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(54) **Title**: HUMANIZED ANTI-N2 ANTIBODIES

(57) **Abstract**: The present invention encompasses humanized antibodies that specifically bind N2 peptide, methods for the preparation thereof and methods for the use thereof.

HUMANIZED, ANTI-N2 ANTIBODIES

RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/777,647, filed March 12, 2013. The entire teachings of the above application are incorporated herein by reference.

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by Grant No. 10388353 from the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

It has been demonstrated that ischemia-reperfusion injury can be initiated by clonally- specific pathogenic IgM that activates the classical pathway of complement (Zhang *et al.* (2004) *Proc. Natl. Acad. Sci.* 101(11):3886-3891). Pathogenic IgM (also referred to as "natural IgM") recognizes and binds to a self-antigen which is an antigen expressed or exposed on damaged tissue, for example, on damaged ischemic tissue. Binding of pathogenic IgM to the self-antigen initiates inflammation by activating complement in the classical pathway. U.S. Patent No. 7,442,783 describes the major epitope for binding of natural IgMs as a conserved region within type II non-muscle myosin heavy chain (NMHC) proteins. This epitope is referred to as the N2 12-mer peptide.

Inhibitors of the interaction between the N2 epitope and pathogenic IgM have been described as useful for the treatment of inflammatory diseases and conditions, including ischemia/reperfusion injury. For example, U.S. Patent No. 8,324,352 describes the murine monoclonal antibody referred to as 21G6. Murine 21G6 (m21G6) was shown to bind to the N2 peptide and provide protection against ischemia/reperfusion injury in animal models. It would be advantageous to develop additional therapeutic agents that bind the N2 peptide and that can be used for treating inflammatory conditions such as ischemia/reperfusion injury.

SUMMARY OF THE INVENTION

The present invention encompasses humanized derivatives of the murine 21G6 antibody that specifically bind N2 peptide. As shown in the Examples below, humanized antibodies have been developed that bind the N2 peptide.

In one embodiment, the invention is directed to an antibody or antigen binding fragment thereof comprising framework regions from a human immunoglobulin and comprising the variable heavy chain (VH) complementarity determining regions (CDRs) of the murine 21G6 antibody and the variable light chain (VL) CDRs of the murine 21G6 antibody.

In some embodiments, the invention is directed to a humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 comprises SEQ ID NO: 3, VH CDR2 comprises SEQ ID NO: 4 and VH CDR3 comprises SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO: 24;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45; and

d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3, wherein the VL CDR1 comprises SEQ ID NO: 6, VL CDR2 comprises SEQ ID NO: 7 and VL CDR3 comprises SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and

d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

In certain additional embodiments, the antibody or antigen-binding fragment has a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In yet additional embodiments, the antibody or antigen-binding fragment has a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

In additional aspects, the antibody or antigen-binding fragment has a VL region that comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In yet additional aspects, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 9 and has a VL region that consists of SEQ ID NO: 12. In other embodiments, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 9 and has a VL region that consists of SEQ ID NO: 13. In another aspect, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 9 and the VL region consists of SEQ ID NO: 14. In a further embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 10 and has a VL region consists of SEQ ID NO: 12. In certain additional aspects, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 10 and has a VL region that consists of SEQ ID NO: 13. In an additional embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 10 and has a VL region that consists of SEQ ID NO: 14. In another aspect of the invention, the antibody or antigen-binding fragment of has a VH region that consists of SEQ ID NO: 11 and a VL region that consists of SEQ ID NO: 12. In another embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 11 and a VL region that consists of SEQ ID NO: 13. In another embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 11 and a VL region that consists of SEQ ID NO: 14. In yet additional embodiments, the antibody or antigen-binding fragment of has a VH region that consists of SEQ ID NO: 49 and a VL region that consists of SEQ ID NO: 12. In another embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 49 and a VL region that consists of

SEQ ID NO: 13. In another embodiment, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 49 and a VL region that consists of SEQ ID NO: 14.

In some embodiments, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 43 and a VL region that consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. In additional embodiments, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 44 and a VL region that consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. In yet other embodiments, the antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 45 and a VL region that consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. In another aspect, the invention is an antibody or antigen-binding fragment has a VH region that consists of SEQ ID NO: 42 and VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In yet additional embodiments, the invention is directed to a humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region comprising a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In an additional embodiment, the invention is directed to a humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region comprising a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

In a further embodiment, the invention is a humanized, anti-N2 antibody or antigen-binding fragment thereof, comprising a light chain variable (VL) region comprising a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In certain embodiments, the invention is directed to a humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 comprises SEQ ID NO: 3, VH CDR2 comprises SEQ ID NO: 4 and VH CDR3 comprises SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:
   a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;
   b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO:24 or SEQ ID NO: 50;
   c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 51 or SEQ ID NO: 52; and
   d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 comprises SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 comprises SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:
   a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;
   b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;
   c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and
   d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

In certain additional embodiments, the antibody or antigen-binding fragment has a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49. In additional aspects, the antibody or antigen-binding fragment has a VL region that comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In yet additional embodiments, the antibody or antigen binding fragment has a VH that consists of the amino acid sequence of SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 12. In yet other embodiments, the antibody or antigen-binding fragment has a VH region that consists of the amino acid sequence of

SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 13. In yet further embodiments, the antibody or antigen-binding fragment has a VH region consists of the amino acid sequence of SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 14. In yet an additional aspect, the antibody or antigen-binding fragment has a VH region that consists of the amino acid sequence of SEQ ID NO: 54 and VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In further embodiments, the invention is directed to a nucleotide encoding the humanized, anti-N2 antibody or antigen-binding fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

FIG. 1 shows a sequence comparison of the murine 21G6 heavy chain variable (VH) region and the humanized heavy chain variable regions (VH) H1, H2 and H3 and also shows a sequence comparison of the murine 21G6 light chain variable (VL) region and the humanized light chain variable regions light chain (VL) regions L1, L2 and L3. The CDR regions are indicated by the boxes.

FIG. 2 shows a sequence comparison of murine 21G6 heavy chain (VH) region and the humanized heavy chain variable region H4.

FIG. 3 shows a nucleotide sequence (SEQ ID NO: 60) encoding the humanized heavy chain variable region H4 that was optimized for the production of the humanized antibody in Chinese hamster ovary (CHO) cells. FIG. 3 also shows the amino acid sequence of the heavy chain variable region H4. Each + indicates where a change was made as compared to SEQ ID NO: 66.

FIG. 4 shows a nucleotide sequence (SEQ ID NO: 69) encoding the humanized light chain variable region L2 that was optimized for the production of the humanized antibody in Chinese hamster ovary (CHO) cells. FIG. 4 also shows the amino acid of the light chain variable region L2. Each + indicates where a change was made as compared to SEQ ID NO: 67.

FIG. 5 is a bar graph showing the levels of recombinant antibody expression on day 5 post-transfection with either the native sequences (pDS2.0; bars on the right) or optimized sequences (pDS-opt; bars on the left). Incorporation of the recombinant antibody into the CHO cells was accomplished by selection in the presence of 10ug/ml puromycin and 100nM methotrexate for transfectants DS5 and DS7 or 20ug/ml puromycin/200nM methotrexate for transfectants DS6 and DS8.

DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention follows.

The words "a" or "an" are meant to encompass one or more, unless otherwise specified.

An "antibody" is a binding molecule including immunoglobulin molecules, antibody fragments, and immunologically active portions of immunoglobulin molecules, for example, molecules that contain an antigen-binding site. Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. An antibody binds specifically to an antigen (or other molecule) if the antibody binds preferentially to the antigen, and, for example, has less than about 30%, preferably less than about 20%, less than about 10%, or less than about 1% cross-reactivity with another molecule. The terms "antibody" and "immunoglobulin" are used interchangeably. "Bind" or "binding" are used herein to refer to detectable relationships or associations (e.g. biochemical interactions) between molecules.

An "isolated" molecule, for example, an isolated antibody or isolated peptide, refers to a condition of being separate or purified from other molecules present in the natural environment or as they occur in nature.

The N2 epitope is an epitope of the self-antigen, the 12 amino acid sequence expressed in non-muscle myosin heavy chain (NMHC) type II. The 12-amino acid sequence is LMKNMDPLNDNV (SEQ ID NO: 47). The N2 epitope is described in detail in U.S. Patent No. 7,442,783, the contents of which are expressly incorporated by reference herein. "Natural IgM" or "pathogenic IgM" refers to an IgM antibody that is naturally produced in a mammal (for example, a human) that binds to the N2 epitope and initiates inflammation by activating complement in the classical pathway.

In some embodiments, antibody or antigen-binding fragment thereof binds to SEQ ID NO: 47. In additional embodiments, the antibody or antigen-binding fragment thereof binds to an epitope wherein the amino acid sequence of the epitope has at least about 80%, 85%, 90%, 95%, or 98% sequence identity to the amino acid sequence of SEQ ID NO: 47. In certain embodiments, the epitope comprises the amino acid sequence LMKNMDPLNDNI (SEQ ID NO: 48).

The hypervariable region of an antibody or fragment thereof refers to the amino acid residues that contribute to antigen-binding. The hypervariable region comprises amino acid residues from the complementarity determining regions (CDRs). The CDRs are specific regions within variable regions of the heavy and the light chain. Generally, the variable region consists of four framework regions (FWR1, FWR2, FWR3, FWR4) and three CDRs arranged as follows: NH_2-FWR1-CDR1-FWR2-CDR2-FWR3-CDR3-FWR4-constant region-C(O)OH. The term "framework regions" refers to those variable domain amino acid residues other than the CDR residues and include, for example, FWR1, FWR2, FWR3, and FWR4.

As described above, the present invention is directed to humanized derivatives of the murine 21G6 antibody described in U.S. Patent No. 8,324,352, the contents of which are expressly incorporated herein. In certain embodiments, the humanized antibodies and fragments thereof bind the N2 peptide. The amino acid sequences of the heavy chain variable region (VH) and the light chain variable region (VL) of the murine 21G6 antibody are shown in FIG. 1 and are below as SEQ ID NOs: 1 and 2:

Murine 21G6 (m21G6) VH
QVQLQQPGAELVKPGASVKLSCKASGYTFTSYYMYWVKQRPGQGLEWIGGINPS
NGGTNFNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCTRWGYDREWFAY
WGQGTLVTVSA (SEQ ID NO: 1).

Murine 21G6 VL
DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQVLIYRM
SNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK
R (SEQ ID NO: 2).

The underlined amino acids represent the complementarity determining regions.

FIG. 1 shows the amino acid sequences of three VH regions encompassed by the invention: H1-21G6, H2-21G6 and H3-21G6. The amino acid sequences of the H1-21G6, H2-21G6 and H3-21G6 VH regions are SEQ ID NOs: 9, 10 and 11, respectively:

H1-21G6 VH
QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYYMYWVKQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATLTVDKSASTAYMELSSLRSEDTAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 9).

H2-21G6 VH
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATMTVDKSTSTAYMELRSLRSDDSAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 10).

H3-21G6 VH
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATITVDKSTSTAYMELSSLRSEDTAVYYCTRWGYDREWFAY
WGQGTLVTVSS (SEQ ID NO: 11).

The underlined amino acids represent the complementarity determining regions.

An additional VH region is also encompassed by the invention: H4-21G6. The amino acid sequence of H4-21G6 is shown below:

H4-21G6 Vh
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWMGGINP
SNGGTNFNEKFKSRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 49)

The Figure also shows the amino acid sequences of three VL regions encompassed by the invention: L1-21G6, L2-21G6 and L3-21G6. The amino acid sequences of L1-21G6, L2-21G6 and L3-21G6 VH regions are SEQ ID NOs: 12, 13 and 14, respectively.

L1-21G6 VL
DIVMTQSPATLSVSPGERATISCRSSKSLLHSNGNTYLYWFQQKPGQPPKVLIYRM
SNLASGVPARFSGSGSGTDFTLTISSVEPEDFATYYCMQHLEYPFTFGGGTKLEIKR
(SEQ ID NO: 12).

L2-21G6 VL
DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMS
NLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIK
R (SEQ ID NO: 13).

L3-21G6 VL
DIVMTQTPLSLSYTPGQPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMS
NLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIK
R (SEQ ID NO: 14).

The underlined amino acids represent the complementarity determining regions.

The names "H1-21G6," "H2-21G6," "H3-21G6" and "H4-21G6" are used interchangeably herein with "H1," "H2," "H3," and "H4," respectively. The names "L1-21G6," "L2-21G6" and "L3-21G6" are used interchangeably with "L1," "L2" and "L3," respectively.

CDR1, CDR2 and CDR3 of the VH regions of the antibodies or fragments of the present invention are SYYMY (SEQ ID NO: 3), GINPSNGGTNFNEKFKS (SEQ ID NO: 4), GYDREWFAY (SEQ ID NO: 5), respectively. CDR1, CDR2 and CDR3 of the VL regions of the antibodies or fragments of the present invention are RSSKSLLHSNGNTYLY (SEQ ID NO: 6), RMSNLAS (SEQ ID NO: 7), and MQHLEYPFT (SEQ ID NO: 8), respectively. The VL region of the antibody or antigen-binding fragments of the present invention includes at least two of the CDRs of m21G6 VL. The VH region of the antibody or antigen-binding fragment of the invention includes at least two CDRs of the m21G6 VH. In some embodiments, the humanized antibodies include all three CDRs of m21G6 VH and/or all three CDRs of the m21G6 VL. The framework regions FWR1, FWR2, FWR3 and FWR4 of the VH region of each of H1-21G6, H2-21G6 and H3-21G6 are shown below:

H1 VH FWR1
QVQLVQSGAEVVKPGASVKLSCKASGYTFT (SEQ ID NO: 15).

H1 VH FWR2
WVKQAPGQGLEWIG (SEQ ID NO: 16).

H1 VH FWR3
KATLTVDKSASTAYMELSSLRSEDTAVYYCTR (SEQ ID NO: 17).

H1 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 18).

H2 VH FWR1
QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 19).

H2 VH FWR2
WVRQAPGQGLEWIG (SEQ ID NO: 20).

H2 VH FWR3
KATMTVDKSTSTAYMELRSLRSDDSAVYYCTR (SEQ ID NO: 21).

H2 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 22).

H3 VH FWR1
QVQLVQSGAEVKKPGSSVKVSCKASGYTFT (SEQ ID NO: 23).

H3 VH FWR2
WVRQAPGQGLEWIG (SEQ ID NO: 24).

H3 VH FWR3
KATITVDKSTSTAYMELSSLRSEDTAVYYCTR (SEQ ID NO: 25).

H3 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 26).

H4 VH FWR1
QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 19).

H4 VH FWR2
WVRQAPGQGLEWMG (SEQ ID NO: 50).

H4 VH FWR3
RVTMTTDTSTSTAYMELRSLRSDDTAVYYCTR (SEQ ID NO: 51).

H4 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 22).

The framework regions FWR1, FWR2, FWR3 and FWR4 of each of the VL region of each of L1-21G6, L2-21G6 and L3-21G6 are shown below:

L1 VL FWR1
DIVMTQSPATLSVSPGERATISC (SEQ ID NO: 27).

L1 VL FWR2
WFQQKPGQPPKVLIY (SEQ ID NO: 28).
L1 VL FWR3
GVPARFSGSGSGTDFTLTISSVEPEDFATYYC (SEQ ID NO: 29).

L1 VL FWR4
FGGGTKLEIKR (SEQ ID NO: 30).

L2 VL FWR1
DIVMTQSPLSLPVTPGEPASISC (SEQ ID NO: 31).

L2 VL FWR2
WFLQKPGQSPQLLIY (SEQ ID NO: 32).

L2 VL FWR3
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 33).

L2 VL FWR4
FGQGTKLEIKR (SEQ ID NO: 34).

L3 VL FWR1
DIVMTQTPLSLSYTPGQPASISC (SEQ ID NO: 35).

L3 VL FWR2
WFLQKPGQSPQLLIY (SEQ ID NO: 36).

L3 VL FWR3
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 37).

L3 VL FWR4
FGQGTKLEIKR (SEQ ID NO: 38).

As described above, the present invention encompasses an antibody or antigen-binding fragment thereof comprising VH CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, respectively, and VL CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively, and further comprising a VH region that comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;
b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24;
c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45; and
d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

and a VL region that comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;
b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;
c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and
d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

The present invention additionally encompasses an antibody or antigen-binding fragment thereof comprising VH CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, respectively, and VL CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively, and further comprising a VH region that comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19, SEQ ID NO: 23;
b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24 or SEQ ID NO: 50;
c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 51 or SEQ ID NO: 52; and
d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

and a VL region that comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;
b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;
c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and
d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

The terms “comprises” and “comprising” permits (but does not require) the inclusion of additional elements. For example, in the context of an amino acid sequence, the terms “comprises” and “comprising” permits the inclusion of additional amino acids at either the N-terminus and/or the carboxy terminal end. In some embodiments, the framework region of the VH and VL regions comprise a specific indicated amino acid sequence and one to three additional amino acids at the N-terminus and/or at the carboxy terminal end.

In certain additional aspects, the antibody or antigen binding fragment of the invention comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

i. the VH comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 consists of SEQ ID NO:3, VH CDR2 consists of SEQ ID NO: 4 and VH CDR3 consists of SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 consists of SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 consists of SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45; and

d. VH FWR4 consists of SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 consists of SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 consists of SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 consists of SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 consists of SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 consists of SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37;

d. VL FWR4 consists of SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

In yet additional embodiments, the present invention additionally encompasses an antibody or antigen-binding fragment thereof comprising VH CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5,

respectively, and VL CDR1, CDR2 and CDR3 having the amino acid sequences SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, respectively, and further comprising a VH region that comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 consists of SEQ ID NO: 15 , SEQ ID NO: 19, SEQ ID NO: 23;
b. The VH FWR2 consists of SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24 or SEQ ID NO: 50;
c. VH FWR3 consists of SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 51 or SEQ ID NO: 52; and
d. VH FWR4 consists of SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

and a VL region that comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

e. the VL FWR1 consists of SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;
f. VL FWR2 consists of SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;
g. VL FWR3 consists of SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and
h. VL FWR4 consists of SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

As described above, FIG. 1 shows the amino acid sequences of three exemplary humanized VH regions that comprise the VH CDRs of m21G6 (H1-21G6, H2-21G6 and H3-21G6; SEQ ID NOs: 9, 10 and 11, respectively). An additional exemplary humanized VH region that comprises the VH CDRs of m21G6 is H4-21G6 (SEQ ID NO: 49 or SEQ ID NO: 54). FIG. 1 also shows the amino acid sequences of three exemplary humanized VL regions (L1-21G6, L2-21G6 and L3-21G6; SEQ ID NOs: 12, 13 and 14, respectively). In some embodiments, the antibody or antigen-binding fragments of the invention comprise a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11. In yet an additional embodiment, the

antibody or antigen-binding fragment comprises a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 49 and SEQ ID NO: 54, and comprises a VL region that comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. In additional embodiments, the antibody or antigen-binding fragments of the invention comprise a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 49 and SEQ ID NO: 54. In additional aspects, the antibody or antigen-binding fragment has a VL region that comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. In yet another embodiment, the antibody or antigen-binding fragment comprises a VH region that comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11 and comprises a VL region that comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

In some embodiments, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 9 and has a VL region that comprises or consists of SEQ ID NO: 12. In other embodiments, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 9 and has a VL region that comprises or consists of SEQ ID NO: 13. In another aspect, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 9 and the VL region comprises or consists of SEQ ID NO: 14. In a further embodiment, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 10 and has a VL region that comprises or consists of SEQ ID NO: 12. In certain additional aspects, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 10 and has a VL region that comprises or consists of SEQ ID NO: 13. In an additional embodiment, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 10 and has a VL region that comprises or consists of SEQ ID NO: 14. In another aspect of the invention, the antibody or antigen-binding fragment of claim 1 has a VH region that comprises or consists of SEQ ID NO: 11 and a VL region that comprises or consists of SEQ ID NO: 12. In another embodiment, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 11 and a VL region that comprises or consists of SEQ ID NO: 13. In another embodiment, the antibody or antigen-binding fragment has a VH region that

comprises or consists of SEQ ID NO: 11 and a VL region that comprises or consists of SEQ ID NO: 14.

In yet additional embodiments, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 49 and has a VL region that comprises or consists of SEQ ID NO: 12. In another aspect, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 49 and the VL region comprises or consists of SEQ ID NO: 13. In a further embodiment, the antibody or antigen-binding fragment has a VH region that comprises or consists of SEQ ID NO: 49 and has a VL region that comprises or consists of SEQ ID NO: 14.

In certain additional embodiments, the antibody or antigen-binding fragment comprises a VH region that comprises a sequence selected from SEQ ID NO: 49 and SEQ ID NO: 54. In yet additional embodiments, the antibody or antigen-binding fragment comprises a VL region that comprises SEQ ID NO: 13 or SEQ ID NO: 55. In a further embodiment, the antibody or antigen-binding fragment comprises a VH region that comprises SEQ ID NO: 49 or SEQ ID NO: 54 and a VL region that comprises SEQ ID NO: 13 or SEQ ID NO: 55. In an additional aspect, the antibody or antigen-binding fragment comprising a VH region that comprises SEQ ID NO: 49 and a VL region that comprises SEQ ID NO: 13.

In certain aspects of the invention, the isotype of the constant region of the antibodies or antigen-binding fragments of the invention is IgG1, IgG2, IgG3, or IgG4. In some embodiments, the isotype of the IgG constant region is IgG1. In other embodiments, the isotype of the IgG constant region is IgG4. In some embodiments, the antibody or antigen-binding fragment thereof have a human IgG1 constant domain or a human IgG4 constant domain. In additional aspects, the antibody or antigen-binding fragment has a human Ig kappa constant domain. The term "isotype" refers to the classification of an antibody's heavy or light chain constant region. The constant domains of antibodies are not involved in binding to antigen, but have various effector functions. Depending on the amino acid sequence of the heavy chain constant region, a human or humanized antibody can be deemed to belong to one of five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. Many of these classes of immunoglobulins, for example the IgG class, can be divided into subclasses (isotypes), for example, IgG1, IgG2, IgG3, and IgG4. Human light chain constant regions are classified into two major classes, kappa and lambda.

When the positions of amino acid residues are referred to by number herein, it is to be understood that Kabat numbering system is used, unless otherwise indicated. Kabat

numbering is described in Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Publication No. 91-3242, National Institutes of Health, National Technical Information Service (hereinafter "Kabat"). Immunoglobulin sequences can be numbered according to Kabat by performing an alignment with the Kabat reference sequence. As such, the Kabat numbering system provides a uniform system for numbering immunoglobulin chains.

The present invention is directed to humanized antibodies wherein the CDRs are from the murine 21G6 antibody and wherein the framework regions are from a human immunoglobulin. It will be understood, that humanized antibodies can comprise amino acid residues that are not found in the recipient antibody or in the donor antibody. For example, such changes in the amino acid sequence can be made to improve binding to the antigen (for example, the N2 peptide) and/or to reduce immunogenicity. Therefore, the present invention encompasses the antibodies or antigen-binding fragments described herein wherein specific amino acids have been substituted, deleted or added. Amino acid substitutions, deletions or additions can be made to the antibodies or antigen-binding fragments thereof to improve or refine the properties of the antibody or fragment, for example amino acid change can be made to inhibit or block inflammation. For example, asparagine at position 297 (Asn 297) of the IgG constant region can be replaced with an alternative amino acid to reduce glycosylation and decrease activation of complement and binding to the Fc receptor. See, for example, Leatherbarrow et al. (1985) Effector functions of a monoclonal aglycosylated mouse IgG2a: binding and activation of complement component C1 and interaction with human monocyte Fc receptor. *Mol Immunol* 22(4):407-415; Tao et al. (1989) Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J Immunol* 143(8):2595-2601; Kabat (1987) Sequences of Proteins of Immunological Interest (In: US Department of Human Services), and Sazinsky et al. (2008), Aglycosylated immunoglobulin G_1 variants productively engage activating Fc receptors, *PNAS* 105(51): 20167–20172, the contents of each of which are expressly incorporated by reference herein. Glycosylation can be reduced, for example, by replacing the asparagine at position 297 (Asn 297) with an alternative amino acid, for example, alanine, glutamine, histidine or glycine. In some embodiments, Asn 297 can be replaced with glutamine. In certain aspects, the antibody or antigen-binding fragment has a human IgG1 constant domain that is aglycosylated.

In some embodiments, the penultimate amino acid in the third framework of the VH (VH FWR3) of each of H1-21G6, H2-21G6 and H3-21G6 (SEQ ID NOs: 17, 21 and 25, respectively) can be changed from threonine to alanine. The amino acid sequences SEQ ID NOs: 39, 40 and 41 are sequences for the VH FWR3 of each of H1, H2 and H3 wherein the penultimate amino acid (threonine) has been replaced with alanine:

H1 VH FWR3 with amino acid mutation to alanine
KATLTVDKSASTAYMELSSLRSEDTAVYYC*A*R (SEQ ID NO: 39).

H2 VH FWR3 with amino acid mutation to alanine
KATMTVDKSTSTAYMELRSLRSDDSAVYYC*A*R (SEQ ID NO: 40).

H3 VH FWR3 with amino acid mutation to alanine
KATITVDKSTSTAYMELSSLRSEDTAVYYC*A*R (SEQ ID NO: 41).

The italicized alanine above represents the change from threonine to alanine. The amino acid sequences SEQ ID NOs: 43, 44, and 45 are sequences for the H1, H2 and H3 VH regions wherein the penultimate amino acid (threonine) of the FWR3 is replaced with alanine:

H1 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYYMYWVKQAPGQGLEWIGGINPS NGGTNFNEKFKSKATLTVDKSASTAYMELSSLRSEDTAVYYC*A*RWGYDREWFA YWGQGTLVTVSS (SEQ ID NO: 43).

H2 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS NGGTNFNEKFKSKATMTVDKSTSTAYMELRSLRSDDSAVYYC*A*RWGYDREWFA YWGQGTLVTVSS (SEQ ID NO: 44).

H3 with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS NGGTNFNEKFKSKATITVDKSTSTAYMELSSLRSEDTAVYYC*A*RWGYDREWFAY WGQGTLVTVSS (SEQ ID NO: 45).

The italicized alanine above represents the change from threonine to alanine.

In an additional embodiment, the penultimate amino acid in the third framework regions of the VH (VH FWR3) of H4-21G6 (SEQ ID NO: 51) can be changed from threonine to alanine. The amino acid sequence SEQ ID NO: 52 is the sequence for the VH

FWR3 of H4 wherein the penultimate amino acid (threonine has been replaced with alanine:

H4 VH FWR3
RVTMTTDTSTSTAYMELRSLRSDDTAVYYC*A*R (SEQ ID NO: 52).

The italicized alanine above represents the change from threonine to alanine. The amino acid sequence SEQ ID NO: 52 is the sequence for the H4 VH regions wherein the penultimate amino acid (threonine) of the FWR3 is replaced with alanine:

H4 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWMGGINP
SNGGTNFNEKFKSRVTMTTDTSTSTAYMELRSLRSDDTAVYYC*A*RWGYDREWF
AYWGQGTLVTVSS (SEQ ID NO: 53).

The italicized alanine above represents the change from threonine to alanine.

Amino acid modifications that may increase stability and/or increase affinity are also contemplated herein. Additional specific amino acid variants contemplated by the invention are variants of H2-21G6 VH and a variant of the murine 21G6 VL kappa chain:

Amino Acid Variant of H2 VH
QVQLVQSGAELVKKPGASLKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINP
SNGGTNFNEKFKGRVTITRDKSTSTAYMELRSLRSEDSAVYYCARWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 42).

Amino Acid Variant of kappa chain (m21G6 VL)
EIVLTQSPGTLSLSP GERATLSCRAS
KSLLHSNGNTYLYWYQQKPGQAPRLLIYRMS NRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYC MQHLEYPFTFGQGTKLEIKR (SEQ ID NO: 46).

Additional amino acid modifications include amino acid variants of the H2 VH amino acid sequence (SEQ ID NO: 42), wherein the amino acid at position 65 is replaced with glycine, the amino acid at position 66 is replaced with arginine, the amino acid at position 67 is replaced with valine or phenylalanine, the amino acid at position 69 is replaced with isoleucine, the amino acid at position 71 is replaced with arginine and/or the amino acid at position 85 can be replaced with glutamic acid.

The invention also encompasses an antibody or antigen-binding fragment thereof wherein an alanine at position 78 (Ala 78) of the VH is replaced with phenylalanine.

In certain embodiments, the antibody or antigen-binding fragment has a human IgG4 constant domain wherein serine at position 228 (Ser 228) is replaced with proline.

Additional modifications can also be made within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, protein stability and/or antigen-dependent cellular cytotoxicity, or lack thereof. In addition, an antibody of the invention can be chemically modified (e.g., one or more chemical moieties can be attached to the antibody). In addition, the class of an antibody can be "switched" by known techniques. Such techniques include, e.g., the use of direct recombinant techniques (see e.g., U.S. Pat. No. 4,816,397) and cell-cell fusion techniques (see e.g., U.S. Pat. No. 5,916,771). For example, an antibody that was originally produced as an IgM molecule may be class switched to an IgG antibody. Class switching techniques also may be used to convert one IgG subclass to another, e.g., from IgG1 to IgG2. Thus, the effector function of the antibodies of the invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses. Exemplary cDNA sequences for constant regions are available from GenBank, for example, each of which incorporated by reference in its entirety, are as follows: Human IgG1 constant heavy chain region: GenBank Accession No.: J00228; Human IgG2 constant heavy chain region: GenBank Accession No.: J00230; Human IgG3 constant heavy chain region: GenBank Accession No.: X04646; Human IgG4 constant heavy chain region: GenBank Accession No.: K01316; and Human kappa light chain constant region: GenBank Accession No.: J00241. The hinge region of CH_1 can also be modified such that the number of cysteine residues in the hinge region is increased or decreased. This approach is described further in U.S. Pat. No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody. The Fc hinge region of an antibody can also be mutated to decrease the biological half-life of the antibody. In another embodiment, the antibody is modified to increase its biological half-life. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Pat. No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Pat. Nos. 5,869,046 and 6,121,022 by Presta et al. In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue

with a different amino acid residue to alter the effecter function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos. 5,624,821 and 5,648,260, both to Winter et al. In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Pat. No. 6,194,551 by Idusogie et al. In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer et al. In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R. L. et al. (2001) J. Biol. Chem. 276:6591-6604).

In addition, the half-life of an antibody molecule can be increased by increasing the affinity of the antibody for the Fc receptor (FcR). The binding of the antibody to the FcR can be improved using art-known techniques, such as by introducing specific mutations in the Fc region (Dall'Acqua, W. F. et al. (2006) J. Biol. Chem. 281: 23514-23524; Petkova, S. B. (2006) et al., Internat. Immunol. 18:1759-1769; U.S. Pat. Nos. 7,785,791, 7,790,858 and 7,371,826; the contents of each of the aforementioned references are incorporated by reference herein). It has been demonstrated that the increased affinity of the antibody for the FcR results in a longer half-life (Petkova et al., 2006). In the case

of amino acid mutagenesis in the Fc domain, it is known that certain amino acid mutations can increase binding to the FcR and extend the half-life. Examples of such amino acid mutations that have been described to increase binding to the Fc receptor include N434A and T307/E380/N434 (Petkova et al., 2006) and as well other mutations M252Y/S254T/T256E, T250Q, M428L, and T250Q/M428L, (Dall'Acqua et al., 2006; Hinton et al., J. Immunol., 176: 346-356).

An antibody or antigen-binding fragment described herein can be chemically modified based on linkage to a polymer. The polymer is typically water soluble so that the antibody to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. The polymer can have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled. An exemplary reactive aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Pat. No. 5,252,714). The polymer can be branched or unbranched. For therapeutic use of the end-product preparation, the polymer is pharmaceutically acceptable. The water soluble polymer, or mixture thereof if desired, can be selected from the group consisting of, for example, polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol.

An antibody fragment or antigen-binding fragment is a derivative of an antibody that is less than full-length. In exemplary embodiments, the antibody fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, scFv, Fv, dsFv, diabody, minibody, Fc, Fd fragments, and single chain antibodies.

Antibody fragments can be produced by methods known in the art. For example, the antibody fragment can be enzymatically or chemically produced by fragmentation of an intact antibody, or the fragment can be produced recombinantly. The antibody fragment can optionally be a single chain antibody fragment. Alternatively, the fragment can comprise multiple chains which are linked together, for instance, by disulfide linkages. In addition, digestion of an antibody with pepsin yields $F(ab')_2$ fragments and multiple small fragments. Mercaptoethanol reduction of an antibody yields individual heavy and light chains. Digestion of an antibody with papain yields individual Fab fragments and the

Fc fragment. The fragment can also optionally be a multimolecular complex. A functional antibody fragment can for example comprise at least about 50 amino acids. In some embodiments, the functional antibody fragment can comprise at least about 200 amino acids.

Humanized antibodies and antigen-binding fragment thereof described herein can be produced using techniques known in the art, including, but not limited to, CDR-grafting (see, for example, European Patent No. EP 239,400; WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, the contents of each of which incorporated by reference), veneering or resurfacing (see, for example, European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, Proc. Natl. Acad. Sci., 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, for example, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, PCT Publication No. WO 9317105, Tan et al., J. Immunol., 169:1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16):10678-84 (1997), Roguska et al, Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp): 5973s-5977s (1995), Couto et al., Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959-73 (1994), each of which is incorporated by reference herein.

The humanized antibody can be produced by, for example, by constructing cDNAs encoding the humanized variable regions, inserting each of them into an expression vector for animal cells comprising genes encoding the heavy chain and light chain of a human antibody to thereby construct a vector for expression of humanized antibody, and introducing it into an animal cell to express and produce the humanized antibody. The invention encompasses a nucleotide sequence that encodes an antibody or antigen-binding fragment described herein.

In additional embodiments, the invention is directed to a nucleotide sequence that encodes an antibody or fragment thereof comprising a heavy chain or VH described herein. In certain embodiments, the nucleotide sequence comprises a sequence that encodes the VH region of H1-21G6, H2-21G6, H3-21G6, or H4-21G6. Exemplary nucleotide sequences include SEQ ID NOs: 56, 57, 58, and 59 respectively (shown below). In some embodiments, the nucleotide sequence that encodes an antibody described herein

comprises a sequence selected from the group consisting of SEQ ID NOs: 56, 57, 58, and 59. In some embodiments, the nucleotide sequence is SEQ ID NO: 59. In certain additional embodiments, the invention encompasses a nucleotide sequence that encodes an antibody of fragment thereof that comprises a nucleotide that encodes a light chain or VL described herein. Exemplary nucleotide sequences encoding the VL regions L1-21G6, L2-21G6 and L3-21G6 are SEQ ID NOs: 61, 62 and 63, respectively (shown below). In some embodiments, the nucleotide sequence that encodes an antibody described herein comprises a sequence selected from the group consisting of SEQ ID NOs: 61, 62 and 63. In yet additional embodiments, the nucleotide sequence that encodes an antibody of antigen-binding fragment thereof comprises a sequence selected from the group consisting of SEQ ID NOs: 56, 57, 58, and 59 and a sequence selected from the group consisting of SEQ ID NOs: 61, 62 and 63. In yet additional embodiments, the nucleotide sequence comprises SEQ ID NO: 59 and SEQ ID NO: 62.

Also encompassed herein are nucleotide sequences that encode antibody, VH and/or VL regions described herein and that are optimized for increased production in specific expression systems. An exemplary method for optimizing expression of mRNA and/or protein is the GENEOPTIMIZER® Process from LifeTechnologies (see, for example, http://www.lifetechnologies.com/us/en/home/life-science/cloning/gene-synthesis/geneart-gene-synthesis/geneoptimizer.html). As shown in FIGs. 3 to 5, SEQ ID NOs: 64, 65, 68 and 69 represent optimized nucleotide sequences for production of the H4-21G6 Vh and L2-21G6 Vl regions in CHO cells. In some embodiments, the invention encompasses a nucleotide sequence of SEQ ID NO: 64. In additional embodiments, the invention encompasses a nucleotide sequence of SEQ ID NO: 65. In yet further embodiments, the nucleotide comprises SEQ ID NO: 64 and SEQ ID NO: 65.

Also encompassed is an expression vector comprising a nucleotide sequence that encodes an antibody or antigen-binding fragment, a VH region and/or a VL region of the invention and an isolated cell comprising said vector. The antibody or antigen-binding fragment, can be produced, for example, by culturing a cell comprising said expression vector, recovering the antibody or fragment thereof from the cultured cells or culture medium. In some embodiments, the antibody or antigen-binding fragment is produced by culturing CHO cells comprising the expression vectors encompassed herein. In certain additional embodiments, the vector comprises a nucleotide sequence comprising SEQ ID NO: 64 and/or SEQ ID NO: 65. In yet additional embodiments, the vector comprises a nucleotide sequence comprising SEQ ID NO: 64 and SEQ ID NO: 65. In yet further

embodiments, the vector comprises a nucleotide sequence comprising SEQ ID NOs: 68 or 69. "Cells" or "host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications can occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

As described above, the antibody and antigen-binding fragment of the invention bind the N2 epitope and can therefore be used for treating a number of inflammatory diseases and conditions that are triggered by binding of natural IgM antibodies. For instance, the antibodies or fragments thereof can be used to treat inflammatory diseases or conditions such as reperfusion injury, ischemia injury, stroke, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, rheumatoid arthritis, celiac disease, hyper-IgG immunodeficiency, arteriosclerosis, coronary artery disease, sepsis, myocarditis, encephalitis, transplant rejection, hepatitis, thyroiditis (e.g., Hashimoto's thyroiditis, Graves disease), osteoporosis, polymyositis, dermatomyositis, drug- or chemotherapy-induced inflammation (e.g., drug or chemotherapy induced nephritis, endocarditis, nephritis), Type I diabetes, gout, dermatitis, alopecia areata, systemic lupus erythematosus, lichen sclerosis, ulcerative colitis, diabetic retinopathy, pelvic inflammatory disease, periodontal disease, arthritis, juvenile chronic arthritis (e.g., chronic iridocyclitis), psoriasis, osteoporosis, nephropathy in diabetes mellitus, asthma, pelvic inflammatory disease, chronic inflammatory liver disease, chronic inflammatory lung disease, lung fibrosis, liver fibrosis, rheumatoid arthritis, chronic inflammatory liver disease, chronic inflammatory lung disease, lung fibrosis, liver fibrosis, Crohn's disease, ulcerative colitis, burn injury (or thermal injury), and other acute and chronic inflammatory diseases of the Central Nervous System (CNS; e.g., multiple sclerosis), gastrointestinal system, the skin and associated structures, the immune system, the hepato-biliary system, or any site in the body where pathology can occur with an inflammatory component.

The invention encompasses methods of inhibiting the activation of an immune response to the N2 antigen in a subject by administering to a subject an antibody described herein. In a further aspect, the invention encompasses methods of treating an inflammatory disease or condition, for example, ischemia-reperfusion injury, in a subject comprising administering to the subject a pharmaceutical composition comprising an antibody or fragment of the invention.

An inflammatory condition such as reperfusion or ischemic injury can result following a naturally occurring episode, including, for example, a stroke or myocardial infarction. Reperfusion or ischemic injury can also occur during and/or following a surgical procedure. Exemplary surgical procedures that cause can cause injury include a vessel-corrective technique selected from the group consisting of angioplasty, stenting procedure, atherectomy, and bypass surgery. In an exemplary embodiment, reperfusion or ischemic injury occurs in a cardiovascular tissue, such as the heart.

Data obtained from animal studies can be used in formulating a range of dosage for use in humans. The dosage of an antibody is within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration. For an antibody or fragment thereof used in the method described herein, the therapeutically effective dose can be estimated initially from *in vitro* assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in *in vitro* assay. This information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, using enzyme linked immunosorbent assay (ELISA).

In some embodiments, an antibody or fragment thereof can be administered prior to, contemporaneously with, or subsequent to a tissue injury. In some embodiments, the pharmaceutical composition can be administered a few hours, a few days or a few weeks after tissue injury. In some embodiments, an antibody or fragment thereof can be administered prior to tissue injury, for example, in subjects at risk for reperfuion injury such as those patients that are about to undergo surgery. In additional embodiments, the antibody or fragment thereof can be administered.

A "therapeutically effective amount" or an "effective amount" is an amount which, alone or in combination with one or more other active agents, can control, decrease, inhibit, ameliorate, prevent or otherwise affect and/or achieve a recited effect. An effective amount of the agent to be administered can be determined using methods well-known in the art. One of skill in the art would take into account the mode of administration, the disease or condition (if any) being treated and the characteristics of the subject, such as general health, other diseases, age, sex, genotype, body weight and tolerance to drugs. A "patient" can refer to a human subject in need of treatment.

The antibody or fragment of the present invention can be provided in pharmaceutically acceptable carriers or formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remmington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. In certain embodiments, the antibody or fragment thereof is provided for transmucosal or transdermal delivery. For such administration, penetrants appropriate to the barrier to be permeated are used in the formulation with the polypeptide. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays or using suppositories. For topical administration, compositions of the invention are formulated into ointments, salves, gels, or creams as generally known in the art.

The pharmaceutical compositions according to the invention are prepared by bringing an antibody or fragment thereof into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include, for example, antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in The Science and Practice of Pharmacy, 20th Edition (Philadelphia College of Pharmacy and Science, 2000) and Remington: The Science and Practice of Pharmacy, 22nd Edition (Pharmaceutical Press and Philadelphia College of Pharmacy at University of the Sciences, 2012), and Remington's Pharmaceutical Sciences, 15th ed. Easton: Mack Publishing Co., 1405-1412, 1461-1487 (1975) and The National Formulary XIV., 14th ed. Washington: American Pharmaceutical Association (1975), the contents of each of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's *The Pharmacological Basis for Therapeutics* (7th ed.) and Goodman and Gilman's *The Pharmacological Basis for Therapeutics*, 12th edition, (McGraw Hill Professional Publishing, 2010).

The pharmaceutical compositions can be prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories and including, for example, alginate based pH dependent release gel caps. For treatment of a subject, depending on activity of the pharmaceutical composition, the manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses can be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or by several smaller dose units and also by multiple administrations of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention can be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject. As discussed above, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, e.g., in Langer, Science, 249: 1527, (1990); Goodman and Gilman's *The Pharmacological Basis for Therapeutics*, 12th edition, (McGraw Hill Professional Publishing, 2010); each of which is herein incorporated by reference.

In one embodiment, the invention provides a pharmaceutical composition useful for administering an antibody or antigen-binding fragment thereof to a subject in need of such treatment. "Administering" the pharmaceutical composition of the invention may be accomplished by any means known to the skilled artisan. A "subject" refers to a mammal, most preferably a human.

The antibody or fragment thereof can be administered parenterally, enterically, by injection, rapid infusion, nasopharyngeal absorption, dermal absorption, rectally and orally. Pharmaceutically acceptable carrier preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers for occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable solid or liquid

pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners and elixirs containing inert diluents commonly used in the art, such as purified water. Where the disease or disorder is a gastrointestinal disorder oral formulations or suppository formulations are preferred.

Sterile injectable solutions can be prepared by incorporating an antibody or antigen-binding fragment thereof in the required amount (e.g., about 10 μg to about 10 mg/kg) in an appropriate solvent and then sterilizing, such as by sterile filtration. Further, powders can be prepared by standard techniques such as freeze drying or vacuum drying.

In another embodiment, antibody or fragment thereof is prepared with a biodegradable carrier for sustained release characteristics for either sustained release in the GI tract or for target organ implantation with long term active agent release characteristics to the intended site of activity. Biodegradable polymers include, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acids, polylactic acids, collagen, polyorthoesters, and poly acetic acid. Liposomal formulation can also be used.

Any route of administration compatible with the active principle can be used. In some embodiments, the route of administration is parenteral administration, such as subcutaneous, intramuscular or intravenous injection. The dose of the antibody or antigen-binding fragment thereof to be administered depends on the basis of the medical prescriptions according to age, weight and the individual response of the patient.

The daily non-weighted dosage for the patient can be between about 2.5-5.0 mg/Kg, e.g., about 2.5-3.0 mg/Kg, about 3.0-3.5 mg/Kg, about 3.5-4.0 mg/Kg, about 4.0-4.5 mg/Kg, and about 4.5-5.0 mg/Kg.

The pharmaceutical composition for parenteral administration can be prepared in an injectable form comprising the active principle and a suitable vehicle. Vehicles for the parenteral administration are well known in the art and comprise, for example, water, saline solution, Ringer solution and/or dextrose. The vehicle can contain small amounts of excipients in order to maintain the stability and isotonicity of the pharmaceutical preparation. The preparation of the cited solutions can be carried out according to the ordinary modalities.

The present invention has been described with reference to the specific embodiments, but the content of the description comprises all modifications and substitutions, including conservative amino acid substitutions, which can be brought by a person skilled in the art without extending beyond the meaning and purpose of the claims. The compositions can, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least about 30%, preferably at least about 40%, more preferably at least about 50%, about 60%, and even more preferably at least about 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences and the percent homology between two sequences is a function of the number of conserved positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity and/or homology between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) J. Mol. Biol. 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available on the internet at the Accelrys website, more specifically at http://www.accelrys.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software

package (available on the world wide web with the extension gcg.com), using a NWSgapdna CMP matrix and a gap weight of 40, 50, 60, 70; or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frame shift gap penalty of 5.

The percent identity and/or homology between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

"Stringency hybridization" or "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" is used herein to describe conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6:3.6, which is incorporated by reference. Aqueous and non-aqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified. Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as described above.

The amino acid sequences referred to in the present application are listed below with the corresponding sequence identifier (SEQ ID NO):

m21G6 VH
QVQLQQPGAELVKPGASVKLSCKASGYTFTSYYMYWVKQRPGQGLEWIGGINPS NGGTNFNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCTRWGYDREWFAY WGQGTLVTVSA (SEQ ID NO: 1).

m21G6 VL
DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQVLIYRM
SNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIK
R (SEQ ID NO: 2).

VH CDR1
SYYMY (SEQ ID NO: 3).

VH CDR2
GINPSNGGTNFNEKFKS (SEQ ID NO: 4).

VH CDR3
GYDREWFAY (SEQ ID NO: 5).

VL CDR1
RSSKSLLHSNGNTYLY (SEQ ID NO: 6).

VL CDR2
RMSNLAS (SEQ ID NO: 7).

VL CDR3
MQHLEYPFT (SEQ ID NO: 8).

H1-21G6 Vh
QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYYMYWVKQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATLTVDKSASTAYMELSSLRSEDTAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 9).

H2-21G6 Vh
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATMTVDKSTSTAYMELRSLRSDDSAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 10).

H3-21G6 Vh
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATITVDKSTSTAYMELSSLRSEDTAVYYCTRWGYDREWFAY
WGQGTLVTVSS (SEQ ID NO: 11).

H4-21G6 Vh
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWMGGINP
SNGGTNFNEKFKSRVTMTTDTSTSTAYMELRSLRSDDTAVYYCTRWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 49).

L1-21G6 Vl (PopVk, CLL)
DIVMTQSPATLSVSPGERATISCRSSKSLLHSNGNTYLYWFQQKPGQPPKVLIYRM
SNLASGVPARFSGSGSGTDFTLTISSVEPEDFATYYCMQHLEYPFTFGGGTKLEIKR
(SEQ ID NO: 12).

L2-21G6 Vl
DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMS
NLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIK
R (SEQ ID NO: 13).

L3-m21G6 Vl
DIVMTQTPLSLSYTPGQPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMS
NLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIK
R (SEQ ID NO: 14).

H1 VH FWR1
QVQLVQSGAEVVKPGASVKLSCKASGYTFT (SEQ ID NO: 15).

H1 VH FWR2
WVKQAPGQGLEWIG (SEQ ID NO: 16).

H1 VH FWR3
KATLTVDKSASTAYMELSSLRSEDTAVYYCTR (SEQ ID NO: 17).

H1 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 18).

H2 VH FWR1
QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 19).

H2 VH FWR2
WVRQAPGQGLEWIG (SEQ ID NO: 20).

H2 VH FWR3
KATMTVDKSTSTAYMELRSLRSDDSAVYYCTR (SEQ ID NO: 21).

H2 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 22).

H3 VH FWR1
QVQLVQSGAEVKKPGSSVKVSCKASGYTFT (SEQ ID NO: 23).

H3 VH FWR2
WVRQAPGQGLEWIG (SEQ ID NO: 24).

H3 VH FWR3
KATITVDKSTSTAYMELSSLRSEDTAVYYCTR (SEQ ID NO: 25).

H3 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 26).

H4 VH FWR1
QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 19)

H4 VH FWR2
WVRQAPGQGLEWMG (SEQ ID NO: 50).

H4 VH FWR3
RVTMTTDTSTSTAYMELRSLRSDDTAVYYCTR (SEQ ID NO: 51).

H4 VH FWR4
WGQGTLVTVSS (SEQ ID NO: 22).

L1 VL FWR1
DIVMTQSPATLSVSPGERATISC (SEQ ID NO: 27).

L1 VL FWR2
WFQQKPGQPPKVLIY (SEQ ID NO: 28).

L1 VL FWR3
GVPARFSGSGSGTDFTLTISSVEPEDFATYYC (SEQ ID NO: 29).

L1 VL FWR4
FGGGTKLEIKR (SEQ ID NO: 30).

L2 VL FWR1
DIVMTQSPLSLPVTPGEPASISC (SEQ ID NO: 31).

L2 VL FWR2
WFLQKPGQSPQLLIY (SEQ ID NO: 32).

L2 VL FWR3
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 33).

L2 VL FWR4
FGQGTKLEIKR (SEQ ID NO: 34).

L3 VL FWR1
DIVMTQTPLSLSYTPGQPASISC (SEQ ID NO: 35).

L3 VL FWR2
WFLQKPGQSPQLLIY (SEQ ID NO: 36).

L3 VL FWR3
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 37).

L3 VL FWR4
FGQGTKLEIKR (SEQ ID NO: 38).

H1 VH FWR3 with amino acid mutation to alanine
KATLTVDKSASTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 39).

H2 VH FWR3 with amino acid mutation to alanine
KATMTVDKSTSTAYMELRSLRSDDSAVYYCAR (SEQ ID NO: 40).

H3 VH FWR3 with amino acid mutation to alanine
KATITVDKSTSTAYMELSSLRSEDTAVYYCAR (SEQ ID NO: 41).

H4 VH FWR3
RVTMTTDTSTSTAYMELRSLRSDDTAVYYC*A*R (SEQ ID NO: 52)

Amino Acid Variant of H2 Vh
QVQLVQSGAEVKKPGASLKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKGRVTITRDKSTSTAYMELRSLRSEDSAVYYCARWGYDREWFAY
WGQGTLVTVSS (SEQ ID NO: 42).

H1 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYYMYWVKQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATLTVDKSASTAYMELSSLRSEDTAVYYCARWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 43).

H2 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATMTVDKSTSTAYMELRSLRSDDSAVYYCARWGYDREWFA
YWGQGTLVTVSS (SEQ ID NO: 44).

H3 with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPS
NGGTNFNEKFKSKATITVDKSTSTAYMELSSLRSEDTAVYYCARWGYDREWFAY
WGQGTLVTVSS (SEQ ID NO: 45).

H4 VH with amino acid mutation to alanine in FWR3
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWMGGINP
SNGGTNFNEKFKSRVTMTTDTSTSTAYMELRSLRSDDTAVYYC*A*RWGYDREWF
AYWGQGTLVTVSS (SEQ ID NO: 53).

Amino Acid Variant of kappa chain (m21G6 Vl)
EIVLTQSPGTLSLSP GERATLSCRAS
KSLLHSNGNTYLYWYQQKPGQAPRLLIYRMS NRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYC MQHLEYPFTFGQGTKLEIKR (SEQ ID
NO: 46).

N2 peptide

LMKNMDPLNDNV (SEQ ID NO: 47).

Peptide sequence

LMKNMDPLNDNI (SEQ ID NO: 48).

H4-21G6 Vh with leader sequence and the constant region of human IgG1

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMY
WVRQAPGQGLEWMGGINPSNGGTNFNEKFKSRVTMTTDTSTSTAYMELRSLRSD
DTAVYYCTRWGYDREWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 54).

L2-21G6 Vl with leader sequence and the constant region of the human kappa light chain

MGWSCIILFLVATATGVHGDIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGNTYL
YWFLQKPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC
MQHLEYPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC (SEQ ID NO: 55).

Nucleotide sequence encoding H1-21G6 Vh

CAGGTCCAACTGGTGCAGtCTGGGGCTGAAGTGGTGAAGCCTGGGGCTTCAGT
GAAGTTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATGTACTG
GGTGAAGCAGGCGCCTGGACAAGGCCTTGAGTGGATTGGGGGGATTAATCCT
AGCAATGGTGGTACTAACTTCAATGAGAAGTTCAAGAGCAAGGCCACACTGA
CTGTAGACAAATCCGCCAGCACAGCCTACATGGAACTCAGCAGCCTGAGATCT
GAGGACACTGCGGTCTATTACTGTACAAGATGGGGTTACGACAGGGAGTGGTT
TGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA (SEQ ID NO: 56).

Nucleotide sequence encoding H2-21G6 Vh

CAGGTCCAACTGGTGCAGTCTGGGGCTGAAGTGAAGAAGCCTGGGGCTTCAG
TGAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATGTACT
GGGTGAGGCAGGCGCCTGGACAAGGCCTTGAGTGGATTGGGGGGATTAATCC
TAGCAATGGTGGTACTAACTTCAATGAGAAGTTCAAGAGCAAGGCCACAATG
ACTGTAGACAAATCCACCAGCACAGCCTACATGGAACTCCGCAGCCTGAGATC
TGACGACACTGCGGTCTATTACTGTACAAGATGGGGTTACGACAGGGAGTGGT
TTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA (SEQ ID NO: 57).

Nucleotide sequence encoding H3-21G6 Vh

CAGGTCCAACTGGTGCAGTCTGGGGCTGAAGTGAAGAAGCCTGGGTCTTCAGT
GAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATGTACT
GGGTGAGGCAGGCGCCTGGACAAGGCCTTGAGTGGATTGGGGGGATTAATCC
TAGCAATGGTGGTACTAACTTCAATGAGAAGTTCAAGAGCAAGGCCACAATC
ACTGTAGACAAATCCACCAGCACAGCCTACATGGAACTCAGCAGcCTGAGATC
TGAGGACACTGCGGTCTATTACTGTACAAGATGGGGTTACGACAGGGAGTGGT
TTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA (SEQ ID NO: 58).

Nucloetide sequence encoding H4-21G6 Vh

CAGGTCCAACTGGTGCAGTCTGGGGCTGAAGTGAAGAAGCCTGGGGCTTCAG
TGAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATGTACT
GGGTGAGGCAGGCGCCTGGACAAGGCCTTGAGTGGATGGGGGGGATTAATCC
TAGCAATGGTGGTACTAACTTCAATGAGAAGTTCAAGAGCAGGGTCACAATG
ACTACAGACACATCCACCAGCACAGCCTACATGGAACTCCGCAGCCTGAGATC
TGACGACACTGCGGTCTATTACTGTACAAGATGGGGTTACGACAGGGAGTGGT
TTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCA (SEQ ID NO: 59).

Optimized nucleotide sequence encoding H4-21G6 Vh including intron from vector, leader sequence and IgG1 constant region

CACTATAGGGCGAATTGAAGGAAGGCCGTCAAGGCCGCATCCTAGGGCCACC
ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTCGCCACCGCCACCGGCGTGCA
CTCCCAGGTCCAGCTGGTCCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGCCT
CCGTGAAGGTGTCCTGCAAGGCCTCCGGCTACACCTTCACCAGCTACTACATG
TACTGGGTCCGACAGGCCCCAGGCCAGGGACTGGAATGGATGGGCGGCATCA
ACCCCTCCAACGGCGGCACCAACTTCAACGAGAAGTTCAAGTCCAGAGTGAC
CATGACCACCGACACCTCCACCTCCACCGCCTACATGGAACTGCGGTCCCTGC
GGAGCGACGACACCGCCGTGTACTACTGCACCAGATGGGGCTACGACAGAGA
GTGGTTCGCCTACTGGGGCCAGGGCACCCTGGTCACAGTGTCCTCCGCTTCCA
CCAAGGGCCCCTCCGTGTTCCCTCTGGCCCCCTCCAGCAAGTCCACCTCTGGC
GGCACCGCTGCCCTGGGCTGCCTGGTCAAAGACTACTTCCCCGAGCCCGTGAC
CGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTCCCTGCCG
TGCTGCAGTCTTCCGGCCTGTACTCCCTGTCCTCCGTGGTCACCGTGCCCTCCA
GCTCTCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAAC
ACCAAGGTGGACAAGCGGGTGGAACCCAAGTCCTGCGACAAGACCCACACCT
GTCCCCCCTGCCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTCC
CCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGC
GTGGTGGTGGACGTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGT
GGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAGTA
CCAGTCCACCTACCGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGC
TGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCC
CATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTG
TACACACTGCCCCCTAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGA
CCTGTCTGGTCAAAGGCTTCTACCCCTCCGACATTGCCGTGGAATGGGAGTCC
AACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCG
ACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAG
CAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTA
CACCCAGAAGTCCCTGTCCCTGAGCCCCGGCAAGTGATGAGTATACCTGGGCC
TCATGGGCCTTCCTTTCACTGCCCGCTTTCCAG (SEQ ID NO: 60).

Nucleotide sequence encoding L1-21G6 Vl

GATATTGTGATGACTCAGTCTCCAGCCACTCTATCTGTCTCTCCTGGAGAGCGA
GCAACCATCTCCTGCAGGGCTAGTAAGAGTCTCCTGCATAGTAATGGCAACAC

TTACTTGTATTGGTTCCAGCAGAAGCCAGGCCAGCCTCCTAAGGTCCTGATAT
ATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGCCAGGTTCAGTGGCAGTGGG
TCAGGAACTGATTTCACACTGACAATCAGTTCggtgGAGccTGAGGATTTTGCTA
CTTATTACTGTATGCAACATCTAGAATATCCATTCACGTTCGGCGGGGGGACA
AAGTTGGAAATAAAACG (SEQ ID NO: 61).

Nucleotide sequence encoding L2-21G6 Vl

GATATTGTGATGACTCAGTCTCCACTCTCTCTACCTGTCACTCCTGGAGAGcCA
GCATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAACAC
TTACTTGTATTGGTTCCTGCAGAAGCCAGGCCAGTCTCCTCAGcTCCTGATATA
TCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTGGCAGTGGGT
CAGGAACTGcTTTCACACTGAAAATCAGTAGAGTGGAGGCTGAGGATGTGGGT
GTTTATTACTGTATGCAACATCTAGAATATCCATTCACGTTCGGCCAGGGGAC
AAAGCTGGAAATAAAACG (SEQ ID NO: 62).

Nucleotide sequence encoding L3-21G6 Vl

GATATTGTGATGACTCAGACTCCACTCTCTCTAtCTGTCACTCCTGGAcAGcCAG
CATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAACACT
TACTTGTATTGGTTCCTGCAGAAGCCAGGCCAGTCTCCTCAGcTCCTGATATAT
CGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTGGCAGTGGGTC
AGGAACTGaTTTCACACTGAAAATCAGTAGAGTGGAGGCTGAGGATGTGGGTG
TTTATTACTGTATGCAACATCTAGAATATCCATTCACGTTCGGCCAGGGGACA
AAGCTGGAAATAAAACG (SEQ ID NO: 63).

Optimized nucleotide sequence encoding H4-21G6 Vh

CAGGTCCAGCTGGTCCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGCCTCCGT
GAAGGTGTCCTGCAAGGCCTCCGGCTACACCTTCACCAGCTACTACATGTACT
GGGTCCGACAGGCCCCAGGCCAGGGACTGGAATGGATGGGCGGCATCAACCC
CTCCAACGGCGGCACCAACTTCAACGAGAAGTTCAAGTCCAGAGTGACCATG
ACCACCGACACCTCCACCTCCACCGCCTACATGGAACTGCGGTCCCTGCGGAG
CGACGACACCGCCGTGTACTACTGCACCAGATGGGGCTACGACAGAGAGTGG
TTCGCCTACTGGGGCCAGGGCACCCTGGTCACAGTGTCCTCC (SEQ ID NO: 64).

Optimized nucleotide sequence encoding L2-21G6 Vl

GACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACACCTGGCGAGCC
TGCCTCCATCTCCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCAATAC
CTACCTGTACTGGTTCCTGCAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCT
ACCGGATGTCCAACCTGGCCTCCGGCGTGCCCGACAGATTCTCCGGCTCTGGC
TCTGGCACAGCCTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGACGTGGG
CGTGTACTACTGCATGCAGCACCTGGAATACCCCTTCACCTTCGGCCAGGGCA
CCAAGCTGGAAATCAAGCGG (SEQ ID NO: 65).

Nucleotide sequence encoding H4-21G6 Vh including leader sequence and constant region

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTCCAC
TCCCAGGTCCAACTGGTGCAGTCTGGGGCTGAAGTGAAGAAGCCTGGGGCTTC
AGTGAAGGTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATGT
ACTGGGTGAGGCAGGCGCCTGGACAAGGCCTTGAGTGGATGGGGGGGATTAA
TCCTAGCAATGGTGGTACTAACTTCAATGAGAAGTTCAAGAGCAGGGTCACAA
TGACTACAGACACATCCACCAGCACAGCCTACATGGAACTCCGCAGCCTGAG
ATCTGACGACACTGCGGTCTATTACTGTACAAGATGGGGTTACGACAGGGAGT
GGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCAGCGTCGACC
AAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCTGTGACGG
TCTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC
CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
ACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACAT
GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTC
CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATG
CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
ACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGG
CTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC
GACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
ACACGCAGAAGAGCCTCTCCCTGTCCCCGGGTAAATGA (SEQ ID NO: 66).

Nucleotide sequence encoding L2-21G6 Vl including leader sequence and human kappa light chain

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACAT
GGGGATATTGTGATGACTCAGTCTCCACTCTCTCTACCTGTCACTCCTGGAGAG
CCAGCATCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAA
CACTTACTTGTATTGGTTCCTGCAGAAGCCAGGCCAGTCTCCTCAGCTCCTGAT
ATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTGGCAGTG
GGTCAGGAACTGCTTTCACACTGAAAATCAGTAGAGTGGAGGCTGAGGATGT
GGGTGTTTATTACTGTATGCAACATCTAGAATATCCATTCACGTTCGGCCAGG
GGACAAAGCTGGAAATAAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCT
GAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC
CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 67).

Optimized nucleotide sequence encoding H4-21G6 Vh with leader sequence and IgG1 constant region

ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTCGCCACCGCCACCGGCGTGCA
CTCCCAGGTCCAGCTGGTCCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGCCT
CCGTGAAGGTGTCCTGCAAGGCCTCCGGCTACACCTTCACCAGCTACTACATG
TACTGGGTCCGACAGGCCCCAGGCCAGGGACTGGAATGGATGGGCGGCATCA
ACCCCTCCAACGGCGGCACCAACTTCAACGAGAAGTTCAAGTCCAGAGTGAC
CATGACCACCGACACCTCCACCTCCACCGCCTACATGGAACTGCGGTCCCTGC
GGAGCGACGACACCGCCGTGTACTACTGCACCAGATGGGGCTACGACAGAGA
GTGGTTCGCCTACTGGGGCCAGGGCACCCTGGTCACAGTGTCCTCCGCTTCCA
CCAAGGGCCCCTCCGTGTTCCCTCTGGCCCCCTCCAGCAAGTCCACCTCTGGC
GGCACCGCTGCCCTGGGCTGCCTGGTCAAAGACTACTTCCCCGAGCCCGTGAC
CGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTCCCTGCCG
TGCTGCAGTCTTCCGGCCTGTACTCCCTGTCCTCCGTGGTCACCGTGCCCTCCA
GCTCTCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAAC
ACCAAGGTGGACAAGCGGGTGGAACCCAAGTCCTGCGACAAGACCCACACCT
GTCCCCCCTGCCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTCCTGTTCC
CCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGC
GTGGTGGTGGACGTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGT
GGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAGTA
CCAGTCCACCTACCGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGC
TGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCC
CATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTG
TACACACTGCCCCCTAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGA
CCTGTCTGGTCAAAGGCTTCTACCCCTCCGACATTGCCGTGGAATGGGAGTCC
AACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCG
ACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAG
CAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTA
CACCCAGAAGTCCCTGTCCCTGAGCCCCGGCAAGTGATGAGTATACCTGGGCC
TCATGGGCCTTCCTTTCACTGCCCGCTTTCCAG (SEQ ID NO: 68).

Optimized nucleotide sequence encoding L2-21G6 Vl including leader sequence and human kappa light chain

CGAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCAGAGCTCGATATCG
CCACCATGGGCTGGTCCTGCATCATCCTGTTTCTGGTCGCCACCGCCACCGGC
GTGCACGGCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACACC
TGGCGAGCCTGCCTCCATCTCCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAA
CGGCAATACCTACCTGTACTGGTTCCTGCAGAAGCCCGGCCAGTCCCCTCAGC
TGCTGATCTACCGGATGTCCAACCTGGCCTCCGGCGTGCCCGACAGATTCTCC
GGCTCTGGCTCTGGCACAGCCTTCACCCTGAAGATCTCCCGGGTGGAAGCCGA
GGACGTGGGCGTGTACTACTGCATGCAGCACCTGGAATACCCCTTCACCTTCG
GCCAGGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTC
ATCTTCCCACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTCTG
CCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGAC
AACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTCACCGAGCAGGACTCCA
AGGACAGCACCTACTCCCTGTCTTCCACCCTGACCCTGTCCAAGGCCGACTAC

GAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCC
CCGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGATGATTAATTAAGGTACCT
GGAGCACAAGACTGGCCTCATGGGCCTTCCGCTCACTGC (SEQ ID NO: 69).

The invention is illustrated by the following non-limiting examples.

EXEMPLIFICATION

Example 1: Humanization of murine antibody 21G6

Murine 21G6 is an IgG1 heavy chain and kappa light chain that was raised against the non-muscle myosin neo-epitope N2 12mer sequence: LMKNMDPLNDNV (SEQ ID NO: 47). The murine 21G6 antibody is described in more detail in U.S. Patent No. 8,324,352, the contents of which are expressly incorporated herein. Using the IMGT database (http://www.imgt.org), a search was performed to identify the human germline antibody sequences with the greatest homology to the murine 21G6 antibody. In addition, a BLAST search was performed to identify homologous human non-germline antibodies. The sequences shown in FIG. 1 were determined to have the highest amino acid homology.

FIG. 1 shows a sequence comparison for the murine 21G6 heavy chain variable (VH) region variable heavy and the humanized heavy chain variable regions (VH) H1-21G6, H2-21G6 and H3-21G6 and also shows a sequence comparison of the murine 21G6 light chain variable (VL) region and the humanized light chain variable regions light chain (VL) regions L1-21G6, L2-21G6 and L3-21G6.

The H1-21G6 and L1-21G6 frameworks were derived from B-cells obtained from lupus and chronic lymphocytic leukemia (CLL) patients for the heavy and light chains, respectively. The remaining sequences represent the germline sequences with the highest homology that encode productive antibody. All of the humanized sequences maintain the murine 21G6 CDR regions which are shown inside the boxes in FIG. 1. The humanized variable regions were cloned into a vector containing wild type human IgG1, human IgG1 containing a mutation at amino acid 297 (Asn 297 to Q297), human IgG4 containing a mutation at amino acid 228 (serine to proline) and human kappa light chain. Each antibody (heavy and light) combination was expressed by transient co-transfection in 293A cells (in the presence of low Ig serum). The antibody containing supernatants were collected and analyzed for binding to the N2 peptide by ELISA and Biacore. All

experiments were performed using a Biacore X100 system. For antibody capture experiments, a CM5 chip was prepared by 10 ul/minute injection of EDC/NHS (*N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS) for 7 minutes, followed by a 10 ul/minute injection of anti-human Fc (GE Lifesciences) at a concentration of 25ug/ml in sodium acetate at pH 5 for 3 minutes. Ethanolamine-HCl was injected for 7 minutes at 10 ul/minute. The chimeric or humanized antibodies were captured onto flow cell 2 and N2 peptide at varying concentrations was flowed over flow cells 1 and 2 at a rate of 30ul/minute with a contact time of 120 seconds and a dissociation period of 120 seconds. Complete removal of captured antibody was accomplished by regeneration with 3M MgCl2 for 30 seconds at a flow rate of 10ul/minute. For peptide immobilized experiments, a CM5 chip was prepared by 10ul/minute injection of EDC/NHS (*N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS) for 7 minutes, followed by a 10ul/minute injection of Neutravidin (ThermoFisher Pierce) at a concentration of 5ug/ml in sodium acetate, pH 5 to an immobilization response of 100-200RU. Ethanolamine-HCl was injected for 7 minutes at 10ul/minute. Biotin-labeled N2 peptide was captured on flow channel 2 with the goal of an experimental Rmax of about 50-100RU. Purified chimeric or humanized antibodies were flowed over both flow channels at a rate of 30ul/min for a contact time of 120 seconds and allowed to dissociate for 600 seconds. Regeneration was achieved with glycine pH 1.7 for 30 seconds thus retaining an active surface. The apparent affinity constants and antibody on/off rates are shown in Tables 1A, 1B, 2, 3A and 3B below:

Table 1A: Apparent Affinity Constants for variable regions and wild type human IgG1 heavy chain (Immobilized Antibody, N2 peptide in solution)

Heavy Chain	**Light Chain**	**Kd (uM) by IgG capture**
H1	L2	17
H1	L3	20.9

H1	L1	6.6-7.7
H2	L2	6.4-8.4
H2	L3	9.56
H2	L1	9.8-11
H3	L2	11.5
H3	L3	9.38
H3	L1	7.9
m21G6	m21G6	4-6
CHIgG1 (human heavy chain constant region IgG1)	Ch	6-7
CHIgG4 (human heavy chain constant region IgG4)	Ch	4.1

Table 1B: Apparent Affinity Constants for variable regions and human IgG1 heavy chain with mutation at position 297 from Asn to Gln (Immobilized Antibody, N2 peptide in solution)

Heavy Chain (N297Q)	**Light Chain**	**Kd (uM) by IgG capture**

H1	L2	TBD
H1	L3	TBD
H1	L1	9
H2	L2	3.1-3.7
H2	L3	8.8
H2	L1	TBD
H3	L2	TBD
H3	L1	20
H3	L3	TBD
CHIgG1 (human heavy chain constant region IgG1)	CH	5.6

(TBD indicated "to be determined")

Table 2: Antibody on- and off-rates (immobilized N2, antibody in solution)

Heavy Chain	**Light Chain**	**Ka (e+4) (on-rate)**	**kd (e-3) (off-rate)**	**Affinity (nM) kd/ka (monovalent)**	**Steady state affinity (nM)**
H1	L3	0.63	9.93	1580	1000
H1	L1	1.5 - 4.3	4.3 - 6.5	100-430	320
H2	L2	2.0 - 3.2	8.6 - 9.1	280-420	240

H2	L3	2.4	3.5	140	680
H2	L1	0.7	4.63	660	NA
H3	L2	1.2	7.2	640	NA
H3	L3	0.60	5.61	900	NA
H3	L1	2.0 - 5.2	3 – 6.5	300	280
ch21G6 N297Q (human heavy chain constant region IgG1 with Q297 mutation)	ch21G6	7-10	7-19	100-200	196-240
ch21G6 IgG4 (human heavy chain constant region IgG4)	ch21G6	5	40	800	113
m21G6	m21G6	1 – 11	5 - 53	200-400	313-540

Table 3A: Apparent Affinity Constant for variable region H4 and IgG1 heavy chain with mutation at position 297 from Asn to Gln (Immobilized Antibody, N2 peptide in solution)

Heavy Chain (N297Q)	**Light Chain**	**Kd (uM) by IgG capture and direct immobilization**
H4	L2	6-11

Table 3B: Antibody on- and off-rates (immobilized N2, antibody in solution)

Heavy Chain	Light Chain	Ka (e+4) (on-rate)	kd (e-3) (off-rate)	Affinity (nM) kd/ka (monovalent)	Steady state affinity (nM)
H4	L2	2	2	100	TBD

As determined by t-test, there was no significant difference between murine 21G6 and H1/L1 and murine 21G6 and H2/L2. (Murine 21G6: n =4; 9r/9r: n=3; 69/9r: n=2).

Example 2: Optimization of nucleotide sequences for increased production in CHO cells

Optimization was performed in order to use codon preferences of Cricetulus griseus (Chinese hamster) for optimal expression of the humanized antibody. The GeneOptimizer program also optimized sequence to prevent aberrant mRNA splicing, eliminate undesirable polyA binding motifs, optimize GC content of the gene and prevent unwanted secondary RNA structures that might decrease protein translation efficiency, thereby increasing overall antibody expression. Amino acid and nucleotides sequences for H4-21G6 and L2-21G6 (SEQ ID NOs: 54, 66, 55 and 67, respectively) were sent to Life Technologies for optimization using the GENEOPTIMIZER® Process described at http://www.lifetechnologies.com/us/en/home/life-science/cloning/gene-synthesis/geneart-gene-synthesis/geneoptimizer.html (the contents of which are expressly incorporated by reference herein). FIG. 3 shows a nucleotide sequence encoding the humanized heavy chain variable region H4 that was optimized for the production of the humanized antibody in Chinese hamster ovary (CHO) cells where each + indicates where a change was made as compared to SEQ ID NO: 66. FIG. 4 shows a nucleotide sequence encoding the humanized light chain variable region L2 that was optimized for the production of the humanized antibody in Chinese hamster ovary (CHO) cells. Each + indicates where a change was made as compared to SEQ ID NO: 67. Figure 5 depicts the levels of recombinant antibody expression on day 5 post-transfection with either native (red) or optimized (blue) antibody sequences. Incorporation of the recombinant antibody sequence into the CHO cells was accomplished by selection in the presence of 10ug/ml puromycin

and 100mM methotrexate for transfectants DS5 and DS7 or 20ug/ml puromycin and 200mM methotrexate for transfectants DS6 and DS8.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 comprises SEQ ID NO: 3, VH CDR2 comprises SEQ ID NO: 4 and VH CDR3 comprises SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45; and

d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 comprises SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 comprises SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and

d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

2. The antibody or antigen-binding fragment of claim 1, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 consists of SEQ ID NO:3 , VH CDR2 consists of SEQ ID NO: 4 and VH CDR3 consists of SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 consists of SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 consists of SEQ ID NO: 16, SEQ ID NO: 20 or SEQ ID NO:24;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44 or SEQ ID NO: 45; and

d. VH FWR4 consists of SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 consists of SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 consists of SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 consists of SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 consists of SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 consists of SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and

d. VL FWR4 consists of SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

3. The antibody or antigen-binding fragment of claim 1, wherein the VH region comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

4. The antibody or antigen-binding fragment of claim 1, wherein the VL region comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

5. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

6. The antibody or antigen-binding fragment of claim 1, wherein the VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO:13 and SEQ ID NO: 14.

7. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 9 and the VL region consists of the amino acid sequence of SEQ ID NO: 12.

8. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 9 and the VL region consists of the amino acid sequence of SEQ ID NO: 13.

9. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 9 and the VL region consists of the amino acid sequence of SEQ ID NO: 14.

10. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 10 and the VL region consists of the amino acid sequence of SEQ ID NO: 12.

11. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 10 and the VL region consists of the amino acid sequence of SEQ ID NO: 13.

12. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 10 and the VL regions consists of the amino acid sequence of SEQ ID NO: 14.

13. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 11 and the VL region consists of the amino acid sequence of SEQ ID NO: 12.

14. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 11 and the VL region consists of the amino acid sequence of SEQ ID NO: 13.

15. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 11 and the VL region consists of the amino acid sequence of SEQ ID NO: 14.

16. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 43 and VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

17. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 44 and VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

18. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 45 and VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

19. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 42 and VL region consists of a

sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

20. A humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region comprising a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

21. A humanized, anti-N2 antibody or antigen-binding fragment thereof, comprising a light chain variable (VL) region comprising a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

22. The antibody or antigen-binding fragment of any one of claims 1 to 21, wherein the isotype of the constant region is IgG1, IgG2, IgG3, or IgG4.

23. The antibody or antigen-binding fragment of claim 21, wherein the isotype of the IgG constant region is IgG1.

24. The antibody or antigen-binding fragment of claim 21, wherein the isotype of the IgG constant region is IgG4.

25. The antibody or antigen-binding fragment of any one of claims 1 to 21 having a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgG1 constant domain and a human IgG4 constant domain.

26. The antibody or antigen-binding fragment of any one of claims 1 to 21 having a human Ig kappa constant domain.

27. The antibody or antigen-binding fragment of any one claims 1 to 26, wherein the antibody is aglycosylated.

28. The antibody or antigen-binding fragment of claims 1 to 21 having a human IgG1 constant domain that is aglycosylated by replacing the amino acid corresponding to asparagine (Asn) 297 of the constant region heavy chain with an alternative amino acid residue.

29. The antibody or antigen-binding fragment of claim 28, wherein the Asn 297 is replaced with glutamine, alanine, histidine or glycine.

30. The antibody or antigen-binding fragment of claim 29, wherein the Asn 297 is replaced with glutamine.

31. The antibody or antigen-binding fragment of any one of claims 1 to 26, wherein the heavy chain immunoglobulin constant domain is a human IgG4 constant domain wherein serine 228 is replaced with proline.

32. The antibody or antigen-binding fragment of claim 1, which is a scFv, diabody, Fab, minibody or scFv-Fc.

33. A nucleotide sequence encoding the antibody or antigen-binding fragment of any one claims 1 to 32.

34. A vector comprising the nucleotide sequence of claim 33.

35. An isolated cell comprising the vector of claim 34.

36. A method for producing a humanized antibody, or antigen-binding fragment thereof, comprising culturing the cell of claim 35 and recovering the antibody, or fragment thereof, from the culture medium or from said cultured cells.

37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antigen-binding fragment of any one of claims 1 to 32.

38. A method of treating an inflammatory disease or disorder comprising administering to a subject an effective amount of the antibody or antigen-binding fragment of any one of claims 1 to 32.

39. The method of claim 38, wherein the inflammatory disease or disorder is ischemia-reperfusion injury.

40. The method of claim 38, wherein the subject is a mammal.

41. The method of claim 40, wherein the mammal is a human.

42. The method of claim 39, wherein the ischemia-reperfusion results after myocardial infarction, stroke or a surgical procedure.

43. A humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 comprises SEQ ID NO: 3, VH CDR2 comprises SEQ ID NO: 4 and VH CDR3 comprises SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 comprises SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 comprises SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO:24 or SEQ ID NO: 50;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 51 or SEQ ID NO: 52; and

d. VH FWR4 comprises SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 comprises SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 comprises SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 comprises SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 comprises SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 comprises SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and

d. VL FWR4 comprises SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

44. The antibody or antigen-binding fragment of claim 43, wherein:

i. the VH region comprises three complementarity determining regions (CDRs) VH CDR1, VH CDR2 and VH CDR3 wherein the VH CDR1 consists of SEQ ID NO:3 , VH CDR2 consists of SEQ ID NO: 4 and VH CDR3 consists of SEQ ID NO: 5;

ii. the VH region comprises four framework regions (FWR) VH FWR1, VH FWR2, VH FWR3 and VH FWR4 wherein:

a. the VH FWR1 consists of SEQ ID NO: 15 , SEQ ID NO: 19 or SEQ ID NO: 23;

b. The VH FWR2 consists of SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO:24 or SEQ ID NO: 50;

c. VH FWR3 comprises SEQ ID NO: 17, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 51 or SEQ ID NO: 52; and

d. VH FWR4 consists of SEQ ID NO: 18, SEQ ID NO: 22, or SEQ ID NO: 26;

iii. the VL region comprises three complementarity determining regions (CDRs) VL CDR1, VL CDR2 and VL CDR3 wherein the VL CDR1 consists of SEQ ID NO: 6, VH CDR2 comprises SEQ ID NO: 7 and VH CDR3 consists of SEQ ID NO: 8;

iv. the VL region comprises four framework regions (FWR) VL FWR1, VL FWR2, VL FWR3 and VL FWR4 wherein:

a. the VL FWR1 consists of SEQ ID NO: 27, SEQ ID NO: 31, or SEQ ID NO: 35;

b. VL FWR2 consists of SEQ ID NO: 28, SEQ ID NO: 32, or SEQ ID NO: 36;

c. VL FWR3 consists of SEQ ID NO: 29, SEQ ID NO: 33, or SEQ ID NO: 37; and

d. VL FWR4 consists of SEQ ID NO: 30, SEQ ID NO: 34, or SEQ ID NO: 38.

45. The antibody or antigen-binding fragment of claim 43, wherein the VH region comprises a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

46. The antibody or antigen-binding fragment of claim 43, wherein the VL region comprises a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

47. The antibody or antigen-binding fragment of claim 43, wherein the VH region consists of a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

48. The antibody or antigen-binding fragment of claim 43, wherein the VL region consists of a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO:13 and SEQ ID NO: 14.

49. The antibody or antigen-binding fragment of claim 43, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 12.

50. The antibody or antigen-binding fragment of claim 43, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 13.

51. The antibody or antigen-binding fragment of claim 1, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 49 and the VL region consists of the amino acid sequence of SEQ ID NO: 14.

52. The antibody or antigen-binding fragment of claim 43, wherein the VH region consists of the amino acid sequence of SEQ ID NO: 54 and VL region consists of a

sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

53. A humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region comprising a sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

54. The antibody or antigen-binding fragment of any one of claims 43 to 53, wherein the isotype of the constant region is IgG1, IgG2, IgG3, or IgG4.

55. The antibody or antigen-binding fragment of claim 54, wherein the isotype of the IgG constant region is IgG1.

56. The antibody or antigen-binding fragment of claim 54, wherein the isotype of the IgG constant region is IgG4.

57. The antibody or antigen-binding fragment of any one of claims 43 to 53 having a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgG1 constant domain and a human IgG4 constant domain.

58. The antibody or antigen-binding fragment of any one of claims 43 to 53 having a human Ig kappa constant domain.

59. The antibody or antigen-binding fragment of any one claims 43 to 58, wherein the antibody is aglycosylated.

60. The antibody or antigen-binding fragment of claims 43 to 55 having a human IgG1 constant domain that is aglycosylated by replacing the amino acid corresponding to asparagine (Asn) 297 of the constant region heavy chain with an alternative amino acid residue.

61. The antibody or antigen-binding fragment of claim 60, wherein the Asn 297 is replaced with glutamine, alanine, histidine or glycine.

62. The antibody or antigen-binding fragment of claim 61, wherein the Asn 297 is replaced with glutamine.

63. The antibody or antigen-binding fragment of any one of claims 43 to 58, wherein the heavy chain immunoglobulin constant domain is a human IgG4 constant domain wherein serine 228 is replaced with proline.

64. The antibody or antigen-binding fragment of claim 43, which is a scFv, diabody, Fab, minibody or scFv-Fc.

65. A nucleotide sequence encoding the antibody or antigen-binding fragment of any one claims 43 to 64.

66. A nucleotide sequence encoding a VH region, wherein the VH region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 49.

67. The nucleotide sequence of claim 66, wherein the VH region comprises SEQ ID NO: 49.

68. A nucleotide sequence encoding a VL region, wherein the VL region comprises an amino acid selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

69. A vector comprising the nucleotide sequence of any one of claims 65 to 68.

70. An isolated cell comprising the vector of claim 69.

71. A method for producing a humanized antibody, or antigen-binding fragment thereof, comprising culturing the cell of claim 70 and recovering the antibody, or fragment thereof, from the culture medium or from said cultured cells.

72. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antigen-binding fragment of any one of claims 43 to 64.

73. A method of treating an inflammatory disease or disorder comprising administering to a subject an effective amount of the antibody or antigen-binding fragment of any one of claims 43 to 64.

74. The method of claim 73, wherein the inflammatory disease or disorder is ischemia-reperfusion injury.

75. The method of claim 73, wherein the subject is a mammal.

76. The method of claim 75, wherein the mammal is a human.

77. The method of claim 73, wherein the ischemia-reperfusion results after myocardial infarction, stroke or a surgical procedure.

Sequence comparison of m21G6 and humanized derivatives

m21G6 VH

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYYMYWVKQRPGQGLEWIGGINPSNGGTNFNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCTRWGYDREWFAYWGQGTLVTVSA

H1-21G6 VH (21/28 CLL)

QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYYMYWVKQAPGQGLEWIGGINPSNGGTNFNEKFKSKATLTVDKSASTAYMELSSLRSEDTAVYYCTRWGYDREWFAYWGQGTLVTVSS

H2-21G6 VH (Germline HuIgH1-18*01)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPSNGGTNFNEKFKSKATMTVDKSTSTAYMELRSLRSDDSAVYYCTRWGYDREWFAYWGQGTLVTVSS

H3-21G6 VH (Germline HuIgH1-69*06)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPSNGGTNFNEKFKSKATITVDKSTSTAYMELSSLRSEDTAVYYCTRWGYDREWFAYWGQGTLVTVSS

m21G6 VL

DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKLEIKR

L1-21G6 VL (PopVk, CLL)

DIVMTQSPATLSVSPGERATISCRSSKSLLHSNGNTYLYWFQQKPGQPPKVLIYRMSNLASGVPARFSGSGSGTDFTLTISSVEPEDFATYYCMQHLEYPFTFGGGTKLEIKR

L2-21G6 VL (Germline HuIgKV2-28*01)

DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIKR

L3-21G6 VL (Germline HuIgKV2-29*02)

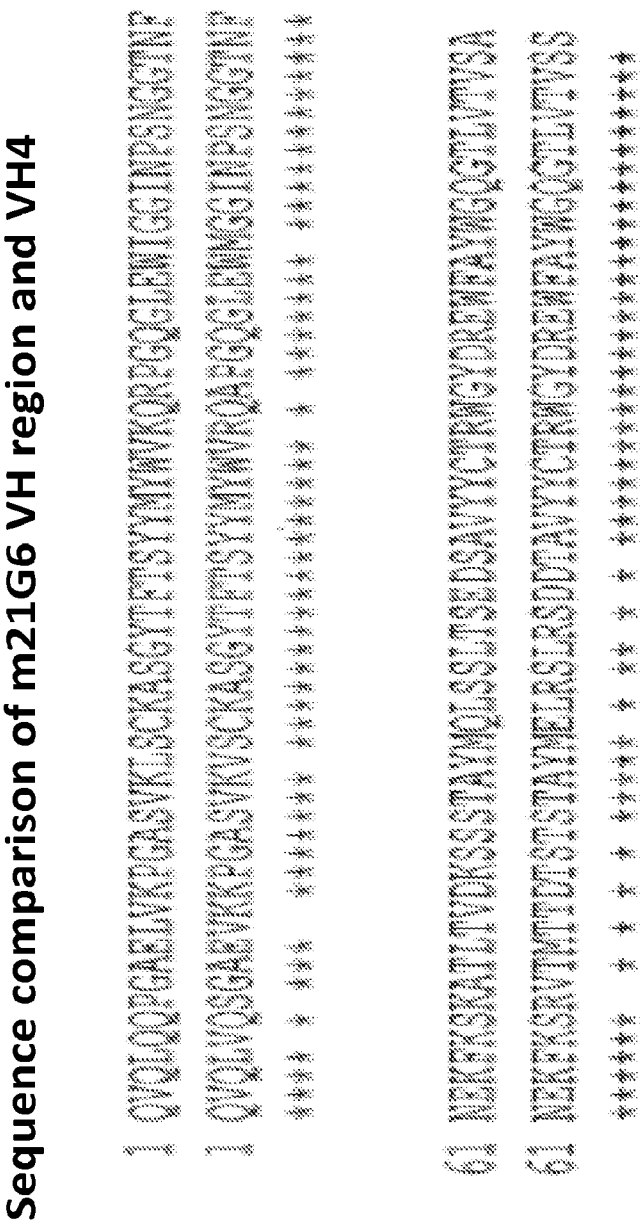
DIVMTQTPLSLSYTPGQPASISCRSSKSLLHSNGNTYLYWFLQKPGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGQGTKLEIKR

Bold = amino acid differences between germline sequence and parental m21g6

Shaded = variable zones

Boxes indicates CDR regions

FIG. 1


Sequence comparison of m21G6 VH region and VH4
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VH4
m21G6
VH4

FIG. 2

optimized for Cricetulus griseus

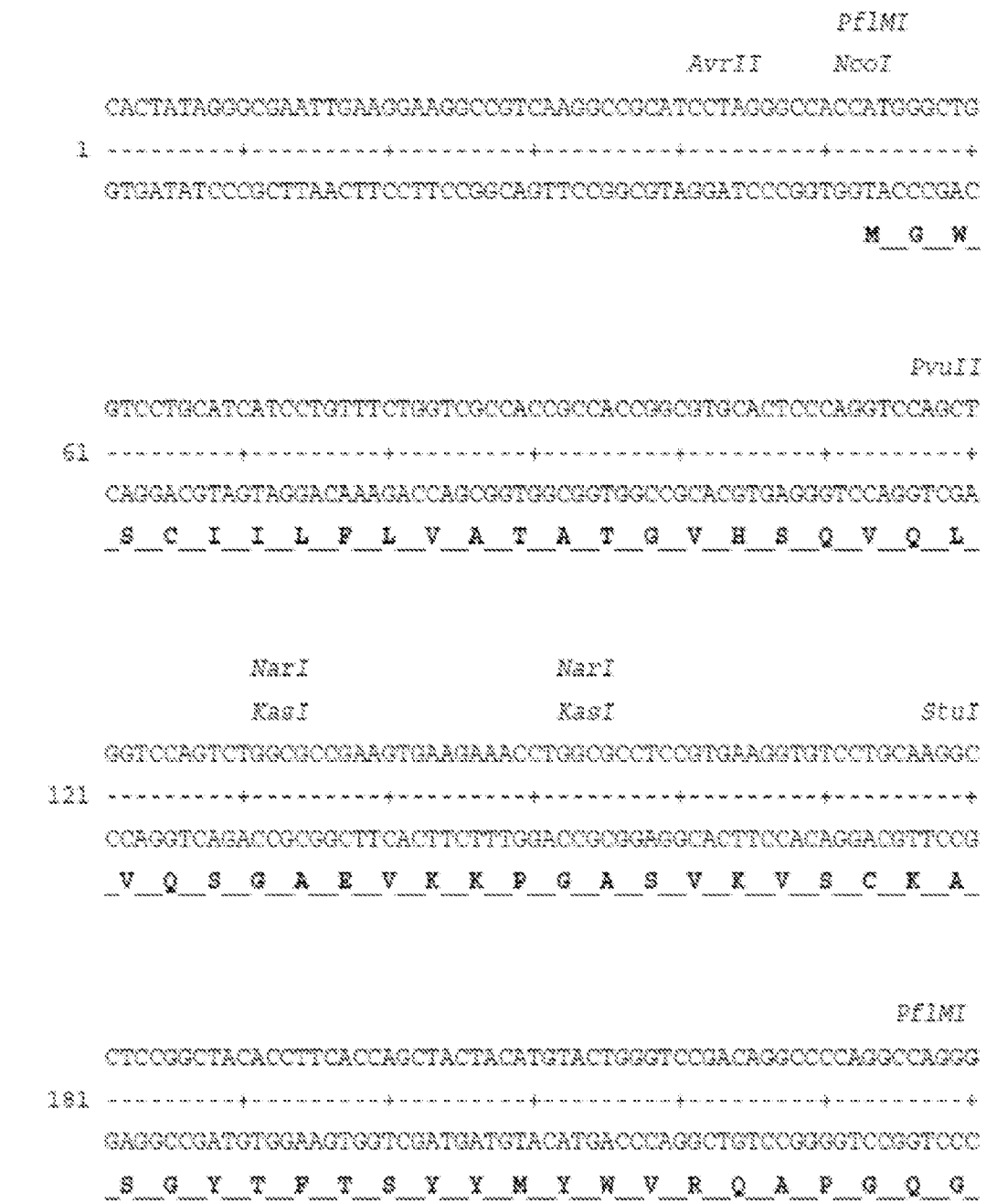
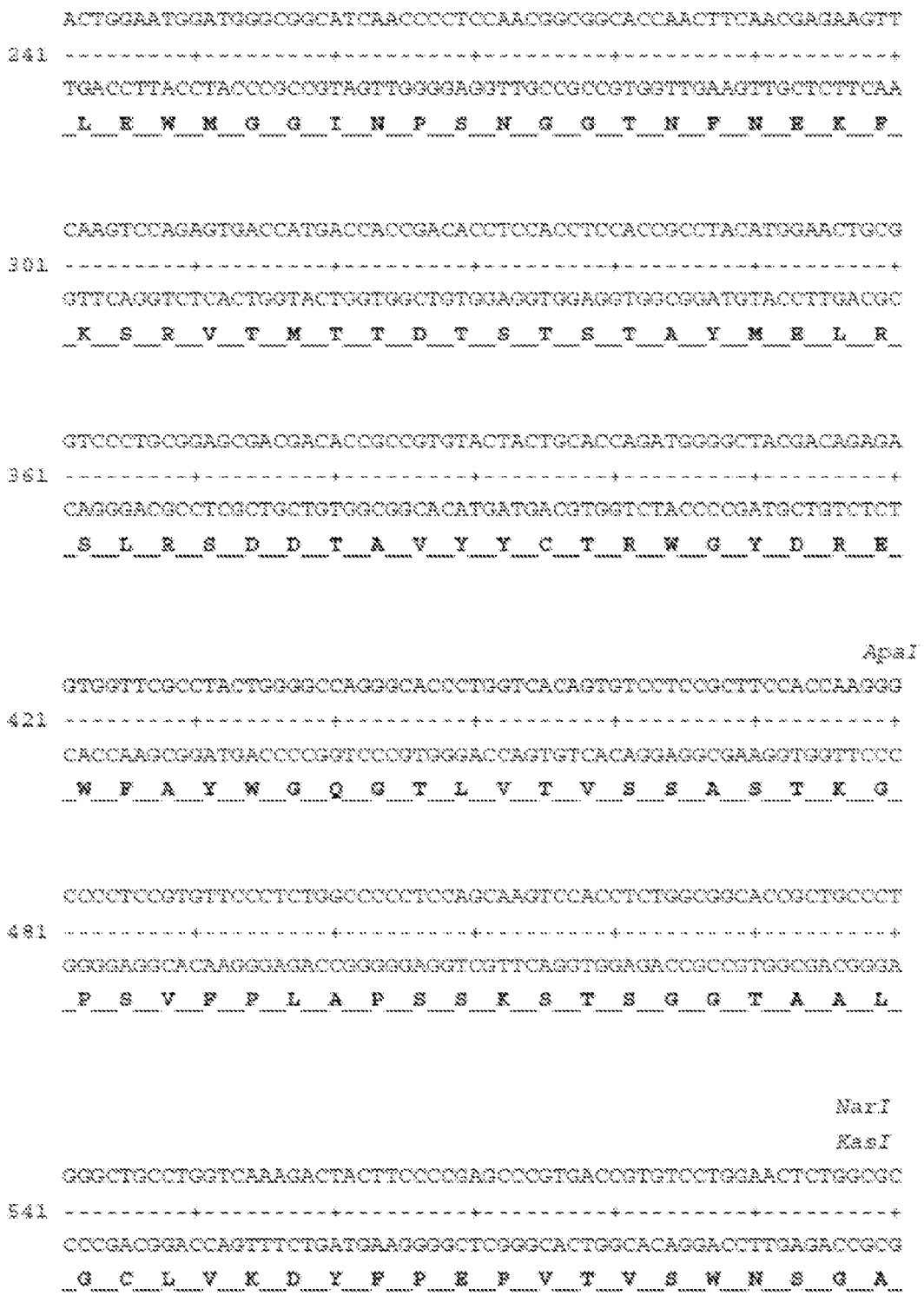


FIG. 3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301
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361
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S L R S D D T A V Y Y C T R W G Y D R E
ApaI
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421
CACCAAGCGGATGACCCCGGTCCCGTGGGACCAGTGTCACAGGAGGCGAAGGTGGTTCCC
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CCCCTCCGTGTTCCCTCTGGCCCCCTCCAGCAAGTCCACCTCTGGCGGCACCGCTGCCCT
481
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P S V F P L A P S S K S T S G G T A A L
NarI
KasI
GGGCTGCCTGGTCAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAACTCTGGCGC
541
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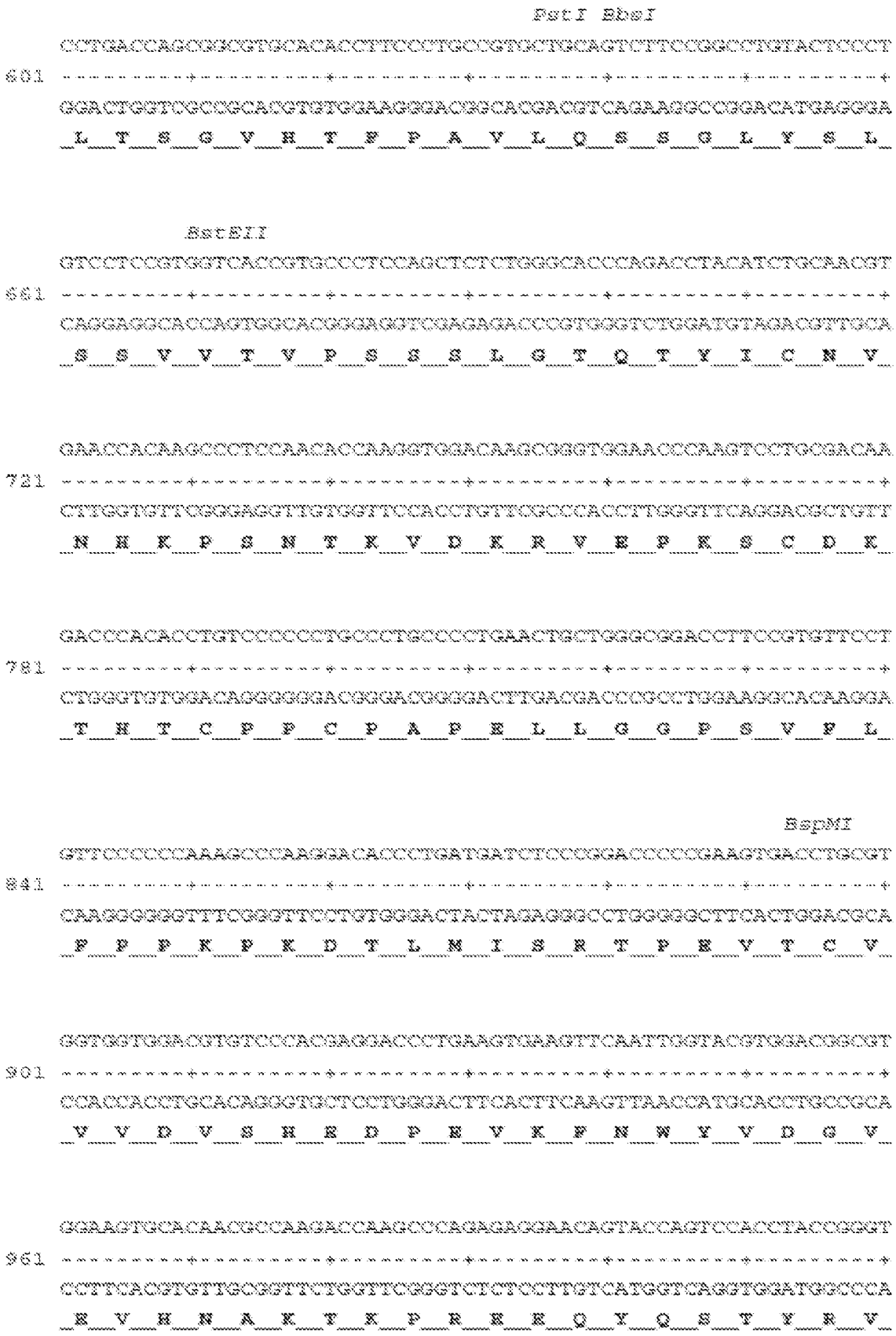
FIG. 3 (cont.)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781
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841
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901
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961
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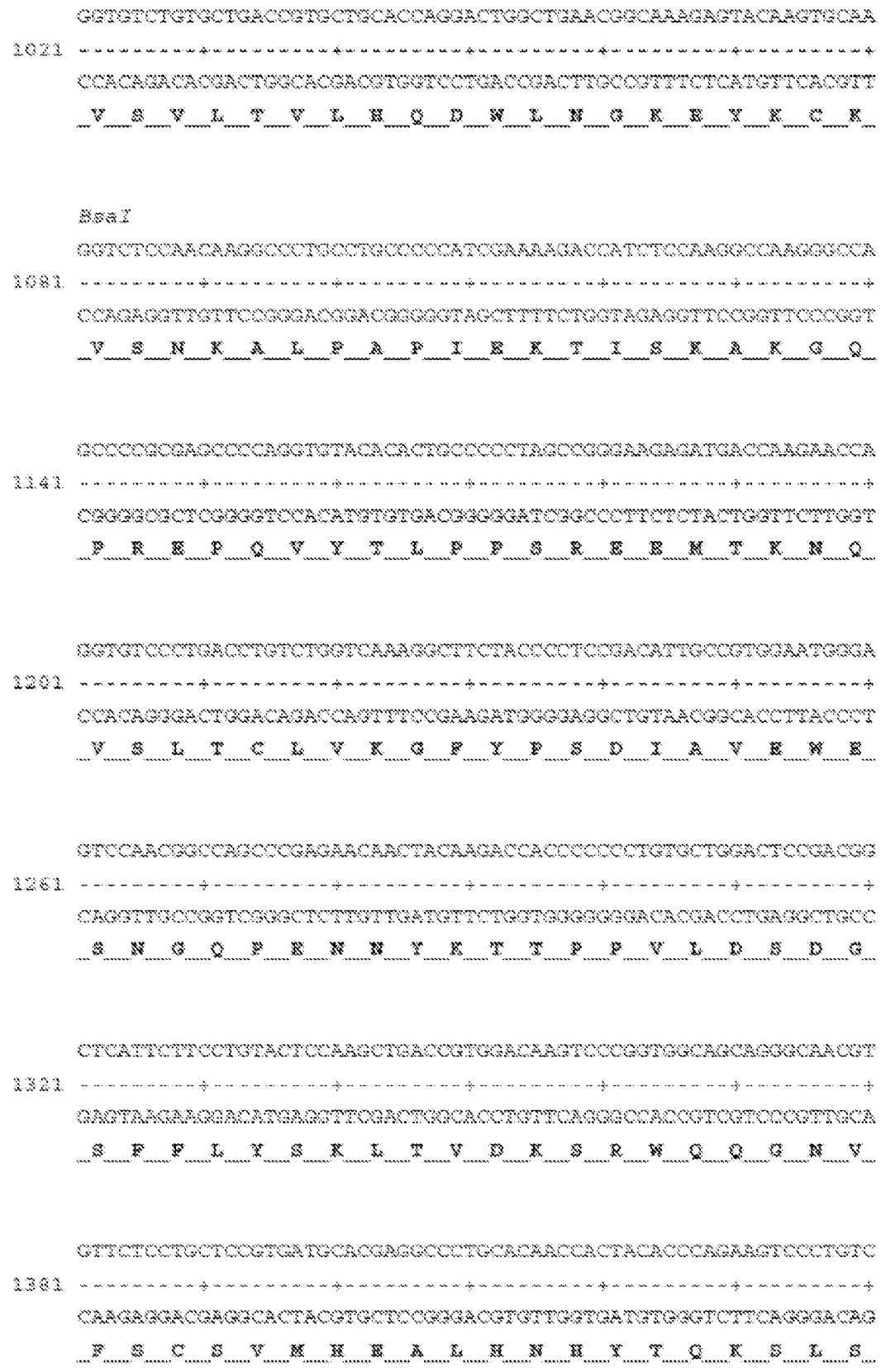
FIG. 3 (cont.)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V S N K A L P A P I E K T I S K A K G Q
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1141
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P R E P Q V Y T L P P S R E E M T K N Q
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1201
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1261
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S F F L Y S K L T V D K S R W Q Q G N V
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1381
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FIG. 3 (cont.)

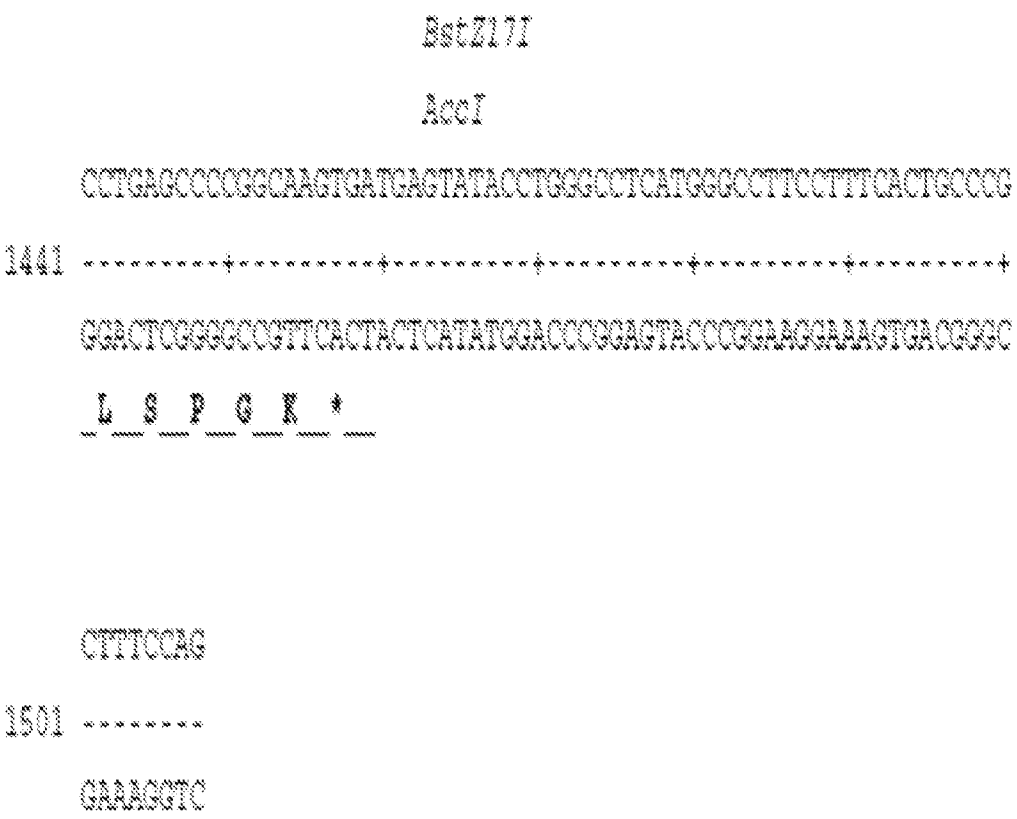
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FIG. 3 (cont.)

optimized for Cricetulus griseus

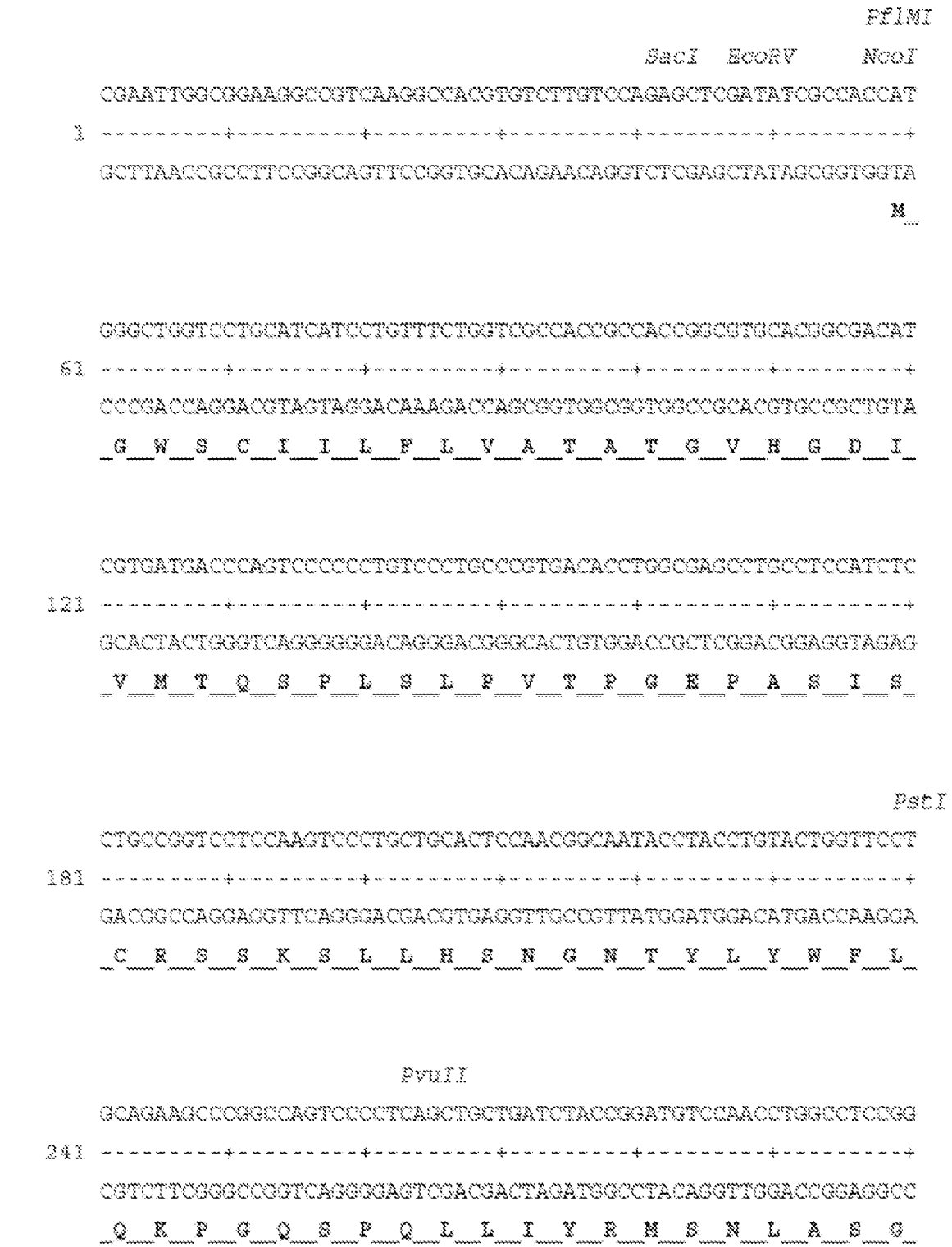
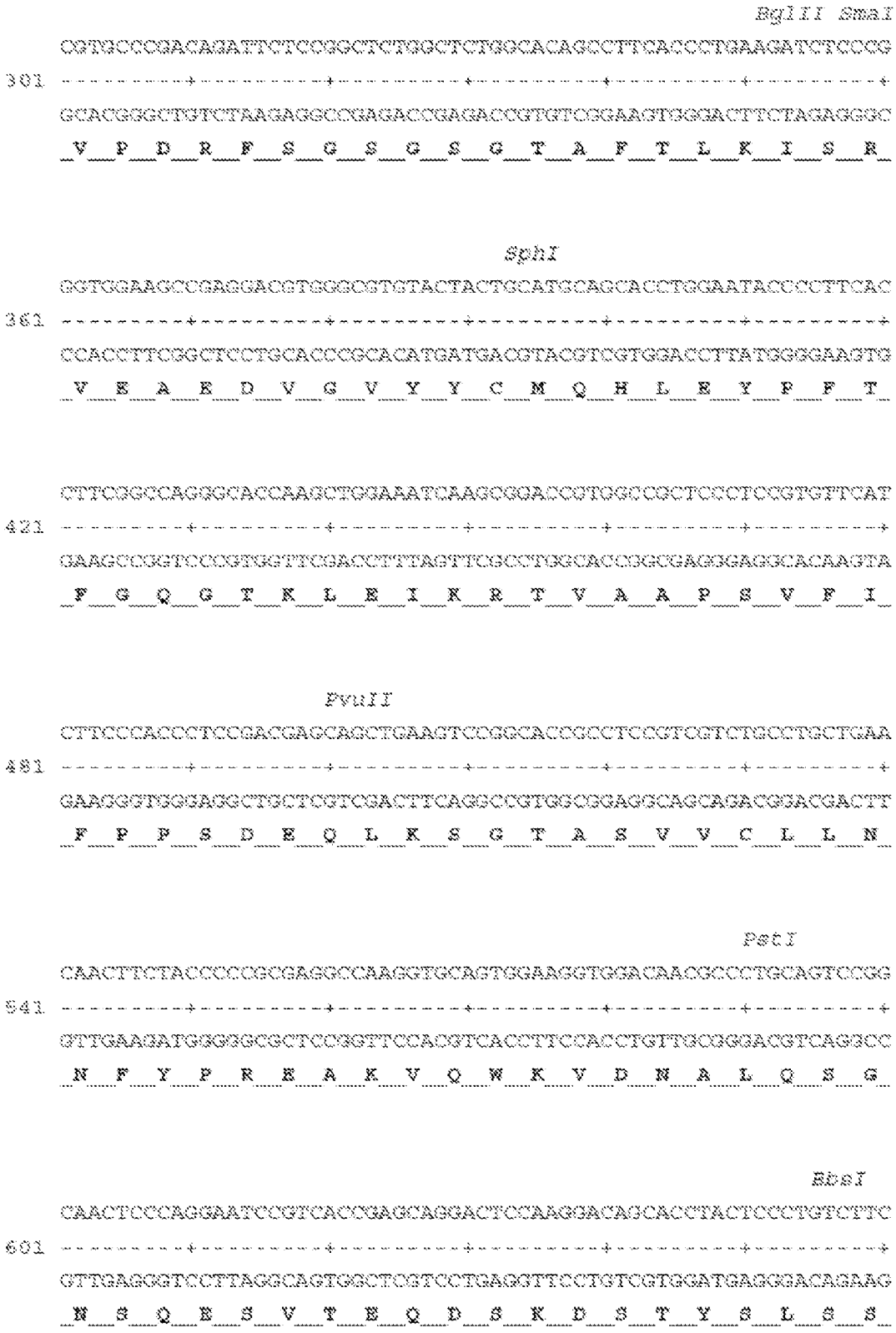


FIG. 4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SphI
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361
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V E A E D V G V Y Y C M Q H L E Y P F T
CTTCGGCCAGGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCAT
421
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CTTCCCACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTCTGCCTGCTGAA
481
GAAGGGTGGGAGGCTGCTCGTCGACTTCAGGCCGTGGCGGAGGCAGCAGACGGACGACTT
F P P S D E Q L K S G T A S V V C L L N
PstI
CAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGG
541
GTTGAAGATGGGGGCGCTCCGGTTCCACGTCACCTTCCACCTGTTGCGGGACGTCAGGCC
N F Y P R E A K V Q W K V D N A L Q S G
EbsI
CAACTCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTTC
601
GTTGAGGGTCCTTAGGCAGTGGCTCGTCCTGAGGTTCCTGTCGTGGATGAGGGACAGAAG
N S Q E S V T E Q D S K D S T Y S L S S

FIG. 4 (cont.)

```
    CACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTCAC
661 ---------+---------+---------+---------+---------+---------+
    GTGGGACTGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCCACATGCCGACGCTTCACTG
     T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T

                                                          PacI
    CCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGATGATT
721 ---------+---------+---------+---------+---------+---------+
    GGTGGTCCCGGACAGGTCGGGGCACTGGTTCAGGAAGTTGGCCCCGCTCACGACTACTAA
     H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  *  *

          KpnI
          ^
    AATTAAGGTACCTGGAGCACAAGACTGGCCTCATGGCCCTTCCGCTCACTGC
781 ---------+---------+---------+---------+---------+--
    TTAATTCCATGGACCTCGTGTTCTGACCGGAGTACCGGGAAGGCGAGTGACG
```

FIG. 4 (cont.)

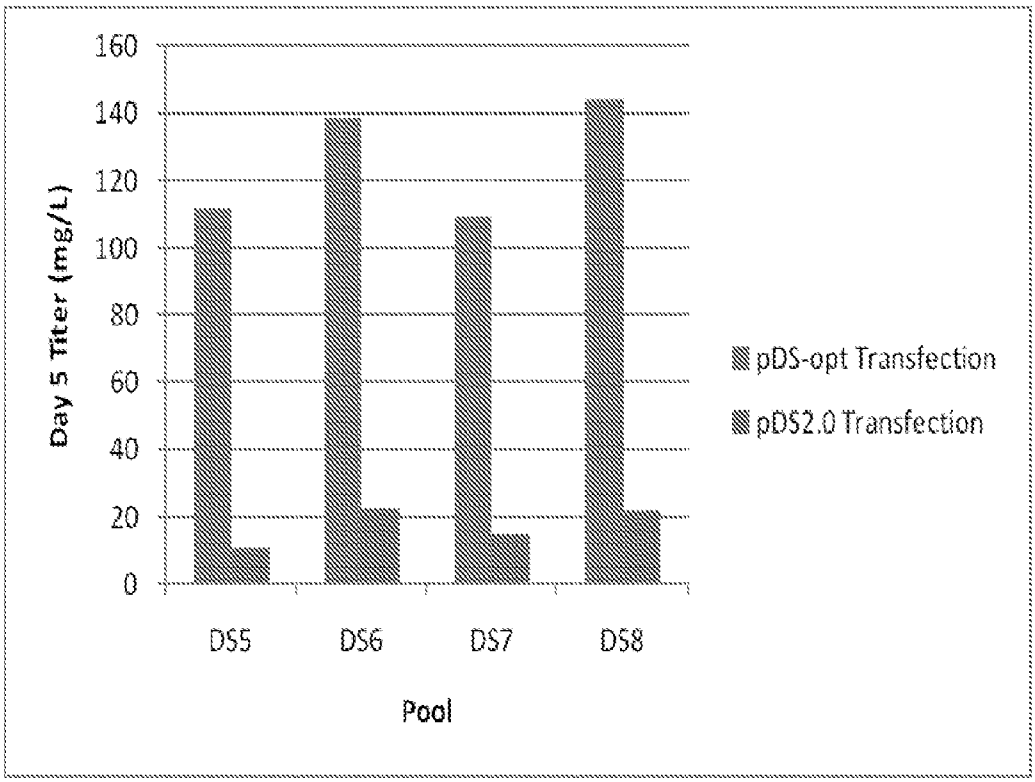
160
140
120
100
80
60
40
20
0
Day 5 Titer (mg/L)
DS5
DS6
DS7
DS8
Pool
pDS-opt Transfection
pDS2.0 Transfection

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/24445

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 16/00, A61K 39/395 (2014.01)
USPC - 424/133.1, 530/387.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 16/00, A61K 39/395 (2014.01)
USPC - 424/133.1, 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/139.1, 530/387.9
CPC - A61K 2039/505, C07K 2317/24

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWEST (PGPB, USPT, EPAB, JPAB), Google Scholar, NCBI BLAST
Search terms: N2, anti-N2, antibody, peptide, polypeptide, protein, epitope, humanized, heavy chain variable, VH, light chain variable, VL, complementarity determining region, CDR, framework region, FWR, IgG1, IgG4, scFv, diabody, Fab, minibody, scFv-Fc

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0093835 A1 (CARROLL) 19 April 2012 (19.04.2012) para [0005], [0076], [0078], [0091], [0093]; SEQ ID NO:14	1-7, 20, 21, 22/(1-7,20,21), 23, 24, (25-26)/(1-7,20,21), (28-30)/(1-7,20,21), 32, 43-48, 53, (54-58)/(43-48,53), 64, 66, 68
A	US 2007/0135998 A1 (VAN VLIJMEN et al.) 14 June 2007 (14.06.2007) para [0020]; SEQ ID NO:4	1-7, 20, 22/(1-7,20), 23, 24, (25-26)/(1-7,20), (28-30)/(1-7,20), 32, 43-48, 53, (54-58)/(43-48,53), 64, 66
A	US 2008/0260731 A1 (BERNETT et al.) 23 October 2008 (23.10.2008) para [0046], [0102], [0104]; SEQ ID NO:96	1-7, 21, 22/(1-7, 21), 23, 24, (25-26)/(1-7,21), (28-30)/(1-7,21), 32, 43-48, (54-58)/(43-48), 64
A	WO 2011/071883 A1 (CARROLL) 16 June 2011 (16.06.2011) SEQ ID NO:14	20, 22/20, (25-26)/20, (28-30)/20, 53, (54-58)/53, 66

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2014 (26.08.2014)	09 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/24445

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/0098448 A1 (KORTH et al.) 28 April 2011 (28.04.2011) SEQ ID NO:3	20, 22/20, (25-26)/20, (28-30)/20, 53, (54-58)/53, 66
A	US 7863419 B2 (TAYLOR et al.) 4 January 2011 (04.01.2011) SEQ ID NO:8	20, 22/20, (25-26)/20, (28-30)/20, 53, (54-58)/53, 66
A	US 2010/0272723 A1 (BERNETT et al.) 28 October 2010 (28.10.2012) SEQ ID NO:96	21, 22/21, 23, 24, (25-26)/21, (28-30)/21, 68
A	US 2012/0082664 A1 (BERNETT et al.) 5 April 2012 (05.04.2012) SEQ ID NO:96	21, 22/21, 23, 24, (25-26)/21, (28-30)/21, 68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/24445

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 27, 31, 33-42, 59-63, 65, and 69-77
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

---plase see extra sheet---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 20-21, 22-26 (in part), 28-30 (in part), 32, 43-48, 53, 54-58 (in part), 64, 66, and 68 restricted to SEQ ID NOs: 3-9, 12, 15-18, 27-30

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/24445

Continuation of: Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-26, 28-30, 32, 43-58, 64, 66-68 drawn to a humanized, anti-N2 antibody or antigen-binding fragment thereof.

Group I+ will be searched to the extent that it reads on the first named humanized anti-N2 antibody or antigen-binding fragment thereof, without fee. The first named invention comprises a humanized anti-N2 antibody or antigen-binding fragment thereof comprising the CDRs and FWRs sequences that comprise VH region SEQ ID NO: 9 and VL region SEQ ID NO:12. Specifically, the CDRs and FWRs that comprise these VH and VL regions are as follows:
- VH region SEQ ID NO:9 comprises
  i. VH CDR1 SEQ ID NO: 3, VH CDR2 SEQ ID NO: 4 and VH CDR3 SEQ ID NO: 5; and
  ii. VH FWR1 SEQ ID NO: 15, VH FWR2 SEQ ID NO: 16, VH FWR3 SEQ ID NO: 17, VH FWR4 SEQ ID NO: 18; and
- VL region SEQ ID NO:12 comprises
  iii. VL CDR1 SEQ ID NO: 6, VL CDR2 SEQ ID NO: 7 and VL CDR3 SEQ ID NO: 8; and
  iv. VL FWR1 SEQ ID NO: 27, VL FWR2 SEQ ID NO: 28, VL FWR3 SEQ ID NO: 29, and VL FWR4 SEQ ID NO: 30.

It is believed that claims 1-7, 20-21, 22-26 (in part), 28-30 (in part), 32, 43-48, 53, 54-58 (in part), 64, 66, and 68 read on this first named invention. [Note that claims 8-19 and 49-52 are not included as they require an antibody having different VH and VL regions not limited to SEQ ID NO: 9 and SEQ ID NO:12. Each of the VH region SEQ ID NOs: 9, 10, 11, 42, 43, 44, 45, 49, 54 comprise different combinations of FWR regions. Similarly, each of the VL region SEQ ID NOs: 12, 13, 14, all comprise different combinations of FWR regions]. Applicants must indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: a humanized anti-N2 antibody or antigen-binding fragment thereof comprising VH region SEQ ID NO:9 and VL region SEQ ID NO:13 (note that SEQ ID NO:13 comprises the unique combination of FWRs: VL FWR1 SEQ ID NO: 31, VL FWR2 SEQ ID NO: 32, VL FWR3 SEQ ID NO: 33, and VL FWR4 SEQ ID NO: 34), i.e. claims 1-6, 8, 20-21, 22-26 (in part), 28-30 (in part), 32, 43-48, 53, 54-58 (in part), 64, 66, and 68.

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

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the specific humanized, anti-N2 antibody or antigen-binding fragment thereof recited therein. Each unique antibody or fragment thereof is based on different VH and VL regions, each having uniquely different combinations of framework regions (FWRs).

Common Technical Features

The feature shared by inventions of Group I+ is a humanized, anti-N2 antibody or antigen-binding fragment thereof comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region comprises three complementarity determining regions (CDRs) and four framework regions (FWR), and wherein the VL region comprises three complementarity determining regions (CDRs), and four framework regions (FWR). However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2012/0093835 A1 (Carroll) in view of US 2011/0243948 A1 to Lee et al. (hereinafter 'Lee'). Carroll discloses a humanized (para [0093]), anti-N2 antibody or antigen-binding fragment thereof (para [0005]) comprising a heavy chain variable (VH) region and a light chain variable (VL) region (para [0005]), wherein the VH region comprises three complementarity determining regions (CDRs) (para [0078] VH CDR1, VH CDR2, VH CDR3) and human framework regions (FWR) (para [0091]), and wherein the VL region comprises three complementarity determining regions (CDRs) (para [0061] VL CDR1, VL CDR2, VL CDR3), and human framework regions (FWR) (para [0091], [0093]). Carroll does not specifically teach that the human framework regions of the VH and VL comprise four framework regions. However, Carroll does teach that the humanized antibody is created by donating non-human CDRs to recipient human frameworks (para [0091]). Lee discloses similar humanized antibodies that are created by grafting donor CDRs onto human framework regions (para [0130], [0320]). Lee further teaches that human framework regions of both the VH region and the VL region comprise four framework regions (para [0024] heavy chain variable region comprising FWR1, FWR2, FWR3, and FWR4; para [0025] light chain variable region comprising FWR1, FWR2, FWR3, and FWR4). Therefore since the humanized antibody of Carroll comprises human framework regions, and Lee teaches that human framework regions comprise four framework regions, one of ordinary skill in the art would have found it obvious that the humanized antibody of Carroll would comprise four framework regions in each of the VH and VL regions of the antibody. As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another feature shared by Group I+ is is a VH region of an anti-N2 antibody that comprises VH CDR1 SEQ ID NO:3, VH CDR2 SEQ ID NO:4, and VH CDR3 SEQ ID NO:5. However, this shared technical feature does not represent a contribution over Carroll, which teaches a VH region comprising VH CDR1 SEQ ID NO:3 (Carroll SEQ ID NO:16, para [0078]), VH CDR2 SEQ ID NO:4 (Carroll SEQ ID NO:18, para [0078]), and VH CDR3 SEQ ID NO:5 (Carroll SEQ ID NO:30, para [0078]). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

---please see continuation on next extra sheet---

Continuation of: Box No. III Observations where unity of invention is lacking

Another feature shared by Group I+ is is a VL region of an anti-N2 antibody comprising VL CDR1 SEQ ID NO:6, VL CDR2 SEQ ID NO:7, and VL CDR3 SEQ ID NO:8. However, this shared technical feature does not represent a contribution over Carroll, which teaches a VL region VL CDR1 SEQ ID NOs: 6 (Carroll SEQ ID NO:22, para [0080]), and VL CDR2 SEQ ID NO:7 (Carroll SEQ ID NO:24, para [0080]). Carroll does not specifically teach VL CDR3 SEQ ID NO:8. However, Carroll does teach a larger sequence that comprises SEQ ID NO:8 (Carroll SEQ ID NO:20, aa 94-102), wherein this larger sequence also comprises the claimed CDR1 and CDR2 sequences upstream of the sequences corresponding to claimed CDR3 SEQ ID NO:8 (para [0098] - "In certain embodiments, the modified antibody may comprise at least the CDR1 region of SEQ ID NO: 20 (SEQ ID NO: 22), or antigen binding portions thereof, and/or at least the CDR2 region of SEQ ID NO: 20 (SEQ ID NO: 24), or antigen binding portions thereof."; SEQ ID NO:20 showing sequences corresponding to Carroll CDR1 SEQ ID NO:22 and Carroll CDR2 SEQ ID NO:24 upstream of the claimed CDR3 sequence). Therefore, one of ordinary skill in the art would have found the claimed VL CDR3 SEQ ID NO:8 obvious in view of Carroll SEQ ID NO:20. As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

A further feature shared by Group I+ is a nucleotide sequence encoding a VH region and a VL region of an anti-N2 antibody (i.e. claims 66 and 68). However, this shared technical feature does not represent a contribution over Carroll, which teaches nucleotide sequence encoding a VH region (para [0051], [0056]) and nucleotide sequence encoding a VL region (para [0053], [0056]). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

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