reactive with other markers
25 (epitopes) characteristic for a disease against which the antibodies are directed or may
have different specificities chosen, for example, to recruit molecules or cells of the
target species, e.g., receptors, target proteins, diseased cells, etc. The hybrid

antibodies or antibody fragments may be administered with such proteins (or parts thereof) as separately administered compositions or as a single composition with the two agents linked by conventional chemical or by molecular biological methods.

Additionally the diagnostic and therapeutic value of the antibodies may be augmented

5 by labeling the antibodies with labels that produce a detectable signal (either in vitro or in vivo) or with a label having a therapeutic property. Some labels, e.g. radionucleotides may produce a detectable signal and have a therapeutic property. Examples of radionuclide labels include ^{125}I , ^{131}I , ^{14}C . Examples of other detectable labels include a fluorescent chromosphere such as green fluorescent protein, fluorescein,

10 phycobiliprotein or tetraethyl rhodamine for fluorescence microscopy, an enzyme which produces a fluorescent or colored product for detection by fluorescence, absorbance, visible color or agglutination, which produces an electron dense product for demonstration by electron microscopy; or an electron dense molecule such as ferritin, peroxidase or gold beads for direct or indirect electron microscopic visualization.

15 Hybrid antibodies or hybrid antibody fragments herein may typically be administered to a patient in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the monoclonal antibodies to the patient, Sterile water, alcohol, fats, waxes, and inert solids may be included in the carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agent) may also be incorporated into the pharmaceutical
20 composition.

The hybrid antibody or hybrid antibody fragment compositions may be administered to a patient in a variety of ways. Preferably, the pharmaceutical compositions may be administered parenterally, e.g., subcutaneously, intramuscularly
25 or intravenously. Thus, compositions for parental administration may include a solution of the antibody, antibody fragment or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g.,

water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody or antibody fragment in these formulations can vary widely, e.g., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 17th Ed., Mack Publishing Company, Easton, Pa (1985), which is incorporated herein by reference.

The following examples are provided by way of illustration and should not be construed or interpreted as limiting any of the subject matter described herein.

EXAMPLE 1

A murine monoclonal antibody directed to human mannose binding lectin (the "initial antibody") was utilized in connection with the techniques described herein. The VH and VL regions were cloned and sequenced, and the individual framework regions designated FR1, FR2, FR3, and FR4 were distinguished from the CDRs using a combined Kabat/Chothia numbering system. See Fig. 4A for the variable light chain sequence of the monoclonal antibody. A BLAST search of the NCBI protein databank was conducted using each individual variable light chain framework region as a query

starting with FR1. Antibody sequence gene identification number 3747016 was selected as having an FR1 with good homology to FR1 of the initial antibody light chain.

See Fig. 4B. 3747016 belongs to human germline family Vk III (see Fig. 1), either member L2 or L16, and its FR1 has 78% homology to FR1 of the initial antibody.

- 5 Antibody sequence gene identification number 5833827 was selected as having an FR2 with good homology (73%) to FR2 of the initial antibody. See Fig. 4C. 5833827 belongs to family Vk III, either members L2 or L16. Antibody sequence gene identification number 722614 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody. See Fig. 4D. 722614 belongs to family Vk III, member L6. Antibody
10 sequence gene identification number 1785870 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody.

- The hybrid humanized variable light chain was constructed by site directed mutagenesis of the initial antibody variable light chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp
15 (Madison, Wisconsin). Fig. 7 depicts the respective nucleic acid and amino acid sequences of the hybrid humanized variable light chain and shows the positions of particular nucleotides and amino acids that were altered as compared to the initial antibody sequences. Framework regions are underlined and altered nucleotides and amino acids are boldface. In summary, according to the Altered Sites II system, cloning
20 and transformation was accomplished by ligating the initial antibody VL with plasmid pALTER-EX2 (which contains the genes for chloramphenicol and tetracycline resistance, the chloramphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured,
25 and resulting plasmids were isolated. The isolated pALTER-EX2-VL plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VL with phosphorylated repair, knockout and

mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable light chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 4F.

Fig. 4G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody light chain (see Fig. 4F) and the light chain of the initial antibody in terms of framework regions alone (81%), CDRs alone (100%) and the whole VL chain (86%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody light chain and the closest human germline family members VkVI (A10/A26) in terms of framework regions alone (70%), CDRs alone (78%) and the Vk chain gene (72%). Also shown is the degree of homology between a humanized light chain constructed by identifying the most similar human rearranged antibody light chain to the initial antibody framework regions and grafting the initial antibody CDRs into this light chain, i.e., human rearranged CDR grafted VL and the initial antibody light chain, is shown in terms of framework regions alone (77%), CDRs alone (100%) and the whole VL chain (83%). Finally, the degree of homology between this human rearranged CDR grafted Vk and the closest germline family member (A14) in terms of framework regions alone (70%), CDRs alone (60%), and the Vk chain gene (67%). As can be seen from the chart, the hybrid antibody light chain exemplified above which was made in accordance with the present disclosure

demonstrates greater homology in both the *framework regions* and the *overall variable heavy chain* as compared to the comparative sequences.

Figs. 4H and 4I show the framework homologies between the most similar antibodies in GenBank while using either the entire initial antibody light chain as a query
5 or the combined framework regions without CDRs.

Fig.5A shows the variable heavy chain sequence of the initial antibody. As above, a BLAST search of the NCBI protein databank was conducted using each *individual variable heavy chain framework region* as a query starting with FR1. Antibody sequence gene identification number 563649 was selected as having an FR1 with good
10 homology (91%) to FR1 of the initial antibody heavy chain. See Fig. 5B. 563649 belongs to human germline family VH4, member 31 (see Fig. 2). Antibody sequence gene identification number 951263 was selected as having an FR2 with good homology (78.5%) to FR2 of the initial antibody heavy chain. See Fig. 5C. 951263 belongs to human germline family VH4, member 31. Antibody sequence gene identification
15 number 484852 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody heavy chain. See Fig. 5D. 484852 belongs to human germline family VH4, members 4 or 31. Antibody sequence gene identification number 2367531 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody heavy chain. See Fig. 5E. 2367531 belongs to VH3, member 23.

20 The hybrid humanized variable heavy chain was constructed by site directed mutagenesis of the initial antibody variable heavy chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 7 depicts the respective nucleic acid and amino acid sequences of the hybrid humanized variable heavy chain and shows the positions of
25 particular nucleotides and amino acids that were altered as compared to the initial antibody sequences. *Framework regions are underlined and altered nucleotides and amino acids are boldface.* In summary, according to the Altered Sites II system, cloning

and transformation was accomplished by ligating the initial antibody VH with plasmid pALTER-EX2 (which contains the genes for chloroamphenicol and tetracycline resistance, the chloroamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VH plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VH with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable heavy chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 5F.

Fig. 5G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody heavy chain (see Fig. 5F) and the heavy chain of the initial antibody in terms of framework regions alone (86.4%), CDRs alone (100%) and the whole VH chain (90%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody and the closest human germline family member VH4-31 in terms of framework regions alone (92.8%), CDRs alone (70%) and the VH chain (86.6%). Also shown is the degree of homology between the initial antibody and a humanized chain constructed by identifying the most similar human

rearranged antibody heavy chain to the initial antibody framework regions and grafting the initial antibody CDRs into this heavy chain, i.e., human rearranged CDR grafted VH, is shown in terms of framework regions alone (80%), CDRs alone (100%) and the whole VH chain (86%). Finally, the degree of homology between this human rearranged CDR grafted VH and the closest germline family member (VH4-31) in terms of framework regions alone (97%), CDRs alone (70%), and the whole VH chain gene (89.6%). As can be seen from the chart, the hybrid antibody exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Figs. 5H and 5I show the framework homologies between the most similar antibodies in GenBank while using either the entire initial antibody light chain as a query or the combined framework regions without CDRs.

Binding affinity, association rate constant and dissociation rate constant are determined for the initial antibody and the hybrid antibody, (h3F8) prepared in accordance with this disclosure using a BIAcore 3000 system (Biacore Inc., Piscataway, N.J.) using mannan-binding lectin (MBL) as the antigen and following the manufacturer's instruction. The results are shown in Figure 12. Two tests using the same hybrid antibody and the average thereof are shown.

EXAMPLE 2

A murine monoclonal antibody directed to h-DC-SIGN (the "initial antibody") was utilized in connection with the techniques described herein. The VH and VL regions were cloned and sequenced, and the individual framework regions designated FR1, FR2, FR3, and FR4 were distinguished from the CDRs using a combined Kabat/Chothia numbering system. See Fig. 9A for the variable light chain sequence of the monoclonal

antibody. A BLAST search of the NCBI protein databank was conducted using each individual variable light chain framework region as a query starting with FR1.

FR1

Antibody sequence gene identification number 441333 was selected as having
5 an FR1 with good homology to FR1 of the initial antibody light chain. See Fig. 9B.
441333 belongs to human germline family Vk II (see Fig. 1), member A17 and its FR1
has 82% homology to FR1 of the initial antibody. Antibody sequence gene identification
number 5578780 was selected as a second antibody having an FR1 with good
homology to FR1 of the initial antibody light chain. See Fig. 9B. 5578780 belongs to
10 human germline family Vk II (see Fig. 1), member A3 or A9, and its FR1 has 78%
homology to FR1 of the initial antibody.

FR2

Antibody sequence gene identification number 4324018 was selected as having
an FR2 with good homology (86%) to FR2 of the initial antibody. See Fig. 9C. 4324018
15 belongs to family Vk II, member A3. Antibody sequence gene identification number
18041766 was selected as a second antibody having an FR2 with good homology to
FR2 of the initial antibody light chain. See Fig. 9B. 18041766 belongs to human
germline family Vk II (see Fig. 1), member A3 and its FR1 has 86% homology to FR1 of
the initial antibody.

20 FR3

Antibody sequence gene identification numbers 553476 and 33251 was selected
as having an FR3 with good homology (93%) to FR3 of the initial antibody. See Fig.
9D. 722614 belongs to family Vk II, member A3.

FR4

Antibody sequence gene identification number 446245 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody. See Figure 9E.

The hybrid humanized variable light chain was constructed by site directed
5 mutagenesis of the initial antibody variable light chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 9F depicts the amino acid sequences of hybrid humanized
variable light chains and shows the positions of particular amino acids that were altered as compared to the initial antibody sequences. Framework regions are boldface and
10 altered amino acids are underlined. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VL with plasmid pALTER-EX2 (which contains the genes for chloramphenicol and tetracycline resistance, the chloramphenicol gene containing a frameshift mutation which
can be restored using the chloramphenicol repair oligonucleotide to provide selection of
15 mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VL plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved
mixing the alkaline-denatured pALTER-EX2-VL with phosphorylated repair, knockout
and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially
20 available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis
buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming
ES1301 mutS competent cells (commercially available from Promega Corp.) with the
25 products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells
(commercially available from Promega Corp.). Purified plasmids from the resulting

JM109 cells were screened by sequencing analysis. The resulting variable light chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 9F.

Fig. 9G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody light chain (see Fig. 9F) and the light chain of the initial antibody in terms of framework regions alone (90%), CDRs alone (100%) and the whole VL chain (93%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody light chain and the closest human germline family members VkII (A17) in terms of framework regions alone (93%), CDRs alone (70%) and the Vk chain gene (87%). Also shown is the degree of homology between a humanized light chain constructed by identifying the most similar human rearranged antibody light chain to the initial antibody framework regions and grafting the initial antibody CDRs into this light chain, i.e., human rearranged CDR grafted VL and the initial antibody light chain, is shown in terms of framework regions alone (85%), CDRs alone (100%) and the whole VL chain (89%). The degree of homology between this human rearranged CDR grafted Vk and the closest germline family member VkII (A17) in terms of framework regions alone (88%), CDRs alone (70%), and the Vk chain gene (84%). Finally, the degree of homology between the hybrid antibody including frameworks with the highest homology germline sequences to the initial antibody in terms of frameworks alone (89%), CDRs alone (100%) and whole VL chain (92%) is shown. As can be seen from the chart, the hybrid antibody light chain exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Fig. 9H shows the framework homologies between the most similar antibodies in GenBank while using the combined framework regions without CDRs as a query.

Fig. 10A shows the variable heavy chain sequence of the initial antibody. As above, a BLAST search of the NCBI protein databank was conducted using each individual variable heavy chain framework region as a query starting with FR1.

FR1

Antibody sequence gene identification number 18698373 was selected as having an FR1 with good homology (80%) to FR1 of the initial antibody heavy chain. See Fig. 10B. 18698373 belongs to human germline family VH7, member 81 (see Fig. 2).

Antibody sequence gene identification number 392677 was selected as a second antibody having an FR1 with good homology to FR1 of the initial antibody heavy chain.

See Fig. 9B. 392677 belongs to human germline family VH1, member 2 (see Fig. 2), and its FR1 has 76% homology to FR1 of the initial antibody.

FR2

Antibody sequence gene identification number 886288 was selected as having an FR2 with good homology (100%) to FR2 of the initial antibody heavy chain. See Fig.

10C. 886288 belongs to human germline family VH1, member 2. Antibody sequence gene identification number 999106 was selected as a second antibody having an FR2 with good homology to FR2 of the initial antibody heavy chain. See Fig. 10B. 999106 belongs to human germline family VH1, member 46 (see Fig. 2), and its FR2 has 100% homology to FR2 of the initial antibody.

FR3

Antibody sequence gene identification number 5542538 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody heavy chain. See Fig. 10D. 5542538 belongs to human germline family VH1, member 2.

FR4

Antibody sequence gene identification number 4530559 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody heavy chain. See Fig. 10E. 4530559 belongs to VH1, member 2.

The hybrid humanized variable heavy chain was constructed by site directed mutagenesis of the initial antibody variable heavy chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 10F depicts the amino acid sequences of the hybrid

5 humanized variable heavy chains and shows the positions of particular nucleotides and amino acids that were altered as compared to the initial antibody sequences.

Framework regions are boldface and altered amino acids are underlined. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VH with plasmid pALTER-EX2 (which contains the genes

10 for chloroamphenicol and tetracycline resistance, the chloroamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair

oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The

isolated pALTER-EX2-VH plasmids were denatured using NaOH (alkaline). Annealing

15 and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VH with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was

heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was

20 incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified

25 plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable heavy chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 10F.

Fig. 10H is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody heavy chain (see Fig. 10F) and the heavy chain of the initial antibody in terms of framework regions alone (87%), CDRs alone (100%) and the whole VH chain (91%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody and the closest human germline family member VH4-31 in terms of framework regions alone (72%), CDRs alone (44%) and the VH chain (64%). Also shown is the degree of homology between the initial antibody and a humanized chain constructed by identifying the most similar human rearranged antibody heavy chain to the initial antibody framework regions and grafting the initial antibody CDRs into this heavy chain, i.e., human rearranged CDR grafted VH, is shown in terms of framework regions alone (80%), CDRs alone (100%) and the whole VH chain (87%). The degree of homology between this human rearranged CDR grafted VH and the closest germline family member (VH1-46) in terms of framework regions alone (69%), CDRs alone (44%), and the whole VH chain gene (62%) is also shown. Finally, the degree of homology between the hybrid antibody having frameworks with highest homology human germline sequences versus the initial antibody in terms of frameworks alone (73%), CDRs alone (100%) and whole VH chain (81.5%) is shown. As can be seen from the chart, the hybrid antibody exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Fig. 10G shows the framework homologies between the most similar antibodies in GenBank while using the combined framework regions without CDRs as a query.

25 Competition ELISA

ELISA plates were coated with 2ug/ml Goat anti-human IgG in Carbonate coating buffer, washed twice with wash buffer. After blocking with blocking buffer at 37°C, the

wells washed twice with wash buffer and then incubated with 0.25ug/ml hDC-SIGN (in blocking buffer) for 1 hr at 37°C, washed 4 times with wash buffer.

For competition assay, either 4ug/ml or 1ug/ml of biotin conjugated AZN-D1 was mixed with different concentrations of AZN-D1 or a hybrid antibody in accordance with the present disclosure (hD1V1) or 5G1.1 antibody (an antibody described in U.S. Patent No. 6,355,245, the disclosure of which is incorporated herein by this reference) in blocking buffer and incubated for 2hrs at RT (room temperature), the wells were then washed 6 times with wash buffer, incubated with 1:1000 SA-HRP (Streptavidin -Horseradish peroxidase) in blocking buffer for 45min at RT. After washing 8 times with wash buffer, the wells were developed by OPD (o-Phenylenediamine) in 0.1M citrate-phosphate buffer, PH5.0 containing 0.03%hydrogen peroxide and read at 492nm.

Anti-hDC-SIGN ELISA REAGENTS

Carbonate coating buffer, pH 9.6

Na₂CO₃ 1.6 g +NaHCO₃ 2.9 g

Add 800 mL H₂O, pH to 9.6 then make to 1 L with H₂O

Blocking buffer

BSA 1 g +PBS 100 mL

Add BSA to PBS and allow to dissolve fully before using. Store at 4.degree. C.

Wash buffer

(0.05% Tween/PBS):Tween 20 0.5 g +PBS 1 L

Add Tween to PBS and mix thoroughly before use

Citrate buffer

Citric Acid. 2.1 g in 50 mL

Sodium Citrate (Dihydrate) 1.47 g in 50 mL

Add solutions together and adjust pH to 4.0-4.2

All incubations can be carried out at 4° C. overnight or at room temperature for 2 hrs OR at 37° C. for 1 hr.

The results of the competition ELISA experiments are shown in Figure 11.

Binding affinity, association rate constant and dissociation rate constant are determined for the initial antibody and two hybrid antibodies (D1V1 and D1V2) prepared in accordance with their disclosure using h-Dc-SIGN as the antigen and following the manufacturer's instruction. The results are shown in Figure 13.

5 It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended herein.

What is claimed is:

1. A method for producing a hybrid antibody or hybrid antibody fragment comprising:

5 providing an initial antibody having specificity for a target;
determining the sequence of a variable region of the initial antibody;
comparing the sequence of the variable region FR4 to sequences contained in a reference database of germline antibody sequences or antibody fragment sequences from a target species;

10 selecting a sequence from an antibody in the database which demonstrates a high degree of homology to the variable region FR4; and
operatively linking the selected framework sequences to the CDR3 region of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

15 2. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 further comprising

selecting a second component of the variable region, the second component being selected from the group consisting of FR1, FR2 and FR3;

20 comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

25 selecting a sequence from the database which demonstrates a high degree of homology to the second component and which is from an antibody which is the same or different than the antibody in the reference database used for selection based on the FR4 region; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

3. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 2 further comprising

selecting a third component of the variable region which is different than the second component, the third component selected from the group consisting of FR1,
5 FR2 and FR3;

comparing the sequence of the third component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

10 selecting a sequence from the database which demonstrates a high degree of homology to the third component and which is from an antibody which is the same or different than the antibodies in the reference database used for selection based on the FR4 and second component; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

15

4. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 3 further comprising selecting a fourth component of the variable region which is different than the second and third components, the fourth component selected from the group consisting of FR1, FR2 and FR3;

20 comparing the sequence of the fourth component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

25 selecting a sequence from the database which demonstrates a high degree of homology to the fourth component and which is from an antibody which is the same or different than the antibodies in the reference database used for selection based on the FR4, second component and third component; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

5. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 2 wherein the second component is a combination of members selected from the group consisting of FR1, FR2 and FR3.

5 6. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the second component is a combination of two or three members of the group consisting of FR1, FR2, FR3, or FR4.

10 7. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the sequences are amino acid sequences or nucleic acid sequences.

15 8. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the antibody fragment is selected from the group consisting of scFv, Fab, Fab', F(ab')₂, Fd, diabodies, antibody light chains and antibody heavy chains.

20 9. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the target species is human.

10. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the step of selecting a sequence from an antibody in the database which demonstrates a high degree of homology to the variable region FR4 comprises selecting a sequence from those which have the highest homology of human JH, Jk, or Jλ family members.

11. A hybrid antibody or hybrid antibody fragment produced in accordance with the method of any of claims 1 through 10.

12. A hybrid antibody or hybrid antibody fragment comprising a first heavy chain framework 4 region from a first antibody, the first antibody being a germline antibody,
5 and a second heavy chain framework region from a second antibody, the second antibody being different from the first antibody.

13. A hybrid antibody or hybrid antibody fragment according to claim 12 further comprising a third heavy chain framework region from an antibody selected from the
10 group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody.

14. A hybrid antibody or hybrid antibody fragment according to claim 12 further comprising a fourth heavy chain framework region from an antibody selected from the
15 group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, the second nor the third antibody.

15. A hybrid antibody or hybrid antibody fragment according to claim 12 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.
20

16. A hybrid antibody or hybrid antibody fragment according to claim 12 wherein the first antibody is chosen from human JH, JK, or Jλ family members.

17. A library of antibodies or antibody fragments comprising hybrid antibodies or
25 hybrid antibody fragments according to any of claims 12 through 16.

18. A hybrid antibody or hybrid antibody fragment comprising one or more variable heavy chain framework sequences from an antibody sequence selected from

the group consisting of germline A1, germline O1, germline VH1-18 and germline VH1-69.

19. A hybrid antibody or hybrid antibody fragment according to claim 18 comprising variable heavy chain framework sequences from two different antibody sequences selected from the group consisting of germline A1, germline O1, germline VH1-18 and germline VH1-69.

20. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to any of claims 18 or 19.

SER. ID NO.

[illegible]

Fig. 1

VH Exon - Amino acid sequence alignment

		H1			H2			SEQ ID NO.			
		FR1			CDR1	FR2		CDR2	FR3		
H1-H2	Locus	1	2	3		4	5	6	7	8	9
		123456789012345678901234567890		1ab2345		67890123456789		012abc3456789012345		678901234567890123456789012345	
VH1	1-3	1-02	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	G--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	41			
	1-3	1-03	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YAMH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	42			
	1-3	1-08	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YDIN	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	43			
	1-2	1-18	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YGIS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	44			
	1-U	1-24	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	E--LSHH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	45			
	1-3	1-45	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	Y--LYLH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	46			
	1-3	1-46	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	47			
	1-3	1-58	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--SAVQ	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	48			
	1-2	1-69	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YALG	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	49			
	1-2	1-e	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YALG	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	50			
	1-2	1-e	QVQLVQSGAEVKKPGATVKISCKVSGTFT	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	51			
VH2	3-1/2-1	2-05	QITLKESGFTLVKPTLTLLCTFSGFSL	TSYGVG	NVRQAPGQGLEWMG	LIIY--WDDKRYSPSLKS	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	52			
	3-1	2-26	QVTLKESGFTLVKPTLTLLCTFSGFSL	MARMGV	NVRQAPGQGLEWMG	LIIY--WDDKRYSPSLKS	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	53			
	3-1	2-70	QVTLKESGFTLVKPTLTLLCTFSGFSL	TSYGVG	NVRQAPGQGLEWMG	LIIY--WDDKRYSPSLKS	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	54			
VH3	1-3	3-07	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	55			
	1-3	3-09	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YAMH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	56			
	1-3	3-11	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	57			
	1-1	3-13	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	58			
	1-U	3-15	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	N--ANNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	59			
	1-3	3-20	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	60			
	1-3	3-21	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	61			
	1-3	3-23	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	62			
	1-3	3-30	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	63			
	1-3	3-30.3	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	64			
	1-3	3-30.5	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	65			
	1-3	3-33	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	66			
	1-3	3-43	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	67			
	1-3	3-48	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	68			
	1-U	3-49	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	69			
	1-1	3-53	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	70			
	1-3	3-64	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	71			
	1-1	3-66	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	72			
	1-4	3-72	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	73			
	1-4	3-73	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	G--SANN	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	74			
	1-3	3-74	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	75			
	1-6	3-d	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	76			
VH4	2-1/1-1	4-04	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	77			
	2-1	4-28	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	78			
	3-1	4-30.1	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	79			
	3-1	4-30.2	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	80			
	3-1	4-30.4	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	81			
	3-1	4-31	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	82			
	3-1	4-34	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	83			
	3-1	4-39	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	84			
	3-1	4-59	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	85			
	3-1	4-61	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	86			
	2-1	4-b	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SS--NNMS	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	87			
VH5	1-2	5-51	EVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	88			
	1-2	5-a	EVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	89			
VH6	3-5	6-01	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	SNSAANN	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	90			
VH7	1-2	7-4.1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YNNH	NVRQAPGQGLEWMG	NINP--NSGGTNYAQKFGQ	RVTITRDTSTSTAYNELSLRAEDTAVYYCAR	91			

Fig. 2

V_h Exon - Amino acid sequence alignment

			FR1	CDR1	FR2	CDR2	FR3	CDR3	SEQ ID NO.
			1	3	4	5	6	7	9
			12345678910111213141516171819202122232425262728293031323334353637383940414243444546474849505152535455565758596061626364656667686970717273747576777879808182838485868788899091929394959697989910010110210310410510610710810911011111211311411511611711811912012112212312412512612712812913013113213313413513613713813914014114214314414514614714814915015115215315415515615715815916016116216316416516616716816917017117217317417517617717817918018118218318418518618718818919019119219319419519619719819920020120220320420520620720820921021121221321421521621721821922022122222322422522622722822923023123223323423523623723823924024124224324424524624724824925025125225325425525625725825926026126226326426526626726826927027127227327427527627727827928028128228328428528628728828929029129229329429529629729829930030130230330430530630730830931031131231331431531631731831932032132232332432532632732832933033133233333433533633733833934034134234334434534634734834935035135235335435535635735835936036136236336436536636736836937037137237337437537637737837938038138238338438538638738838939039139239339439539639739839940040140240340440540640740840941041141241341441541641741841942042142242342442542642742842943043143243343443543643743843944044144244344444544644744844945045145245345445545645745845946046146246346446546646746846947047147247347447547647747847948048148248348448548648748848949049149249349449549649749849950050150250350450550650750850951051151251351451551651751851952052152252352452552652752852953053153253353453553653753853954054154254354454554654754854955055155255355455555655755855956056156256356456556656756856957057157257357457557657757857958058158258358458558658758858959059159259359459559659759859960060160260360460560660760860961061161261361461561661761861962062162262362462562662762862963063163263363463563663763863964064164264364464564664764864965065165265365465565665765865966066166266366466566666766866967067167267367467567667767867968068168268368468568668768868969069169269369469569669769869970070170270370470570670770870971071171271371471571671771871972072172272372472572672772872973073173273373473573673773873974074174274374474574674774874975075175275375475575675775875976076176276376476576676776876977077177277377477577677777877978078178278378478578678778878979079179279379479579679779879980080180280380480580680780880981081181281381481581681781881982082182282382482582682782882983083183283383483583683783883984084184284384484584684784884985085185285385485585685785885986086186286386486586686786886987087187287387487587687787887988088188288388488588688788888989089189289389489589689789889990090190290390490590690790890991091191291391491591691791891992092192292392492592692792892993093193293393493593693793893994094194294394494594694794894995095195295395495595695795895996096196296396496596696796896997097197297397497597697797897998098198298398498598698798898999099199299399499599699799899910001001100210031004100510061007100810091010101110121013101410151016101710181019102010211022102310241025102610271028102910301031103210331034103510361037103810391040104110421043104410451046104710481049105010511052105310541055105610571058105910601061106210631064106510661067106810691070107110721073107410751076107710781079108010811082108310841085108610871088108910901091109210931094109510961097109810991100110111021103110411051106110711081109111011111121131141151161171181191201211221231241251261271281291301311321331341351361371381391401411421431441451461471481491501511521531541551561571581591601611621631641651661671681691701711721731741751761771781791801811821831841851861871881891901911921931941951961971981992002012022032042052062072082092102112122132142152162172182192202212222232242252262272282292302312322332342352362372382392402412422432442452462472482492502512522532542552562572582592602612622632642652662672682692702712722732742752762772782792802812822832842852862872882892902912922932942952962972982993003013023033043053063073083093103113123133143153163173183193203213223233243253263273283293303313323333343353363373383393403413423433443453463473483493503513523533543553563573583593603613623633643653663673683693703713723733743753763773783793803813823833843853863873883893903913923933943953963973983994004014024034044054064074084094104114124134144154164174184194204214224234244254264274284294304314324334344354364374384394404414424434444454464474484494504514524534544554564574584594604614624634644654664674684694704714724734744754764774784794804814824834844854864874884894904914924934944954964974984995005015025035045055065075085095105115125135145155165175185195205215225235245255265275285295305315325335345355365375385395405415425435445455465475485495505515525535545555565575585595605615625635645655665675685695705715725735745755765775785795805815825835845855865875885895905915925935945955965975985996006016026036046056066076086096106116126136146156166176186196206216226236246256266276286296306316326336346356366376386396406416426436446456466476486496506516526536546556566576586596606616626636646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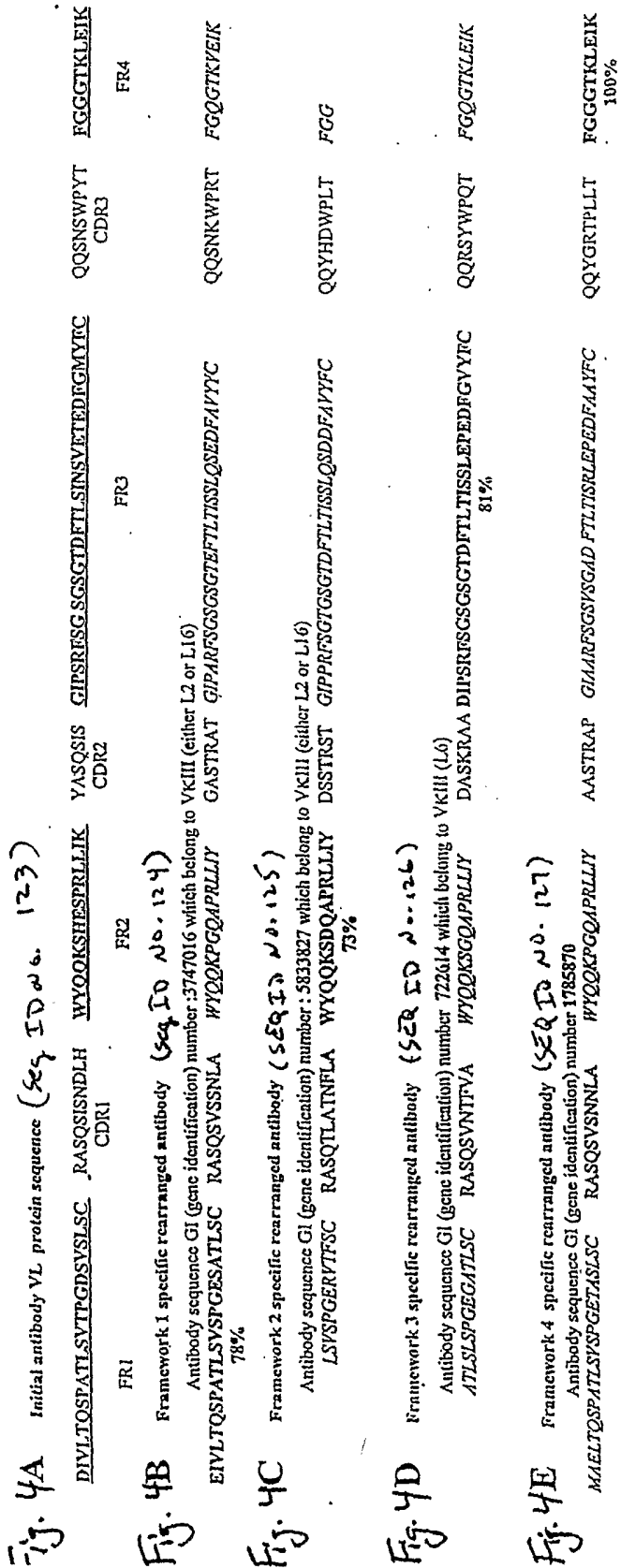


Fig. 4F Hybrid antibody VL sequence (Seq ID No. 128)
 EIVLTQSPATLSVPGESATLSC RASQSIINDLH WYQQKSDQAPRLLIY 78%
 YASQSI DIFSRFSGSGGTDTFTLTSSLEPEDFQVYFC 81%
 QQTNWPTT FGGGTKLEIK 100%

Fig. 4G Sequence homologies of initial, hybrid and germline VL sequences

	Antibody comparisons	Frameworks	CDRs	Whole VL
VL	Hybrid antibody versus initial antibody sequence	(65/80) 81%	(27/27) 100%	(92/107) 86%
VL	Hybrid antibody versus the most similar human germline sequences VκVI (A10/A26)	(49/70) 70%*	(18/25) 72%*	(67/95) 71%*
VL	The most similar human rearranged CDR grafted VL versus initial antibody sequence	(62/80) 77%	(27/27) 100%	(89/107) 83%
VL	The most similar human rearranged CDR grafted VL versus the most similar human germline sequence VκVI (A14)	(49/70) 70%*	(15/25) 60%*	(64/95) 67%*

*Does not include J region sequence

Fig. 4H Search with complete VL of initial antibody (Seq ID No. 129)
 Antibody sequence GI (gene identification) number 418844 which belong to VκVI (A14)
 DYLLTQSPAILSPGERSFSC RASQSIGTSIH WYQQTNGPPRLLIK 67%
 YASQSI GIPSRFSGSGGTDTFTLTSSVESEDIDYYC 81%
 QQTNWPTT FGGGTKLEIK 100%

Fig. 4I Search with VL combined framework(excluding CDRs) of initial antibody (Seq ID No. 130)
 Antibody sequence GI (gene identification) number 418844 which belong to VκVI (A14)
 DYLLTQSPAILSPGERSFSC RASQSIGTSIH WYQQTNGPPRLLIK 67%
 YASQSI GIPSRFSGSGGTDTFTLTSSVESEDIDYYC 81%
 QQTNWPTT FGGGTKLEIK 100%

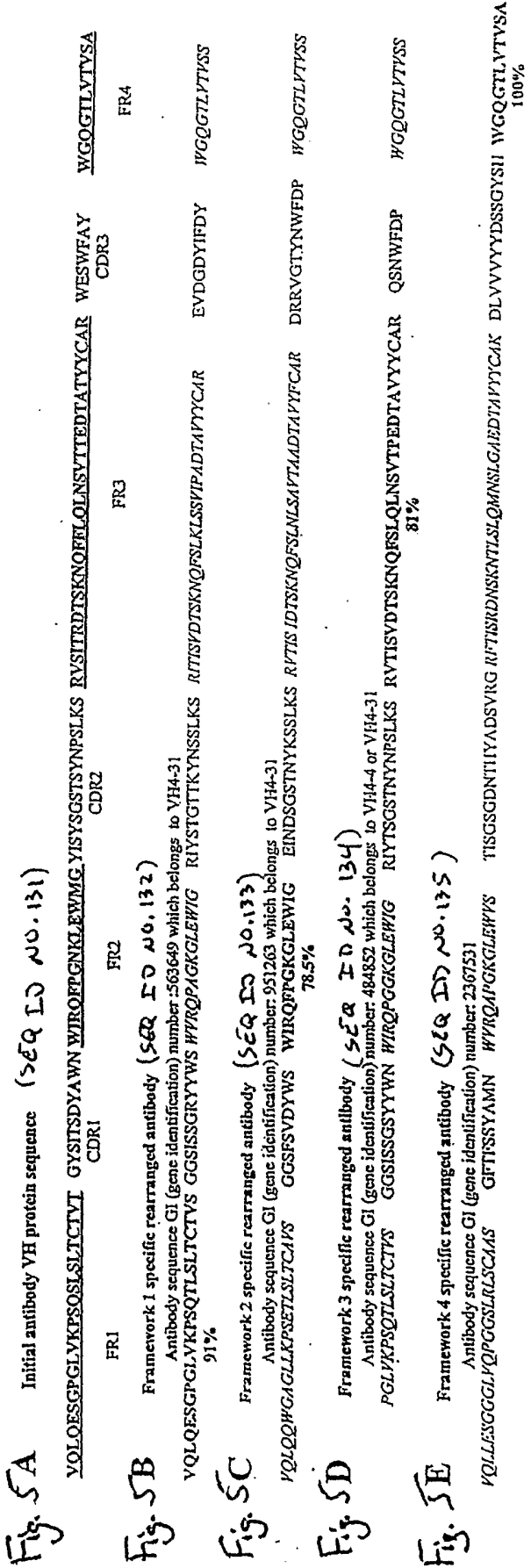


Fig. 5F Hybrid antibody VH sequence (SEQ ID No. 134)
VQLQESGPGLVKPSQTLSTCTVS GYTSIDYAWN WIRQHPGKGLEWIG YISYSGTSYNPSLKS RVTISVDTSKNQPSLQLSNVTPTEDTAVYYCAR WESWEAY WGQGTLPTVSSA
91% 78.5% 81% 100%

Fig. 5G Sequence homologues of initial, hybrid and germline VH sequences

	Antibody comparisons	Frameworks	CDRs	Whole VH
VH	Hybrid antibody versus initial antibody	(70/81) 86.4%	(34/34) 100%	(104/115) 90%
VH	Hybrid antibody versus the most similar human germline sequence (VH4-31)	(65/70) 92.8%*	(19/27) 70%*	(84/97) 86.6%*
VH	The most similar human rearranged CDR grafted VH versus initial antibody	(65/81) 80%	(34/34) 100%	(99/115) 86%
VH	The most similar human rearranged CDR grafted VH versus the most similar human germline sequence (VH4-31)	(68/70) 97%*	(19/27) 70%*	(87/97) 89.6%*

*Does not include D or J region sequence

Fig. 5H Search with complete VH of initial antibody (SEQ ID No. 137)
Antibody sequence name: 4995411
VQLQESGPGLVKPSQTLSTCTVS GGSISGGYYWN WIRQHPGKGLEWIG YIYSGSTYYNPSLKS RVTISVDTSKNQPSLKLSSYTAADTAIYYCAR GLKWGSNHYFDY WGQGTLPTVSS
91% 71% 69% 91%

Fig. 5I Search with VH combined framework(excluding CDRs) of initial antibody (SEQ ID No. 138)
Antibody sequence name: 1791179
VQLQESGPGLVKPSQTLSTCTVS GGSISGGYYWS WIRQHPGKGLEWIG YIYASSTYYKQSLKS RVTISVDTSKNQPSLKLSSYTAADTAIYYCAR GCSEYYFDH WGQGTLPTVSS
91% 71% 72% 91%

JH - Amino acid sequence alignment

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      H3
      -----
      CDR3
      -----
           100           110
           |             |
JH1  ---AEYFQHWGQGTLVTVSS (SEQ ID NO. 137)
JH2  ---YWYFDLWGRGTLVTVSS (SEQ ID NO. 140)
JH3  -----AFDHWGQGTMTVTVSS (SEQ ID NO. 141)
JH4  -----YFDYWGQGTTLVTVSS (SEQ ID NO. 142)
JH5  ---NWFDPWGQGTTLVTVSS (SEQ ID NO. 143)
JH6  YYYYYGMDVWGQGTTVTVSS (SEQ ID NO. 144)

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JK - Amino acid sequence alignment

```

      L3
      -
      CDR3
      --
           100
           |
JK1  WTFGQGTKVEIK (SEQ ID NO. 145)
JK2  YTFGQGTKLEIK (SEQ ID NO. 146)
JK3  FTFGPGTKVDIK (SEQ ID NO. 147)
JK4  LTFGGGTKVEIK (SEQ ID NO. 148)
JK5  ITFGQGTRLEIK (SEQ ID NO. 149)

```

Jλ - Amino acid sequence alignment

```

      CDR3
      --
           100
           |
Jλ1  YVFGTGTKVTVL (SEQ ID NO. 150)
Jλ2  VVFGGGTKLTVL (SEQ ID NO. 151)
Jλ3  YVFGGGTKLTVL (SEQ ID NO. 152)
Jλ7  AVFGGGTQLTVL (SEQ ID NO. 153)

```

Fig. 6

Hybrid antibody variable light chain (VL) and variable heavy chain(VH)
(Frameworks are underlined, changed amino acid and nucleotides are in bold)

VL

GAA ATT GTG CTA ACT CAG TCT CCA GCC ACC CTG TCT GTG AGT CCA GGA GAT AGC GCC
E I V L T O S P A T L S V S P G E S A
 ACT CTT TCC TGC AGG GCC AGC CAA AGT ATT AGC AAC GAC CTA CAC TGG TAT CAA CAA
T L S C R A S Q S I S N D L H W Y Q Q
 AAA TCA GAT CAG GCT CCA AGG CTT CTC ATC TAC TAT GCT TCC CAG TCC ATC TCT GAT
K S D O A P R L L I Y Y A S Q S I S D
 ATC CCC TCC CGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACT CTC ACT ATC AGC
I P S R F S G S G S G T D F T L T I S
 AGT CTG GAG CCT GAA GAT TTT GGA GTG TAT TTC TGT CAA CAG AGT AAC AGC TGG CCG
S L E P E D F G V Y F C Q Q S N S W P
 TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA (SEQ ID. NO. 154)
Y T F G G G T K L E I K (SEQ ID No. 155)

VH

GAT GTG CAG CTT CAG GAG TCG GGA CCT GGC CTG GTG AAA CCT TCT CAG ACT CTG TCC
D V O L O E S G P G L V K P S O T L S
 CTC ACC TGC ACT GTC TCT GGC TAC TCA ATC ACC AGT GAT TAT GCC TGG AAC TGG ATC
L T C T V S G Y S I T S D Y A W N W I
 CGG CAG TTT CCA GGA AAA GGA CTG GAG TGG ATT GGC TAC ATA AGC TAC AGT GGT AGC
R O F P G K G L E W I G Y I S Y S G S
 ACT AGC TAC AAC CCA TCT CTC AAA AGT CGA GTC ACT ATC TCT GTA GAC ACA TCC AAG
T S Y N P S L K S R V T I S V D T S K
 AAC CAG TTC TCC CTG CAG TTG AAT TCT GTG ACT CCT GAG GAC ACA GCC GTA TAT TAC
N O F S L O L N S V T P E D T A V Y Y
 TGT GCA AGA TGG GAG TCC TGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT GTC
C A R W E S W F A Y W G O G T L V T V
 TCT GCA (SEQ ID NO. 156)
S A (SEQ ID NO 157)

Fig. 7

VL

Oligo1 (SEQ ID NO. 158)

5'GATATACCC ATGG GAA ATT GTG CTA ACT CAG ~~ACT~~

Oligo2 (SEQ ID NO. 159)

5'GCC ACC CTG TCT GTG AGT CCA GGA GAT AGC GCC ACT CTT TCC TGC AGG

Oligo3 (SEQ ID NO. 160)

5'TAT CAA CAA AAA TCA GAT CAG GCT CCA AGG CTT CTC ATC

Oligo4 (SEQ ID NO. 161)

5'AGG CTT CTC ATC TAC TAT GCT TCC CAG TCC ATC

Oligo5 (SEQ ID NO. 162)

5'CAG TCC ATC TCT GAT ATC CCC TCC CGG

Oligo6 (SEQ ID NO. 163)

5'ACA GAT TTC ACT CTC ACT ATC AGC AGT CTG GAG CCT GAA GAT TTT

Oligo7 (SEQ ID NO. 164)

5'GAA GAT TTT GGA GTG TAT TTC TGT CAA CAG

VH

Oligo8 (SEQ ID NO. 165)

5'GGC CTG GTG AAA CCT TCT CAG ACT CTG TCC CTC ACC

Oligo9 (SEQ ID NO. 166)

5'CTC ACC TGC ACT GTC TCT GGC TAC TCA ATC ACC

Oligo10 (SEQ ID NO. 167)

5'CAG TTT CCA GGA AAA GGA CTG GAG TGG ATT GGC TAC ATA AGC

Oligo11 (SEQ ID NO. 168)

5'CCA TCT CTC AAA AGT CGA GTC ACT ATC TCT GTA GAC ACA TCC AAG

Oligo12 (SEQ ID NO. 169)

5'TCC AAG AAC CAG TTC TCC CTG CAG TTG AAT TCT

Oligo13 (SEQ ID NO. 170)

5'TTG AAT TCT GTG ACT CCT GAG GAC ACA GCC

Oligo14 (SEQ ID NO. 171)

5'GAG GAC ACA GCC GTA TAT TAC TGT GCA

Fig. 8

D1 light Chain

Fig. 9A Initial antibody VL protein sequence (SEQ ID NO. 172)

*L1,L2,L3: loop regions structural criteria defined by Chothia
CDRs : CDRs are according to Kabat

	l a b c d e f									
			30(V)	32(Y)						
			-----L1-----							
1(D)	10(S)	20(S)								
DVLMTQSPFLSPVSLGDKASISC RSSONIVHSNGDTIYLE WYLQRPQGSPKLLIF KVSNRFS GVPDRFSGSGGTDFILKISRVEAEDLVGYVC										
			-----L2-----							
				50(K)	60(D)	70(D)	80(A)	90(Q)	100(G)	107(K)
				-----L3-----						
								FGGSHVPWT		
								CDR3		
									FGGGTKLEIK	
										FR4

Fig. 9B Framework 1 specific rearranged antibody

Antibody sequence GI (gene identification) number : 4324018 (germline Vkl A17), also the same % with A1 and A17 (SEQ ID NO. 173)

DVVMTQSPFLSPVTLGQASISC TSSQSLVYTDGKTYLN WFOQRPQGSPRLIF KVSNRDS GVPDRFSGSGGTDFTLKISRVEAEDVAIYYC	MQGTHWPGT	FGQGTKVEIKR
82%		
Antibody sequence GI (gene identification) number : 5578780 (germline Vkl A3 or A19), (SEQ ID NO. 174)		
DVVMTQSPFLSPVTPGEPASISC RSSQSLHSGNGYNYFA WYLQKPGSQPLL VYLGSRAS GVPDRFSGS GSGTDFTLKISRVEAEDVGYYC	MQVLQTPYT	FGQGTKLEIS
78%		

Fig. 9C Framework 2 specific rearranged antibody

Antibody sequence GI (gene identification) number : 4324018 (germline Vkl A3, GI:33613) *not chose this one because AA change in position close to CDR2 (SEQ ID NO. 175)

A-----LPVTPGEPASISC RSSQSLHSGNGKNYLD WYLQKPGSQPKLLIY FGSTRAS GVPDRFSGSGGTDFTLKI SRVEAEDVGIIYC MKAQQTPA	FGPGTKVEIK
Antibody sequence GI (gene identification) number : 18041766 (germline Vkl A3, GI:33613) (SEQ ID NO. 176)	
B-----LPVTPGEPASISC RSSQSLPGNGYNYLD WFLQRPQGSPQLLIY LITSNRAS GVPDRFSGSGGTDFTLKI SRVEAEDVGIIYC MQRQTPYI	FGQGTKL
86%	

Fig. 9D Framework 3 specific rearranged antibody

Antibody sequence GI (gene identification) number : 553476 and 33251(Vkl germline Vkl A3) (SEQ ID NO. 177)

DVMTQSPFLSPVTPGEPASISC RSSQSLHSGNGYNYLD WYLQKPGSQPLL IY LGSNRAS GVPDRF SGSGSGTDFTLKISRVEAEDVGIIYC	MQALQTPQT	FGQGTKVEIKR
93%		

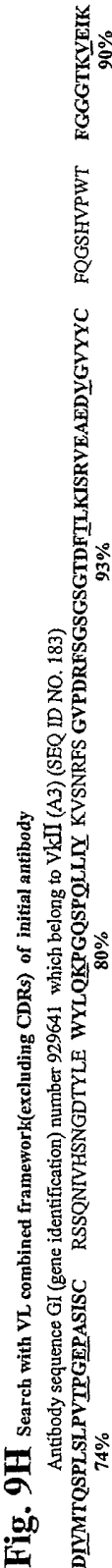
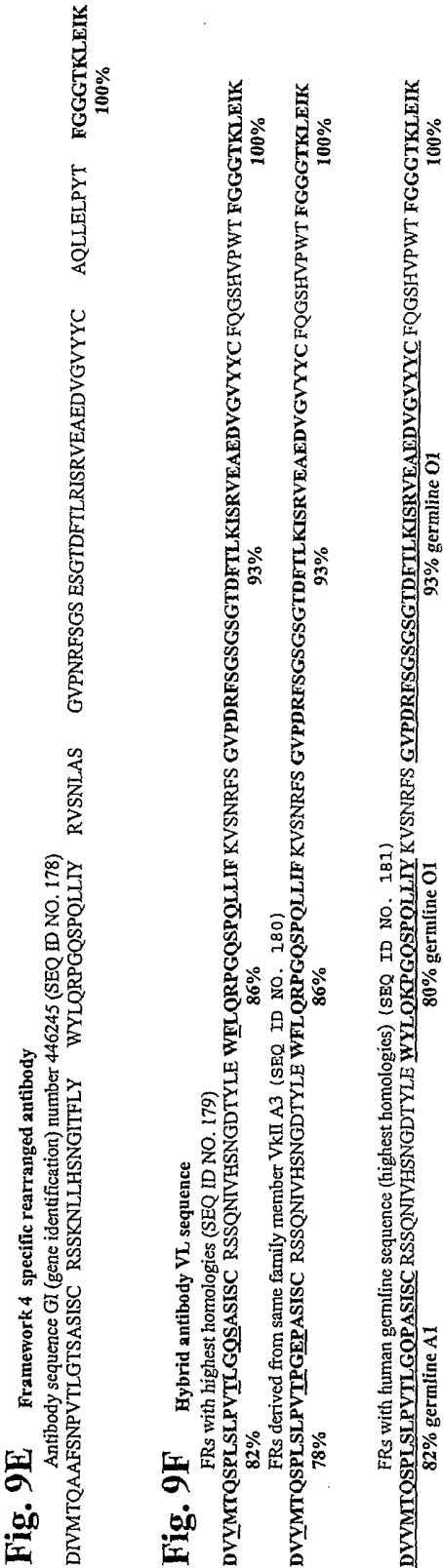


Fig. 9C Sequence homologies of initial, hybrid and germline VL sequences

	Antibody comparisons	Frameworks	CDRs	Whole VL
VL	Hybrid antibody versus initial antibody sequence	(72/80)90%	(32/32)100%	(104/112)93%
VL	Hybrid antibody versus the most similar human germline sequences VkII (A17)	(65/70)93%*	(16/23)70%*	(81/93)87%*
VL	The most similar human rearranged CDR grafted VL versus initial antibody sequence	(68/80)85%	(32/32)100%	(100/112)89%
VL	The most similar human rearranged CDR grafted VL versus the most similar human germline sequence VkII (A17)	(62/70)88%*	(16/23)70%*	(78/93)84%*
VL	Hybrid antibody(frameworks with highest homology human germline sequences) versus initial antibody	(71/80)89%	(32/32)100%	(103/112)92%

*Does not include J region sequence

D1 Heavy Chain

Fig. 10A

Initial antibody VH protein sequence (SEQ ID NO. 183)

*H1,H2,H3: loop regions structural criteria defined by Chothia

CDRs : CDRs are according to Kabat

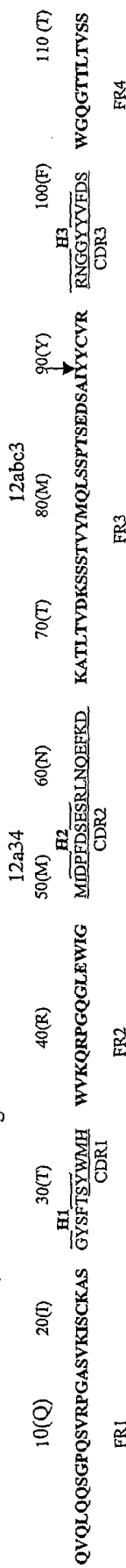


Fig. 10B

Framework 1 specific rearranged antibody

Antibody sequence GI (gene identification) number : 18698373 (closest germline: VH7-81, GI:4512268) (SEQ ID NO. 184)



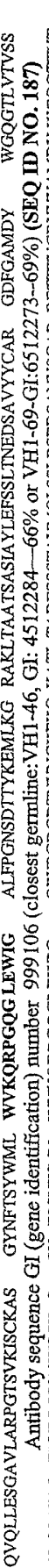
Antibody sequence GI (gene identification) number : 392677 (closest germline: VH1-2, GI:4512302) (SEQ ID NO. 185)



Fig. 10C

Framework 2 specific rearranged antibody

Antibody sequence GI (gene identification) number 886288 (closest germline: VH1-2, GI:4512313) (SEQ ID NO. 186)



Antibody sequence GI (gene identification) number 999106 (closest germline: VH1-46, GI: 4512284--66% or VH1-69-GI:6512273--69%) (SEQ ID NO. 187)



Fig. 10D

Framework 3 specific rearranged antibody

Antibody sequence GI (gene identification) number: 5542538 (closest germline: VH1-2, GI:4512314) (SEQ ID NO. 188)
 QVQLLESQAELVKPGASVKLSCKAS GYFTSYWMH WVKQRPGRGLEWIG MIDPNSGGTKYNEKFKS KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR RDMDY WGAGTTVTYSS
 81%

Fig. 10E

Framework 4 specific rearranged antibody (there are only two antibody having 100% in FR4)

Antibody sequence GI (gene identification) number: 4530559 (closest germline: VH4-34, GI:4512291) (SEQ ID NO. 189)
 QVQLQQWGAELVKPSETLSLTCVVY GGSFGYSWS WIRQSPGKGLEWIG ENHSGSTNYNSLSKS RVTISVDTSKNQFSLKNSVTAAADTAVYYCAR GVVKGMDV WGQGTITLTYS
 Antibody sequence GI (gene identification) number: 5834122 (closest germline: VH3-48, GI:4512283) (SEQ ID NO. 190)
 EVQLVESGGGLVQPGGSLRLSCAAS GFTFSYSMN WVRQAPGKGLEWYS YISSSSTIYYADSVKG RFTI SRDNAKNSLYLQMNLSRAEDTAVYYCAR DWSSQYYYYYGMDV WGQGTITLTYS
 100%
 The closest VH1 family number
 Antibody sequence GI (gene identification) number: 106709 (closest germline: VH1-69, GI:6512273) (SEQ ID NO. 191)
 QVQLVQSGAEVKKPGSSVKVCKAS GGTFSYAIS WVRQAPGQGLEWMGG IPIFGTANYAQKFGG RVITADESTSTAYMELSSLSRSEDVAVYYCAR GYYYYYGMDDV WGQGTITVTYSS
 91%

Fig. 10F

Hybrid antibody VH sequence FRs with highest homologies (SEQ ID NO. 192)

QVQLQSGSELKPKPGASVKISCKAS GYSFTSYWMH WVKQRPQGQGLEWIG MIDPFDESRLNQEFKD KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR RGGYYVFDS WGQGTITLTYS
 80%
 FRs derived from same family VH1 (SEQ ID NO. 193)
 QVQLVQSGPEVKKPGASVKVCKAS GYSFTSYWMH WVKQRPQGQGLEWIG MIDPFDESRLNQEFKD KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR RGGYYVFDS WGQGTITVTYSS
 76%*from VH1-18 100%*from VH1-3
 FRs with human germline sequence (highest homologies) (SEQ ID NO. 196)
 QVQLVQSGAEVKKPGASVKISCKAS GYSFTSYWMH WVRQAPGQGLEWMG MIDPFDESRLNQEFKD RVITADKSTSTAYMELSSLSRSEDVAVYYCAR RGGYYVFDS WGQGTITVTYSS
 72% germline VH1-18 62% germline VH1-69

Fig. 10G

Search with VH combined framework(excluding CDRs) of initial antibody

Antibody sequence GI (gene identification) number: 5542536 (closest germline: VH1-2, GI:4512314) (SEQ ID NO. 194)
 QVQLLESQAELVKPGASVKLSCKAS GYFTSYWMH WVKQRPGRGLEWIG MIDPNSGGTKYNEKFKS KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR RDMDY WGAGTTVTYSS
 76%
 The most similar human rearranged CDR grafted VH (SEQ ID NO. 195)
 QVQLLESQAELVKPGASVKLSCKAS GYSFTSYWMH WVKQRPGRGLEWIG MIDPFDESRLNQEFKD KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR RGGYYVFDS WGAGTTVTYSS

Fig. 10H Sequence homologies of initial, hybrid and germline VH sequences

	Antibody comparisons	Frameworks	CDRs	Whole VH
VH	Hybrid antibody versus initial antibody	(71/82)87%	(37/37)100%	(108/119)91%
VH	Hybrid antibody versus the most similar human germline sequence (VH1-46)	(51/71)72%	(12/27)44%*	(63/98)64%*
VH	The most similar human rearranged CDR grafted VH versus initial antibody	(66/82)80%	(37/37)100%	(103/119)87%
VH	The most similar human rearranged CDR grafted VH versus the most similar human germline sequence (VH1-46)	(49/71)69%*	(12/27)44%*	(61/98)62%*
VH	Hybrid antibody(frameworks with highest homology human germline sequences) versus initial antibody	(60/82) 73%	(37/37)100%	(97/119)81.5%

*does not include D and J regions

Fig. 11

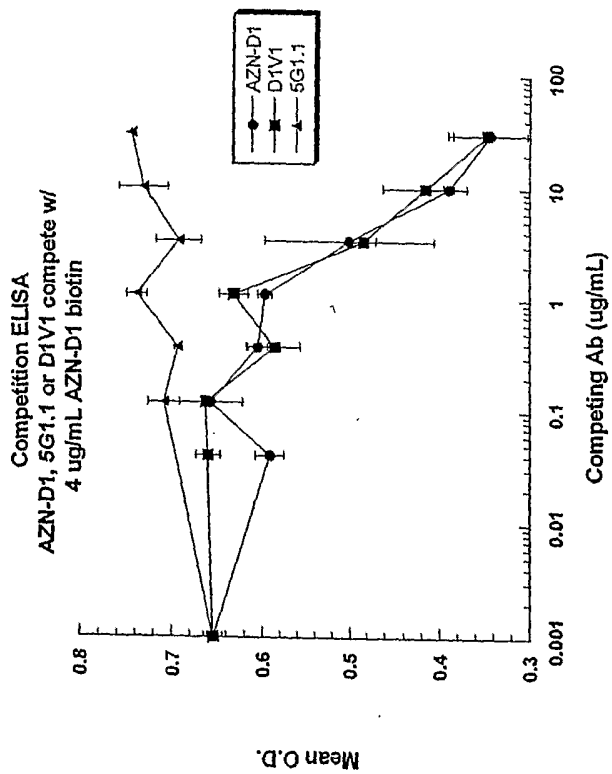


Fig. 12

Binding kinetics of initial antibody and hybrid antibody

Antibody	Kd (10 ⁻¹⁰ M)	Kon (10 ⁵ s ⁻¹ M ⁻¹)	Koff (10 ⁻⁴ s ⁻¹)	Kd (Initial/Hybrid)
Initial Ab	12.4	7.01	1.17	
Hybrid Ab(1)	17.7	0.426	1.11	
Hybrid Ab(2)	4.96	0.85	0.3	
Hybrid Ave	11.33	0.638	0.7	1.09

Kon: Association rate constant
Koff: Dissociation rate constant
Kd: Affinity

The retention of Initial and hybrid antibodies on MBL (Mannan-binding lectin) was determined on BIAcore 3000 system.
Kd results show a 9% increase of hybrid antibody versus Initial antibody

Binding kinetics of anti-hDC-SIGN initial antibody and hybrid antibody

Antibody	Kd (10 ⁻¹⁰ M)	Kon (10 ⁵ s ⁻¹ M ⁻¹)	Koff (10 ⁻⁴ s ⁻¹)	Kd (Initial/Hybrid)
Initial Ab(AZND1)	38.8	0.62	1.17	
Hybrid Ab(D1V1)	37	0.671	1.77	1.38
Hybrid Ab(D1V2)	127	0.335	2.14	0.4

Kon: Association rate constant
Koff: Dissociation rate constant
Kd: Affinity

The retention of Initial and hybrid antibodies on hDC-SIGN-Fc (human Dendritic Cell-Specific, ICAM-3 Grabbing Non-integrin) was determined on BIAcore 3000 system.
Kd results show a 38% increase on D1V1 and 60% drop on D1V2 hybrid antibody versus Initial antibody

Fig. 13