Title: METHODS FOR TREATING HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMA

Abstract: The present invention relates to methods for treating homozygous familial hypercholesterolemia using antibodies against proprotein convertase subtilisin/kexin type 9 (PCSK9).
METHODS FOR TREATING HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMA

REFERENCE TO THE SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled A-1837-WO-PCT-SequenceList062713.txt, created June 27, 2013 which is 322 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods for treatment of homozygous familial hypercholesterolemia using antigen binding proteins, including antibodies, against proprotein convertase subtilisin/kexin type 9 (PCSK9).

BACKGROUND

Homozygous familial hypercholesterolemia is a rare, but serious clinical disorder caused by substantial reduction in low density lipoprotein (LDL) receptor function. As a result, LDL cholesterol levels are severely elevated, leading to cardiovascular disease, and often death, in childhood (Goldstein JL, Hobbs HH, Brown MS, eds. Familial hypercholesterolemia. 8th Edition ed: McGraw-Hill; 2001). Over 95% have a mutation in the LDL receptor, less than 4% in apolipoprotein B and less than 0.5% in proprotein convertase subtilisin/kexin 9 (PCSK9)(Abifadel M, Varret M, Rabes JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia.


Response to conventional therapies, such as statins and ezetimibe, the most commonly used drugs for homozygous familial hypercholesterolemia, is modest, and patients usually also require LDL apheresis when it is available. Reductions in LDL cholesterol with statins tend to correlate with LDL receptor function, although receptor negative patients have shown decreases (Raal FJ, Pappu AS, Illingworth DR, et al. Inhibition of cholesterol synthesis by atorvastatin in homozygous familial hypercholesterolaemia. Atherosclerosis 2000;150:421-8). The improvements in LDL cholesterol with statins appear to reduce cardiovascular disease morbidity and mortality (Raal FJ, Pilcher GJ, Panz VR, et al. Reduction in mortality in subjects with homozygous familial hypercholesterolemia associated with advances in lipid-lowering therapy. Circulation 2011;124:2202-7). Recently two drugs, lomitapide and mipomersen, which both reduce hepatic lipoprotein production, have been approved solely for the treatment of homozygous familial hypercholesterolemia. Even with the introduction of these two new drugs, there remains a need to identify new methods for treating patients diagnosed with homozygous familial hypercholesterolemia.

SUMMARY OF VARIOUS EMBODIMENTS

In some aspects, the invention provided comprises a method of lowering serum LDL cholesterol in a patient diagnosed with homozygous familial hypercholesterolemia comprising administering at least one anti-PCSK9 antibody to the patient in need thereof at a dose of about 120 mg to about 3000 mg, thereby lowering said serum LDL cholesterol level by at least about 10%, as compared to a predose level of serum LDL cholesterol in the patient. In some embodiments of this aspect of the invention, the serum LDL cholesterol level of said patient is lowered by at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at least about 90% as compared to a predose level of serum LDL cholesterol in the patient.
In some embodiments of this aspect of the invention, the anti-PCSK9 antibody is administered to a patient diagnosed with homozygous familial hypercholesterolemia at a dose of about 140 mg to about 3000 mg, of about 140 mg to about 2800 mg, of about 140 mg to about 2500 mg, of about 140 mg to about 2000 mg, of about 140 mg to about 1800 mg, of about 140 mg to about 1400 mg, of about 120 mg to about 1200 mg, of about 120 mg to about 1000 mg, of about 120 mg to about 700 mg, of about 140 mg to about 700 mg, of about 140 mg to about 600 mg, of about 140 mg to about 450 mg, of about 120 mg to about 450 mg, of about 120 mg to about 450 mg, of about 140 mg to about 450 mg, of about 210 mg to about 450 mg, or of about 280 mg to about 450 mg, of about 210 mg to about 420 mg, of about 280 mg to about 420 mg, of about 420 mg to about 3000 mg, of about 700 mg to about 3000 mg, of about 1000 mg to about 3000 mg, of about 1200 to about 3000 mg, of about 1400 mg to about 3000 mg, of about 1800 mg to about 3000 mg, of about 2000 mg to about 3000 mg, of about 2400 mg to about 3000 mg, or about 2800 mg to about 3000 mg. In some embodiments of this aspect, the anti-PCSK9 antibody is administered to a patient at a dose of about 35 mg, of about 45 mg, of about 70 mg, of about 105 mg, of about 120 mg of about 140 mg, of about 150 mg, of about 160 mg, of about 170 mg, of about 180 mg, of about 190 mg, of about 200 mg, of about 210 mg, of about 280 mg, of about 360 mg, of about 420 mg, of about 450 mg, of about 600 mg, of about 700 mg, of about 1200 mg, of about 1400 mg, of about 1800 mg, of about 2000 mg, of about 2500 mg, of about 2800 mg, or about 3000 mg.

In some embodiments of this aspect of the invention the anti-PCSK9 antibody is administered to a patient on a schedule selected from the group consisting of: (1) once a week, (2) once every two weeks, (3) once a month, (4) once every other month, (5) once every three months, (6) once every six months and (7) once every twelve months. In some embodiments of this aspect of the invention the anti-PCSK9 antibody is administered parenterally. In some embodiments of this aspect of the invention, the anti-PCSK9 antibody is administered intravenously. In some embodiments of this aspect of the invention, the anti-PCSK9 antibody is administered subcutaneously.

In some embodiments of this aspect of the invention, the anti-PCSK9 antibody comprises: A) one or more heavy chain complementary determining regions (CDRHs)
selected from the group consisting of: (i) a CDRH1 from a CDRH1 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; (ii) a CDRH2 from a CDRH2 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; (iii) a CDRH3 from a CDRH3 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; and (iv) a CDRH of (i), (ii), and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than 4 amino acids; B) one or more light chain complementary determining regions (CDRLs) selected from the group consisting of: (i) a CDRL1 from a CDRL1 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; (ii) a CDRL2 from a CDRL2 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; (iii) a CDRL3 from a CDRL3 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than 4 amino acids; or C) one or more heavy chain CDRHs of A) and one or more light chain CDRLs of B). In some embodiments, the isolated antigen binding protein comprises at least one CDRH of A) and at least one CDRL of B). In some embodiments, the isolated antigen binding protein comprises at least two CDRH of A) and at least two CDRL of B). In some embodiments, the isolated antigen binding protein comprises at least three CDRH of A) and at least three CDRL of B).

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO: 23, a CDRL2 of the CDRL2 sequence in SEQ ID NO: 23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO: 23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49.

In some embodiments, the isolated antigen bindng protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO: 465, a CDRL2 of the CDRL2 sequence in SEQ ID NO: 465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO: 465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2
sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:12, a CDRL2 of the CDRL2 sequence in SEQ ID NO:12, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:12, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 67, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 67, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:67.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:461, a CDRL2 of the CDRL2 sequence in SEQ ID NO:461, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:461, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 459, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 459, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:459.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:485, a CDRL2 of the CDRL2 sequence in SEQ ID NO:485, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:485, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 483, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 483, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:483.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:582, a CDRL2 of the CDRL2 sequence in SEQ ID NO:582, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:582, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 583, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 583, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:583.

In some embodiments of this aspect of the invention the anti-PCSK9 antibody comprises: a light chain variable region that comprises an amino acid sequence that is
at least 90% identical to that of SEQ ID NO: 23 and a heavy chain variable region that comprises and amino acid sequence that is at least 90% identical to that of SEQ ID NO:49; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 12 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:67; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 461 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:459; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:463; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 485 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:483; or a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 582 and a heavy chain variable region that comprises and amino acid sequence that is at least 90% identical to that of SEQ ID NO:583. In some embodiments of this aspect of the invention the anti-PCSK9 antibody comprises: a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 23, and a heavy chain variable region that comprises and amino acid sequence, SEQ ID NO:49; a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 12, and a heavy chain variable region that comprises an amino acid sequence, SEQ ID NO:67; a light chain variable region that comprises amino acid sequence SEQ ID NO:459; a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463; a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 485 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:483; a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 582, and a heavy chain variable region that comprises and amino acid sequence, SEQ ID NO:583; or a light chain
variable region that comprises an amino acid sequence, SEQ ID NO:591, and a heavy chain variable region that comprises and amino acid sequence, SEQ ID NO:590. In some embodiments of this aspect of the invention the anti-PCSK9 antibody is selected from the group consisting of 21B12, 31H4, 8A3, 11F1 and 8A1.

In some aspects, the invention comprises a method of treating a patient diagnosed with homozygous familial hypercholesterolemia comprising administering at least one anti-PCSK9 antibody to the patient in need thereof at a dose of about 120 mg to about 3000 mg, thereby treating the homozygous familial hypercholesterolemia in said patient. In some embodiments of this aspect, the serum LDL cholesterol level of said patient diagnosed with homozygous familial hypercholesterolemia is lowered by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at least about 90% as compared to a predose level of serum LDL cholesterol in said patient.

In some embodiments of this aspect of the invention, the anti-PCSK9 antibody comprises: A) one or more heavy chain complementary determining regions (CDRHs) selected from the group consisting of: (i) a CDRH1 from a CDRH1 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; (ii) a CDRH2 from a CDRH2 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; (iii) a CDRH3 from a CDRH3 in a sequence selected from the group consisting of SEQ ID NO: 49, 67, 459, 463 and 483; and (iv) a CDRH of (i), (ii), and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than 4 amino acids; B) one or more light chain complementary determining regions (CDRLs) selected from the group consisting of: (i) a CDRL1 from a CDRL1 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; (ii) a CDRL2 from a CDRL2 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; (iii) a CDRL3 from a CDRL3 in a sequence selected from the group consisting of SEQ ID NO: 23, 12, 461, 465, and 485; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than 4 amino acids; or C) one or more heavy chain
CDRHs of A) and one or more light chain CDRLs of B). In some embodiments, the isolated antigen binding protein comprises at least one CDRH of A) and at least one CDRL of B). In some embodiments, the isolated antigen binding protein comprises at least two CDRH of A) and at least two CDRL of B). In some embodiments, the isolated antigen binding protein comprises at least three CDRH of A) and at least three CDRL of B).

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:23, a CDRL2 of the CDRL2 sequence in SEQ ID NO:23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:12, a CDRL2 of the CDRL2 sequence in SEQ ID NO:12, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:12, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 67, a CDRH2 of the CDRH2 sequence in SEQ ID NO:67, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:67.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:461, a CDRL2 of the CDRL2 sequence in SEQ ID NO:461, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:461, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 459, a CDRH2 of the CDRH2
sequence in SEQ ID NO: 459, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:459.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:485, a CDRL2 of the CDRL2 sequence in SEQ ID NO:485, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:485, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 483, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 483, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:483.

In some embodiments, the isolated antigen binding protein comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:582, a CDRL2 of the CDRL2 sequence in SEQ ID NO:582, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:582, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 583, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 583, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:583.

In some embodiments of this aspect of the invention, the anti-PCSK9 antibody comprises: a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 23 and a heavy chain variable region that comprises and amino acid sequence that is at least 90% identical to that of SEQ ID NO:49; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 12 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:67; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 461 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:459; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:463; a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 485 and a heavy chain variable region that
comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 483; or a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 582 and a heavy chain variable region that comprises and amino acid sequence that is at least 90% identical to that of SEQ ID NO: 583. In some embodiments of this aspect of the invention the anti-PCSK9 antibody comprises: a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 23, and a heavy chain variable region that comprises and amino acid sequence, SEQ ID NO: 49; a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 12, and a heavy chain variable region that comprises an amino acid sequence, SEQ ID NO: 67; a light chain variable region that comprises amino acid sequence SEQ ID NO: 461 and a heavy chain variable region that comprises amino acid sequence SEQ ID NO: 459; a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 463; a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 485 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 483; a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 582, and a heavy chain variable region that comprises amino acid sequence, SEQ ID NO: 583; or a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 591, and a heavy chain variable region that comprises amino acid sequence, SEQ ID NO: 590. In some embodiments of this aspect of the invention the anti-PCSK9 antibody is selected from the group consisting of 21B12, 31H4, 8A3, 11F1 and 8A1.

In particular embodiments the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO: 23, a CDRL2 of the CDRL2 sequence in SEQ ID NO: 23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO: 23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49. In some embodiments, the anti-PCSK9 antibody comprises a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 23 and a heavy chain variable region that comprises an amino acid sequence that is
at least 90% identical to that of SEQ ID NO:49. In some embodiments the anti-PCSK9 antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49. In some embodiments the anti-PCSK9 antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:590. In some embodiments, the anti-PCSK9 antibody is 21B12. In a particular embodiment wherein the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:23, a CDRL2 of the CDRL2 sequence in SEQ ID NO:23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:49, a CDRH2 of the CDRH2 sequence in SEQ ID NO:49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:49, or an amino acid sequence that is at least 90% identical to that of SEQ ID NO:23 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:590, or comprises the antibody is 21B12, the anti-PCSK9 antibody is administered to a patient at a dose of about 120 mg to about 450 mg subcutaneously once a week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 3-10 days; is administered to a patient at a dose of about 120 mg subcutaneously once a week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 3-10 days; is administered to a patient at a dose of about 140 mg subcutaneously once a week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 3-10 days; is administered to a patient at a dose of about 120 mg to about 450 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 120 mg subcutaneously
once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 140 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 210 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 280 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 350 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 420 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 280 mg to about 420 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 280 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 350 mg subcutaneously once every four weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 420 mg subcutaneously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days.

In another particular embodiment, wherein the anti-PCS9 antibody comprises a light chain complementarity region (CDR) of the CDR11 sequence in SEQ ID NO:23, a CDR2 of the CDR2 sequence in SEQ ID NO:23, and a CDR3 of the CDR3 sequence in SEQ ID NO:23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49, or comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:23 and a heavy chain variable region that comprises an amino acid sequence that is
at least 90% identical to that of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:590, or comprises the antibody is 21B12, the anti-PCSK9 antibody is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 3-10 days, is administered to a patient at a dose of about 700 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 3-10 days; is administered to a patient at a dose of about 1200 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 3-10 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 3-10 days; is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 7-14 days; is administered to a patient at a dose of about 700 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 7-14 days; is administered to a patient at a dose of about 1200 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 7-14 days; is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days, is administered to a patient at a dose of about 700 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days; is administered to a patient at a dose of about 1200 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days; is administered
to a patient at a dose of about greater than 1200 mg to about 3000 mg intraveneously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 21-31 days.

In another particular embodiment wherein the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:23, a CDRL2 of the CDRL2 sequence in SEQ ID NO:23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49, or comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:23 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49 or the antibody is 21B12, the anti-PCSK9 antibody is administered to a patient at a dose of about 120 mg subcutaneously once a week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 7-10 days; is administered to a patient at a dose of about 140 mg subcutaneously once a week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 7-10 days; is administered to a patient at a dose of about 120 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 10-14 days; is administered to a patient at a dose of about 140 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 10-14 days; is administered to a patient at a dose of about 210 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 10-14 days; is administered to a patient at a dose of about 280 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 10-14 days; is administered to a patient at a dose of about 350 mg subcutaneously once every other week, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 10-14 days; is
administered to a patient at a dose of about 420 mg subcutaneously once every other week; is administered to a patient at a dose of about 280 mg to about 450 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patent is lowered at least about 30-50% for about 24-28 days; is administered to a patient at a dose of about 280 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 24-28 days; is administered to a patient at a dose of about 350 mg subcutaneously once every four weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 24-28 days; is administered to a patient at a dose of about 420 mg subcutaneously every 4 weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days.

In another particular embodiment, wherein the anti-PCSK9 antibody comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:23 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:590, or comprises the antibody is 21B12, the anti-PCSK9 antibody is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 700 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 1200 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is
administered to a patient at a dose of about 700 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about 1200 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days, is administered to a patient at a dose of about 700 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days; is administered to a patient at a dose of about 1200 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days.

In particular embodiments of the invention, the anti-PCSK9 antibody is 8A3, 11F1 and 8A1. In some embodiments the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463. In some embodiments, the anti-PCSK9 antibody comprises a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:463. In some embodiments the anti-PCSK9 antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463. In some embodiments the anti-PCSK9
antibody is 11F1. In a particular embodiment, wherein the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463, or comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:463, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463, or comprises the antibody is 11F1, the anti-PCSK9 antibody is administered to a patient at a dose of about 150 mg subcutaneously once a week wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 3-10 days, is administered to a patient at a dose of about 150 mg subcutaneously once every other week wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 7-14 days; is administered to a patient at a dose of about 150 mg subcutaneously once every four weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about greater than 150 mg to about 200 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 150 mg to about 170 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 450 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 21-31 days; is administered to a patient at a dose of about 150 mg subcutaneously once every six weeks wherein the serum LDL cholesterol level of the patent is lowered at
least about 15-50% for about 31-42 days; is administered to a patient at a dose of about
greater than 150 mg to about 200 mg subcutaneously once every six weeks, wherein the
serum LDL cholesterol level of the patient is lowered at least about 15-50% for about
31-42 days; is administered to a patient at a dose of about 170 mg to about 180 mg
subcutaneously once every six weeks wherein the serum LDL cholesterol level of the
patient is lowered at least about 15-50% for about 31-42 days; is administered to a
patient at a dose of about 150 mg to about 170 mg subcutaneously once every six
weeks wherein the serum LDL cholesterol level of the patient is lowered at least about
15-50% for about 31-42 days; is administered to a patient at a dose of about 450 mg
subcutaneously once every six weeks wherein the serum LDL cholesterol level of the
patient is lowered at least about 15-50% for about 31-42 days; is administered to a
patient at a dose of about 140 mg to about 200 mg subcutaneously every 8 weeks
wherein the serum LDL cholesterol level of the patient is lowered 15-50% for about 45-56
days; is administered to a patient at a dose of about 170 mg to about 180 mg
subcutaneously every 8 weeks wherein the serum LDL cholesterol level of the patient
is lowered 15-50% for about 45-56 days; is administered to a patient at a dose of about
150 mg to about 170 mg subcutaneously every 8 weeks wherein the serum LDL
cholesterol level of the patient is lowered 15-50% for about 45-56 days; is administered
to a patient at a dose of about 450 mg subcutaneously every 8 weeks wherein the serum
LDL cholesterol level of the patient is lowered 15-50% for about 45-56 days; at a dose
of about 600 mg subcutaneously once every 8 weeks wherein the serum LDL
cholesterol level of the patient is lowered at least about 15-50% for about 45-56 days;
at a dose of about 700 mg subcutaneously once every 8 weeks wherein the serum LDL
cholesterol level of the patient is lowered at least about 15-50% for about 45-56 days;
at a dose of about 600 mg subcutaneously once every 12 weeks wherein the serum LDL
cholesterol level of the patient is lowered at least about 15-50% for about 74-84 days;
at a dose of about 700 mg subcutaneously once every 12 weeks wherein the serum
LDL cholesterol level of the patient is lowered at least about 15-50% for about 74-84
days; at a dose of about 600 mg subcutaneously once every 16 weeks wherein the
serum LDL cholesterol level of the patient is lowered at least about 15-50% for about
100-112 days; at a dose of about 700 mg subcutaneously once every 16 weeks wherein
the serum LDL cholesterol level of the patient is lowered at least about 15-50\% for about 100-112 days.

In particular embodiments of the invention wherein the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:463, a CDRH2 of the CDRH2 sequence in SEQ ID NO:463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463. In some embodiments, the anti-PCSK9 antibody comprises, or comprises an amino acid sequence that is at least 90\% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90\% identical to that of SEQ ID NO:463, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463 or comprises the antibody is 11F1, the anti-PCSK9 antibody is administered to a patient at a dose of about 150 mg subcutaneously once a week wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 7-10 days, is administered to a patient at a dose of about 150 mg subcutaneously once every other week wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 10-14 days; is administered to a patient at a dose of about 150 mg subcutaneously once every four weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 24-28 days; is administered to a patient at a dose of about greater than 150 mg to about 200 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 24-28 days; is administered to a patient at a dose of about 170 mg to about 180 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 24-28 days; is administered to a patient at a dose of about 150 mg to about 170 mg subcutaneously once every four weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50\% for about 24-28 days; is administered to a patient at a dose of about 450 mg subcutaneously once every four weeks, wherein the
serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 24-28 days; is administered to a patient at a dose of about 150 mg subcutaneously once every six weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 40-41 days; is administered to a patient at a dose of about greater than 150 mg to about 200 mg subcutaneously once every six weeks, wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 40-41 days; is administered to a patient at a dose of about 170 mg to about 180 mg subcutaneously once every six weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 40-41 days; is administered to a patient at a dose of about 150 mg to about 170 mg subcutaneously once every six weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 40-41 days; is administered to a patient at a dose of about 450 mg subcutaneously once every six weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 40-41 days; is administered to a patient at a dose of about 140 mg to about 200 mg subcutaneously every 8 weeks wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 50-56 days; is administered to a patient at a dose of about 170 mg to about 180 mg subcutaneously every 8 weeks wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 50-56 days; is administered to a patient at a dose of about 150 mg to about 170 mg subcutaneously every 8 weeks wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 50-56 days; is administered to a patient at a dose of about 600 mg subcutaneously once every 8 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 50-56 days; at a dose of about 700 mg subcutaneously once every 8 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 50-56 days; at a dose of about 600 mg subcutaneously once every 12 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 80-84 days; at a dose of about 700 mg subcutaneously once every 12 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 80-84
days; at a dose of about 600 mg subcutaneously once every 16 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 105-112 days; at a dose of about 700 mg subcutaneously once every 16 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 105-112 days.

In particular embodiments of the invention wherein the anti-PCSK9 antibody comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463. In some embodiments, the anti-PCSK9 antibody comprises, or comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:463, or comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463, or the antibody is 11F1, the anti-PCSK9 antibody is administered to a patient the anti-PCSK9 antibody is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 700 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 1200 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 7-10 days; is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about 700 mg intravenously every other week, wherein the serum LDL cholesterol level of the
patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about 1200 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about greater than 1200 mg to about 3000 mg intravenously every other week, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 10-14 days; is administered to a patient at a dose of about 420 mg to about 3000 mg intravenously four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days, is administered to a patient at a dose of about 700 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days; is administered to a patient at a dose of about 1200 mg intravenously every four weeks, wherein the serum LDL cholesterol level of the patient is lowered 30-50% for about 24-28 days; is administered at a dose of about 1000 mg – 3000 mg intravenously once every 24 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 150 to 168 days; is administered at a dose of about 1000 mg – 3000 mg intravenously once every 24 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 160 to 168 days; is administered at a dose of about 1000 mg – 3000 mg intravenously once every 52 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 15-50% for about 350 to 365 days; is administered at a dose of about 1000 mg – 3000 mg intravenously once every 52 weeks wherein the serum LDL cholesterol level of the patient is lowered at least about 30-50% for about 360 to 365 days.

In another aspect of the invention, the at least one anti-PCSK9 antibody is administered to the patient before, after or concurrent with at least one other cholesterol-lowering agent. Cholesterol lowering agents include statins, including, atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, nicotinic acid (niacin), slow release niacin (SLO-NIACIN), laropiprant (CORDAPTIVE), fibric acid (LOPID (Gemfibrozil)), TRICOR
(fenofibrate)), Bile acid sequestrants, such as cholestyramine (QUESTRAN), colesvelam (WELCHOL), COLESTID (colestipol)), cholesterol absorption inhibitor (ZETIA (ezetimibe)), lipid modifying agents, PPAR gamma agonists, PPAR alpha/gamma agonists, squalene synthase inhibitors, CETP inhibitors, anti-hypertensives, anti-diabetic agents, including sulphonyl ureas, insulin, GLP-1 analogs, DDPIV inhibitors, ApoB modulators, MTP inhibitoris and/or arteriosclerosis obliterans treatments, oncostatin M, estrogen, berbine and therapeutic agents for an immune-related disorder.

In some aspects, the invention comprises a method of lowering the serum LDL cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia. The method comprises administering to a patient diagnosed with homozygous familial hypercholesterolemia a dose of about 120 mg to about 3000 mg of at least one anti-PCSK9 antibody described herein. In some embodiments, the dose is about 120 mg to about 450 mg of at least one anti-PCSK9 antibody administered once weekly (QW). In some embodiments, the dose is about 140 mg to about 450 mg of at least one anti-PCSK9 antibody administered once weekly. In some embodiments, the dose is about 280 mg to about 450 mg of at least one anti-PCSK9 antibody administered once weekly. In some embodiments, the dose is about 120 mg to about 450 mg of at least one anti-PCSK9 antibody administered once every 2 weeks (Q2W). In some embodiments, the dose is about 140 mg to about 450 mg of at least one anti-PCSK9 antibody administered once every 2 weeks (Q2W). In some embodiments, the dose is about 280 mg to about 420 mg of at least one anti-PCSK9 antibody administered once every 2 weeks (Q2W). In some embodiments, the dose is about 400 mg to about 450 mg of at least one anti-PCSK9 antibody administered once every 2 weeks (Q2W). In some embodiments, the dose is about 420 mg of at least one anti-PCSK9 antibody administered once every 2 weeks (Q2W). In some embodiments, the dose is about 250 mg to about 480 mg of at least one anti-PCSK9 antibody administered once every 4 weeks (Q4W). In some embodiments, the dose is about 280 mg to about 420 mg of at least one anti-PCSK9 antibody administered once every 4 weeks (Q4W). In some embodiments, the dose is about 350 mg to about 420 mg of at least one anti-PCSK9 antibody administered once every 4 weeks (Q4W). In some
embodiments, the dose is about 420 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every week (QW). In some embodiments, the dose is about 1000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every week (QW). In some embodiments, the dose is about 2000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every other week (Q2W). In some embodiments, the dose is about 1000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every other week (Q2W). In some embodiments, the dose is about 2000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every month (Q4W). In some embodiments, the dose is about 1000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every month (Q4W). In some embodiments, the dose is about 2000 mg to about 3000 mg of at least one anti-PCSK9 antibody administered once every month (Q4W). In some embodiments, the serum LDL cholesterol level is reduced by at least about 10% as compared to a predose serum LDL cholesterol level. In some embodiments, the serum LDL cholesterol level is reduced by at least about 15%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 20%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 25%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 30%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 35%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 40%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 45%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 50%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 55%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%. In some embodiments, the serum LDL cholesterol level is reduced by at least
about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%. % In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%.

In some aspects, the invention comprises a method of lowering the serum LDL cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of:

1. at least about 120 mg every week (QW); 2. at least an amount of about 140 mg every week (QW); 3. at least an amount of about 120 mg every two weeks or every other week (Q2W); 4. at least an amount of about 140 mg every two weeks or every other week (Q2W); 5. at least an amount of about 150 mg every two weeks or every other week (Q2W) 6. at least an amount of about 280 mg every two weeks or every other week (Q2W); 7. at least an amount of about 350 mg every two weeks or every other week (Q2W); 8. at least an amount of about 420 mg every two weeks or every other week (Q2W); and 9. at least an amount of about 150 mg every four weeks (Q4W); 10. at least an amount of about 160 mg every four weeks (Q4W); 11. at least an amount of about 170 mg every four weeks (Q4W); 12. at least an amount of about 180 mg every four weeks (Q4W); 13. at least an amount of about 190 mg every four weeks (Q4W); 14. at least an amount of about 200 mg every four weeks (Q4W); 15. at least an amount of about 280 mg every four weeks (Q4W); 16. at least an amount of about 350 every four weeks (Q4W); 17. at least an amount of about 420 mg every four weeks (Q4W); 18. at least an amount of about 1000 mg every four weeks (Q4W); 19. at least an amount of about 2000 mg every four weeks (Q4W); 20. at least an amount of about 3000 mg every four weeks (Q4W).

In some embodiments, the serum LDL cholesterol level is reduced by at least about 10% as compared to a predose serum LDL cholesterol level. In some embodiments, the serum LDL cholesterol level is reduced by at least about 10% as compared to a predose serum LDL cholesterol level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 15%. In some embodiments, the serum LDL cholesterol level is reduced by at least
about 20%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 25%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 30%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 35%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 40%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 45%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 50%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 55%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 65%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%.

In some aspects, the invention comprises a method of lowering the serum PCSK9 level in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of: (1) at least about 120 mg every week (QW); (2) at least an amount of about 140 mg every week (QW); (3) at least an amount of about 120 mg every two weeks or every other week (Q2W); (4) at least an amount of about 140 mg every two weeks or every other week (Q2W); (5) at least an amount of about 150 mg every two weeks or every other week (Q2W) (6) at least an amount of about 280 mg every two weeks or every other week (Q2W); (7) at least an amount of about 350 mg every two weeks or every other week (Q2W); (8) at least an amount of about 420 mg every two weeks or every other week (Q2W); and (9) at least an amount of about 150 mg every four weeks (Q4W); (10) at least an amount of about 160 mg every four weeks (Q4W); (11) at least an amount of about 170 mg every four weeks (Q4W); (12) at least an amount of about 180 mg every four weeks (Q4W); (13) at
least an amount of about 190 mg every four weeks (Q4W); (14) at least an amount of about 200 mg every four weeks (Q4W); (15) at least an amount of about 280 mg every four weeks (Q4W); (16) at least an amount of about 350 every four weeks (Q4W); (17) at least an amount of about 420 mg every four weeks (Q4W); (18) at least an amount of about 1000 mg every four weeks (Q4W); (19) at least an amount of about 2000 mg every four weeks (Q4W); and (20) at least an amount of about 3000 mg every four weeks (Q4W). In some embodiments, the serum PCSK9 value is reduced by at least about 20% as compared to a predose serum PCSK9 level. In some embodiments, the serum PCSK9 value is reduced by at least about 30%. In some embodiments, the serum PCSK9 value is reduced by at least about 40%. In some embodiments, the serum PCSK9 value is reduced by at least about 50%. In some embodiments, the serum PCSK9 value is reduced by at least about 60%. In some embodiments, the serum PCSK9 value is reduced by at least about 65%. In some embodiments, the serum PCSK9 value is reduced by at least about 70%. In some embodiments, the serum PCSK9 value is reduced by at least about 75%. In some embodiments, the serum PCSK9 value is reduced by at least about 80%. In some embodiments, the serum PCSK9 value is reduced by at least about 85%. In some embodiments, the serum PCSK9 value is reduced by at least about 90%.

In some aspects, the invention comprises a method of lowering the total cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of: (1) at least about 120 mg every week (QW); (2) at least an amount of about 140 mg every week (QW); (3) at least an amount of about 120 mg every two weeks or every other week (Q2W); (4) at least an amount of about 140 mg every two weeks or every other week (Q2W); (5) at least an amount of about 150 mg every two weeks or every other week (Q2W) (6) at least an amount of about 280 mg every two weeks or every other week (Q2W); (7) at least an amount of about 350 mg every two weeks or every other week (Q2W); (8) at least an amount of about 420 mg every two weeks or every other week (Q2W); and (9) at least an amount of about 150 mg every four weeks.
(Q4W); (10) at least an amount of about 160 mg every four weeks (Q4W); (11) at least an amount of about 170 mg every four weeks (Q4W); (12) at least an amount of about 180 mg every four weeks (Q4W); (13) at least an amount of about 190 mg every four weeks (Q4W); (14) at least an amount of about 200 mg every four weeks (Q4W); (15) at least an amount of about 280 mg every four weeks (Q4W); (16) at least an amount of about 350 mg every four weeks (Q4W); (17) at least an amount of about 420 mg every four weeks (Q4W); (18) at least an amount of about 1000 mg every four weeks (Q4W); (19) at least an amount of about 2000 mg every four weeks (Q4W); and (20) at least an amount of about 3000 mg every four weeks (Q4W). In some embodiments, the total cholesterol level is reduced by at least about 20% as compared to a predose total cholesterol level. In some embodiments, the total cholesterol level is reduced by at least about 25%. In some embodiments, the total cholesterol level is reduced by at least about 30%. In some embodiments, the total cholesterol level is reduced by at least about 35%. In some embodiments, the total cholesterol level is reduced by at least about 40%. In some embodiments, the total cholesterol level is reduced by at least about 45%. In some embodiments, the total cholesterol level is reduced by at least about 50%. In some embodiments, the total cholesterol level is reduced by at least about 55%. In some embodiments, the total cholesterol level is reduced by at least about 60%.

In some aspects, the invention comprises a method of lowering the non-HDL cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of:

(1) at least about 120 mg every week (QW); (2) at least an amount of about 140 mg every week (QW); (3) at least an amount of about 120 mg every two weeks or every other week (Q2W); (4) at least an amount of about 140 mg every two weeks or every other week (Q2W); (5) at least an amount of about 150 mg every two weeks or every other week (Q2W) (6) at least an amount of about 280 mg every two weeks or every other week (Q2W); (7) at least an amount of about 350 mg every two weeks or every other week (Q2W); (8) at least an amount of about 420 mg every two weeks or every
other week (Q2W); and (9) at least an amount of about 150 mg every four weeks (Q4W); (10) at least an amount of about 160 mg every four weeks (Q4W); (11) at least an amount of about 170 mg every four weeks (Q4W); (12) at least an amount of about 180 mg every four weeks (Q4W); (13) at least an amount of about 190 mg every four weeks (Q4W); (14) at least an amount of about 200 mg every four weeks (Q4W); (15) at least an amount of about 280 mg every four weeks (Q4W); (16) at least an amount of about 350 mg every four weeks (Q4W); (17) at least an amount of about 420 mg every four weeks (Q4W); (18) at least an amount of about 1000 mg every four weeks (Q4W); (19) at least an amount of about 2000 mg every four weeks (Q4W); and (20) at least an amount of about 3000 mg every four weeks (Q4W). In some embodiments, the non-HDL cholesterol level is reduced by at least about 30% as compared to a predose non-HDL cholesterol level. In some embodiments, the non-HDL cholesterol level is reduced by at least about 35%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 40%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 45%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 50%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 55%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 60%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 65%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 70%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 75%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 80%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 85%.

In some aspects, the invention comprises a method of lowering ApoB levels in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of: (1) at least about 120 mg every week (QW); (2) at least an amount of about 140 mg every week (QW); (3) at least an amount of about 120 mg every two weeks or every other week (Q2W); (4) at least an amount of about 140 mg every two weeks or every other week (Q2W); (5) at least an amount of about
150 mg every two weeks or every other week (Q2W); (6) at least an amount of about 280 mg every two weeks or every other week (Q2W); (7) at least an amount of about 350 mg every two weeks or every other week (Q2W); (8) at least an amount of about 420 mg every two weeks or every other week (Q2W); and (9) at least an amount of about 150 mg every four weeks (Q4W); (10) at least an amount of about 160 mg every four weeks (Q4W); (11) at least an amount of about 170 mg every four weeks (Q4W); (12) at least an amount of about 180 mg every four weeks (Q4W); (13) at least an amount of about 190 mg every four weeks (Q4W); (14) at least an amount of about 200 mg every four weeks (Q4W); (15) at least an amount of about 280 mg every four weeks (Q4W); (16) at least an amount of about 350 mg every four weeks (Q4W); (17) at least an amount of about 420 mg every four weeks (Q4W); (18) at least an amount of about 1000 mg every four weeks (Q4W); (19) at least an amount of about 2000 mg every four weeks (Q4W); and (20) at least an amount of about 3000 mg every four weeks (Q4W).

In some embodiments, the ApoB level is reduced by at least about 10% as compared to a predose ApoB level. In some embodiments, the ApoB level is reduced by at least about 15%. In some embodiments, the ApoB level is reduced by at least about 20%. In some embodiments, the ApoB level is reduced by at least about 25%. In some embodiments, the ApoB level is reduced by at least about 30%. In some embodiments, the ApoB level is reduced by at least about 35%. In some embodiments, the ApoB level is reduced by at least about 40%. In some embodiments, the ApoB level is reduced by at least about 45%. In some embodiments, the ApoB level is reduced by at least about 50%. In some embodiments, the ApoB level is reduced by at least about 55%. In some embodiments, the ApoB level is reduced by at least about 60%. In some embodiments, the ApoB level is reduced by at least about 65%. In some embodiments, the ApoB level is reduced by at least about 70%. In some embodiments, the ApoB level is reduced by at least about 75%.

In some aspects, the invention comprises a method of lowering Lipoprotein A ("Lp(a)") levels in a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient in need thereof, a dose of at least one anti-PCSK9 antibody, and wherein the dose of anti-PCSK9 antibody is administered on a schedule selected from the group consisting of: (1) at least about 120 mg every week
(QW); (2) at least an amount of about 140 mg every week (QW); (3) at least an amount of about 120 mg every two weeks or every other week (Q2W); (4) at least an amount of about 140 mg every two weeks or every other week (Q2W); (5) at least an amount of about 150 mg every two weeks or every other week (Q2W) (6) at least an amount of about 280 mg every two weeks or every other week (Q2W); (7) at least an amount of about 350 mg every two weeks or every other week (Q2W); (8) at least an amount of about 420 mg every two weeks or every other week (Q2W); and (9) at least an amount of about 150 mg every four weeks (Q4W); (10) at least an amount of about 160 mg every four weeks (Q4W); (11) at least an amount of about 170 mg every four weeks (Q4W); (12) at least an amount of about 180 mg every four weeks (Q4W); (13) at least an amount of about 190 mg every four weeks (Q4W); (14) at least an amount of about 200 mg every four weeks (Q4W); (15) at least an amount of about 280 mg every four weeks (Q4W); (16) at least an amount of about 350 every four weeks (Q4W); (17) at least an amount of about 420 mg every four weeks (Q4W); (18) at least an amount of about 1000 mg every four weeks (Q4W); (19) at least an amount of about 2000 mg every four weeks (Q4W); and (20) at least an amount of about 3000 mg every four weeks (Q4W).

In some embodiments, the Lp(a) level is reduced by at least about 10% as compared to a predose Lp(a) level. In some embodiments, the Lp(a) level is reduced by at least about 15%. In some embodiments, the Lp(a) level is reduced by at least about 20%. In some embodiments, the Lp(a) level is reduced by at least about 25%. In some embodiments, the Lp(a) level is reduced by at least about 30%. In some embodiments, the Lp(a) level is reduced by at least about 35%. In some embodiments, the Lp(a) level is reduced by at least about 40%. In some embodiments, the Lp(a) level is reduced by at least about 45%. In some embodiments, the Lp(a) level is reduced by at least about 50%. In some embodiments, the Lp(a) level is reduced by at least about 55%. In some embodiments, the Lp(a) level is reduced by at least about 60%. In some embodiments, the Lp(a) level is reduced by at least about 65%.

In some aspects, the invention comprises a method for treating a patient diagnosed with homozygous familial hypercholesterolemia, the method comprising administering to a patient diagnosed with homozygous familial hypercholesterolemia a dose of about 120 mg to about 3000 mg of at least one anti-PCSK9 antibody described herein. In
some embodiments, the dose is about 120 mg to about 450 mg of at least one anti-PCS9 antibody administered once weekly (QW). In some embodiments, the dose is about 140 mg to about 450 mg of at least one anti-PCS9 antibody administered once weekly. In some embodiments, the dose is about 120 mg to about 450 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 140 mg to about 420 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 105 mg to about 350 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 140 mg to about 280 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 150 mg to about 280 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 150 mg to about 200 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 400 mg to about 450 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 420 mg of at least one anti-PCS9 antibody administered once every two weeks (Q2W). In some embodiments, the dose is about 120 mg to about 480 mg of at least one anti-PCS9 antibody administered once every four weeks (Q4W). In some embodiments, the dose is about 150 mg to about 420 mg of at least one anti-PCS9 antibody administered once every four weeks (Q4W). In some embodiments, the dose is about 400 mg to about 450 mg of at least one anti-PCS9 antibody administered once every four weeks (Q4W). In some embodiments, the dose is about 250 mg to about 480 mg of at least one anti-PCS9 antibody administered once every four weeks (Q4W). In some embodiments, the dose is about 280 mg to about 420 mg of at least one anti-PCS9 antibody administered once every four weeks (Q4W). In some embodiments, the dose is about 350 mg to about 420 mg of at least one anti-PCS9 antibody administered once every four weeks. In some embodiments, the dose is about 1000 mg every four weeks (Q4W). In some embodiments, the dose is about 2000 mg every four weeks (Q4W). In some embodiments, the dose is about 3000 mg every four weeks (Q4W). In some embodiments, the serum LDL cholesterol level is reduced by at least
about 10% as compared to a predose serum LDL cholesterol level. In some embodiments, the serum LDL cholesterol level is reduced by at least about 15%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 20%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 25%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 30%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 35%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 40%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 45%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 50%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 55%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 65%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%.

In some embodiments, the anti-PCSK9 antibody is 21B12, 26H5, 31H4, 8A3, 11F1 and/or 8A1.

Other embodiments of this invention will be readily apparent from the disclosure provided herewith.
BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A depicts an amino acid sequence of the mature form of the PCSK9 with the pro-domain underlined.

FIGs. 1B1-1B4 depict amino acid and nucleic acid sequences of PCSK9 with the pro-domain underlined and the signal sequence in bold.

FIGs. 2A-2D are sequence comparison tables of various light chains of various antigen binding proteins. FIG. 2C continues the sequence started in FIG. 2A. FIG. 2D continues the sequence started on FIG. 2B.

FIGs. 3A-3D are sequence comparison tables of various heavy chains of various antigen binding proteins. FIG. 3C continues the sequence started in FIG. 3A. FIG. 3D continues the sequence started on FIG. 3B.

FIGs. 3E-3JJ depict the amino acid and nucleic acid sequences for the variable domains of some embodiments of the antigen binding proteins.

FIG. 3KK depicts the amino acid sequences for various constant domains.

FIGs. 3LL-3BBB depict the amino acid and nucleic acid sequences for the variable domains of some embodiments of the antigen binding proteins.

FIGs. 3CCC-3JJJ are sequence comparison tables of various heavy and light chains of some embodiments of the antigen binding proteins.

FIG. 3LLL is a set of ABP sequences identifying various differences between the human ABP sequences and the ABP sequences that were raised in E. coli. (U.S.P. 8,030,457).

FIG. 4A is a binding curve of an antigen binding protein to human PCSK9.

FIG. 4B is a binding curve of an antigen binding protein to human PCSK9.

FIG. 4C is a binding curve of an antigen binding protein to cynomolgus PCSK9.

FIG. 4D is a binding curve of an antigen binding protein to cynomolgus PCSK9.

FIG. 4E is a binding curve of an antigen binding protein to mouse PCSK9.

FIG. 4F is a binding curve of an antigen binding protein to mouse PCSK9.

FIG. 5A depicts the results of an SDS PAGE experiment involving PCSK9 and various antigen binding proteins demonstrating the relative purity and concentration of the proteins.
FIG. 5B and 5C depict graphs from Biacore solution equilibrium assays for 21B12.

FIG. 5D depicts the graph of the kinetics from a Biacore capture assay.

FIG. 6A is an inhibition curve of antigen binding protein 31H4 IgG2 to PCSK9 in an in vitro PCSK9:LDLR binding assay.

FIG. 6B is an inhibition curve of antigen binding protein 31H4 IgG4 to PCSK9 in an in vitro PCSK9:LDLR binding assay.

FIG. 6C is an inhibition curve of antigen binding protein 21B12 IgG2 to PCSK9 in an in vitro PCSK9:LDLR binding assay.

FIG. 6D is an inhibition curve of antigen binding protein 21B12 IgG4 to PCSK9 in an in vitro PCSK9:LDLR binding assay.

FIG. 7A is an inhibition curve of antigen binding protein 31H4 IgG2 in the cell LDL uptake assay showing the effect of the ABP to reduce the LDL uptake blocking effects of PCSK9.

FIG. 7B is an inhibition curve of antigen binding protein 31H4 IgG4 in the cell LDL uptake assay showing the effect of the ABP to reduce the LDL uptake blocking effects of PCSK9.

FIG. 7C is an inhibition curve of antigen binding protein 21B12 IgG2 in the cell LDL uptake assay showing the effect of the ABP to reduce the LDL uptake blocking effects of PCSK9.

FIG. 7D is an inhibition curve of antigen binding protein 21B12 IgG4 in the cell LDL uptake assay showing the effect of the ABP to reduce the LDL uptake blocking effects of PCSK9.

FIG. 8A is a graph depicting the serum cholesterol lowering ability in mice of ABP 31H4, changes relative to the IgG control treated mice (* p< 0.01).

FIG. 8B is a graph depicting the serum cholesterol lowering ability in mice of ABP 31H4, changes relative to time = zero hours (# p, 0.05).

FIG. 8C is a graph depicting the effect of ABP 31H4 on HDL cholesterol levels in C57B1/6 mice (* p< 0.01).

FIG. 8D is a graph depicting the effect of ABP 31H4 on HDL cholesterol levels in C57Bl/6 mice (# p< 0.05).
FIG. 9 depicts a western blot analysis of the ability of ABP 31H4 to enhance the amount of liver LDLR protein present after various time points.

FIG. 10A is a graph depicting the ability of an antigen binding protein 31H4 to lower total serum cholesterol in wild type mice, relative.

FIG. 10B is a graph depicting the ability of an antigen binding protein 31H4 to lower HDL in wild type mice.

FIG. 10C is a graph depicting the serum cholesterol lowering ability of various antigen binding proteins 31H4 and 16F12.

FIG. 11A depicts an injection protocol for testing the duration and ability of antigen binding proteins to lower serum cholesterol.

FIG. 11B is a graph depicting the results of the protocol in FIG. 11A.

FIG. 12A depicts LDLR levels in response to the combination of a statin and ABP 21B12 in HepG2 cells.

FIG. 12B depicts LDLR levels in response to the combination of a statin and ABP 31H4 in HepG2 cells.

FIG. 12C depicts LDLR levels in response to the combination of a statin and ABP 25A7.1, a non-neutralizing antibody, (in contrast the “25A7” a neutralizing antibody) in HepG2 cells.

FIG. 12D depicts LDLR levels in response to the combination of a statin and ABP 21B12 in HepG2 cells over expressing PCSK9.

FIG. 12E depicts LDLR levels in response to the combination of a statin and ABP 31H4 in HepG2 cells over expressing PCSK9.

FIG. 12F depicts LDLR levels in response to the combination of a statin and ABP 25A7.1, a non-neutralizing antibody, (in contrast the “25A7” a neutralizing antibody) in HepG2 cells over expressing PCSK9.

FIG. 13A depicts the various light chain amino acid sequences of various ABPs to PCSK9. The dots (.) indicate no amino acid.

FIG. 13B depicts a light chain cladogram for various ABPs to PCSK9.

FIG. 13C depicts the various heavy chain amino acid sequences of various ABPs to PCSK9. The dots (.) indicate no amino acid.

FIG. 13D depicts a heavy chain dendrogram for various ABPs to PCSK9.
FIG. 13E depicts a comparison of light and heavy CDRs and designation of
groups from which to derive consensus.

FIG. 13F depicts the consensus sequences for Groups 1 and 2.
FIG. 13G depicts the consensus sequences for Groups 3 and 4.

FIG. 13H depicts the consensus sequences for Groups 1 and 2. The dots (.)
indicated identical residues.

FIG. 13I depicts the consensus sequences for Group 2. The dots (.) indicated
identical residues.

FIG. 13J depicts the consensus sequences for Groups 3 and 4. The dots (.)
indicated identical residues.

FIG. 14 is a graph illustrating the binding specificity of 11F1 in a competition
assay with PCSKP, PCSK2, PCSK1 PCSK7 and Furin with OD_{450} plotted on the
vertical axis and concentration of PCSK9 (ug/ml) plotted on the horizontal axis.

FIG. 15 is a graph showing the dose response curve for inhibition of
LDLR:D374Y PCSK9 binding by 11F1 in a competition assay with OD_{450} plotted on the
vertical axis and Log [11F1] (pM) plotted on the horizontal axis.

FIG. 16 is a graph depicting the dose response curve for the inhibition of LDLR:
WT PCSK9 binding by 11F1 in a competition assay with OD_{450} plotted on the vertical
axis and Log [11f1] (pM) plotted on the horizontal axis.

FIG. 17 is a graph depicting the dose response curve for the ability of 11F1 to
block human D374Y PCSK9-mediated reduction of LDL uptake in HepG2 cells with
relative fluorescence units (x10^4) plotted on the vertical axis and Log [11F1] (nM)
plotted on the horizontal axis.

FIG. 18 is a graph depicting the dose response curve for the ability of 11F1 to
block human WT PCSK9-mediated reduction of LDL uptake in HepG2 cells with
relative fluorescence units plotted (x10^4) on the vertical axis and Log [11F1] (nM)
plotted on the horizontal axis.

FIG. 19 is a bar graph depicting the effect of 11F1 and 8A3 on serum non-HDL
cholesterol in mice expressing human PCSK9 by AAV with non-HDL-C serum
concentration (mg/ml) on the vertical axis and time following injection (days) plotted
on the horizontal axis.
FIG. 20 is a bar graph depicting the effect of 11F1 and 8A3 on Serum Total Cholesterol in mice expressing human PCSK9 by AAV with Serum Total Cholesterol (mg/ml) on the vertical axis and time following injection (days) plotted on the horizontal axis.

FIG. 21 is a bar graph depicting the effect of 11F1 and 8A3 on Serum HDL Cholesterol (HDL-C) in mice expressing human PCSK9 by AAV with HDL-C (mg/ml) on the vertical axis and time following injection (days) plotted on the horizontal axis.

FIG. 22 is a graph depicting IgG2, 8A3 and 11F1 antibody concentration profiles in mice expressing human PCSK9 by AAV with serum antibody concentration (ng/mL) plotted on the vertical axis and time following injection in days plotted on the horizontal axis.

FIG. 23 is a table summarizing PK parameters for IgG2, 11F1 and 8A3 in mice expressing human PCSK9 by AAV.

FIG. 24 is a graph depicting the effect of a single subcutaneous administration of an ant-KLH antibody (control), 21B12, 8A3 and 11F1 on serum LDL concentration (LDL-C) in cynomolgus monkeys with LDL-C (mg/dl) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 25 is a graph depicting the effect of a single subcutaneous administration of an ant-KLH antibody (control), 21B12, 8A3 and 11F1 on Serum Total Cholesterol in cynomolgus monkeys with Total Cholesterol concentration (mg/dl) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 26 is a graph depicting the effect of a single subcutaneous administration of an ant-KLH antibody (control), 21B12, 8A3 and 11F1 on Serum HDL Cholesterol in cynomolgus monkeys with HDL-C (mg/dl) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 27 is a graph depicting the effect of a single subcutaneous administration of an ant-KLH antibody (control), 21B12, 8A3 and 11F1 on Serum Triglycerides in cynomolgus monkeys with Serum Triglyceride concentration (mg/dl) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 28 is a graph depicting the effect of a single subcutaneous administration of an ant-KLH antibody (control), 21B12, 8A3 and 11F1 on Apolipoprotein B (ApoB)
in cynomolgus monkeys with APOB concentration (mg/dl) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 29 is a graph depicting the mean pharmacokinetic profiles for the anti--KLH antibody (control), 21B12, 8A3 and 11F1 in cynomolgus monkeys with antibody concentrations (ng/ml) plotted on the vertical axis and time following administration in days on the horizontal axis.

FIG. 30 is a table summarizing PK parameters for the anti--KLH antibody (control), 21B12, 8A3 and 11F1 in cynomolgus monkeys.

FIG. 31A depicts a comparison of light chain amino acid sequences of 8A1, 8A3 and 11F1, as well as a consensus sequence derived from the comparison. CDR sequences are underlined.

FIG. 31B depicts a comparison of heavy chain amino acid sequences of 8A1, 8A3 and 11F1, as well as a consensus sequence derived from the comparison. CDR sequences are underlined.

DETAILED DESCRIPTION OF CERTAIN EXEMPLARY EMBODIMENTS

Provided herein are methods of treating a patient diagnosed with homozygous familial hypercholesterolemia are also included, said method comprises administering at least one antigen binding protein, including antibodies, against proprotein convertase subtilisin/kexin type 9 (PCSK9) to the patient. Moreover, methods of lowering serum LDL cholesterol in a patient diagnosed with homozygous familial hypercholesterolemia using antigen binding proteins, including antibodies, against proprotein convertase subtilisin/kexin type 9 (PCSK9) are provided herein.

For convenience, the following sections generally outline the various meanings of the terms used herein. Following this discussion, general aspects regarding antigen binding proteins are discussed, followed by specific examples demonstrating the properties of various embodiments of the antigen binding proteins and how they can be employed.

Definitions and Embodiments

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not
restrictive of the invention as claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including”, as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise. Also, the use of the term “portion” can include part of a moiety or the entire moiety.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term “proprotein convertase subtilisin kexin type 9” or “PCSK9” refers to a polypeptide as set forth in SEQ ID NO: 1 and/or 3 or fragments thereof, as well as related polypeptides, which include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants including the addition of an N-terminal methionine, fusion polypeptides, and interspecies homologs. In certain embodiments, a PCSK9 polypeptide includes terminal residues, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues. “PCSK9” has also been referred to as FH3, NARC1, HCHOLA3, proprotein convertase subtilisin/kexin type 9, and neural apoptosis regulated convertase 1. The PCSK9 gene encodes a proprotein convertase protein that belongs to the proteinase K subfamily of the secretory subtilase family. The term “PCSK9” denotes both the proprotein and the product generated following autocatalysis of the proprotein. When only the autocatalyzed product is being referred to (such as for an antigen binding protein that selectively binds to the cleaved PCSK9), the protein can be referred to as the “mature,” “cleaved”, “processed” or “active”
PCSK9. When only the inactive form is being referred to, the protein can be referred to as the “inactive”, “pro-form”, or “unprocessed” form of PCSK9. The term PCSK9 as used herein also includes naturally occurring alleles, such as the mutations D374Y, S127R and F216L. The term PCSK9 also encompasses PCSK9 molecules incorporating post-translational modifications of the PCSK9 amino acid sequence, such as PCSK9 sequences that have been glycosylated, PEGylated, PCSK9 sequences from which its signal sequence has been cleaved, PCSK9 sequence from which its pro domain has been cleaved from the catalytic domain but not separated from the catalytic domain (e.g., FIGs. 1A and 1B).

The term “PCSK9 activity” includes any biological effect of PCSK9. In certain embodiments, PCSK9 activity includes the ability of PCSK9 to interact or bind to a substrate or receptor. In some embodiments, PCSK9 activity is represented by the ability of PCSK9 to bind to a LDL receptor (LDLR). In some embodiments, PCSK9 binds to and catalyzes a reaction involving LDLR. In some embodiments, PCSK9 activity includes the ability of PCSK9 to alter (e.g., reduce) the availability of LDLR. In some embodiments, PCSK9 activity includes the ability of PCSK9 to increase the amount of LDL in a subject. In some embodiments, PCSK9 activity includes the ability of PCSK9 to decrease the amount of LDLR that is available to bind to LDL. In some embodiments, “PCSK9 activity” includes any biological activity resulting from PCSK9 signaling. Exemplary activities include, but are not limited to, PCSK9 binding to LDLR, PCSK9 enzyme activity that cleaves LDLR or other proteins, PCSK9 binding to proteins other than LDLR that facilitate PCSK9 action, PCSK9 altering APOB secretion (Sun X-M et al, “Evidence for effect of mutant PCSK9 on apolipoprotein B secretion as the cause of unusually severe dominant hypercholesterolemia, Human Molecular Genetics 14: 1161-1169, 2005 and Ouguerram K et al, “Apolipoprotein B100 metabolism in autosomal-dominant hypercholesterolemia related to mutations in PCSK9, Arterioscler thromb Vasc Biol. 24: 1448-1453, 2004), PCSK9’s role in liver regeneration and neuronal cell differentiation (Seidah NG et al, “The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation” PNAS 100: 928-933, 2003), and PCSK9’s role in hepatic glucose metabolism (Costet et al., “Hepatic PCSK9 expression is

The term “hypercholesterolemia,” as used herein, refers to a condition in which cholesterol levels are elevated above a desired level. In some embodiments, this denotes that serum cholesterol levels are elevated. In some embodiments, the desired level takes into account various “risk factors” that are known to one of skill in the art (and are described or referenced herein).

The term “homozygous familial hypercholesterolemia” or “HoFH” as used herein, refers a cholesterol-related disorder that is determined by genetic confirmation or clinical diagnosis (such as history of an untreated LDL-cholesterol concentration greater than 13 mmol/L plus either xanthoma before 10 years of age or evidence of heterozygous familial hypercholesterolaemia in both parents).

The term “polynucleotide” or “nucleic acid” includes both single-stranded and double-stranded nucleotide polymers. The nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine and inosine derivatives, ribose modifications such as 2’3’-dideoxyribose, and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate.

The term “oligonucleotide” means a polynucleotide comprising 200 or fewer nucleotides. In some embodiments, oligonucleotides are 10 to 60 bases in length. In other embodiments, oligonucleotides are 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 nucleotides in length. Oligonucleotides can be single stranded or double stranded, e.g., for use in the construction of a mutant gene. Oligonucleotides can be sense or antisense oligonucleotides. An oligonucleotide can include a label, including a radiolabel, a fluorescent label, a hapten or an antigenic label, for detection assays. Oligonucleotides can be used, for example, as PCR primers, cloning primers or hybridization probes.

An “isolated nucleic acid molecule” means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in
nature, or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that “a nucleic acid molecule comprising” a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules “comprising” specified nucleic acid sequences can include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty other proteins or portions thereof, or can include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or can include vector sequences.

Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence discussed herein is the 5’ end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5’ direction. The direction of 5’ to 3’ addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5’ to the 5’ end of the RNA transcript are referred to as “upstream sequences;” sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3’ to the 3’ end of the RNA transcript are referred to as “downstream sequences.”

The term “control sequence” refers to a polynucleotide sequence that can affect the expression and processing of coding sequences to which it is ligated. The nature of such control sequences can depend upon the host organism. In particular embodiments, control sequences for prokaryotes can include a promoter, a ribosomal binding site, and a transcription termination sequence. For example, control sequences for eukaryotes can include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, and transcription termination sequence. “Control sequences” can include leader sequences and/or fusion partner sequences.

The term “vector” means any molecule or entity (e.g., nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell.

The term “expression vector” or “expression construct” refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more
heterologous coding regions operatively linked thereto. An expression construct can include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto.

As used herein, “operably linked” means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions. For example, a control sequence in a vector that is “operably linked” to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

The term “host cell” means a cell that has been transformed, or is capable of being transformed, with a nucleic acid sequence and thereby expresses a gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent cell, so long as the gene of interest is present.

The term “transfection” means the uptake of foreign or exogenous DNA by a cell, and a cell has been “transfected” when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham et al., 1973, Virology 52:456; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, supra; Davis et al., 1986, Basic Methods in Molecular Biology, Elsevier; Chu et al., 1981, Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term “transformation” refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain new DNA or RNA. For example, a cell is transformed where it is genetically modified from its native state by introducing new genetic material via transfection, transduction, or other techniques. Following transfection or transduction, the transforming DNA can recombine with that of the cell by physically integrating into a chromosome of the cell, or can be maintained transiently as an episomal element without being replicated, or
can replicate independently as a plasmid. A cell is considered to have been “stably transformed” when the transforming DNA is replicated with the division of the cell.

The terms “polypeptide” or “protein” means a macromolecule having the amino acid sequence of a native protein, that is, a protein produced by a naturally-occurring and non-recombinant cell; or it is produced by a genetically-engineered or recombinant cell, and comprise molecules having the amino acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The term also includes amino acid polymers in which one or more amino acids are chemical analogs of a corresponding naturally-occurring amino acid and polymers. The terms “polypeptide” and “protein” specifically encompass PCSK9 antigen binding proteins, antibodies, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of antigen-binding protein. The term “polypeptide fragment” refers to a polypeptide that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion as compared with the full-length native protein. Such fragments can also contain modified amino acids as compared with the native protein. In certain embodiments, fragments are about five to 500 amino acids long. For example, fragments can be at least 5, 6, 8, 10, 14, 20, 50, 70, 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long. Useful polypeptide fragments include immunologically functional fragments of antibodies, including binding domains. In the case of a PCSK9-binding antibody, useful fragments include but are not limited to a CDR region, a variable domain of a heavy and/or light chain, a portion of an antibody chain or just its variable region including two CDRs, and the like.

The term “isolated protein” referred means that a subject protein (1) is free of at least some other proteins with which it would normally be found, (2) is essentially free of other proteins from the same source, e.g., from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (6) does not occur in nature. Typically, an “isolated protein” constitutes at least about 5%, at least about 10%, at
least about 25%, or at least about 50% of a given sample. Genomic DNA, cDNA, mRNA or other RNA, of synthetic origin, or any combination thereof can encode such an isolated protein. Preferably, the isolated protein is substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic, research or other use.

The term “amino acid” includes its normal meaning in the art.

A “variant” of a polypeptide (e.g., an antigen binding protein, or an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants include fusion proteins.

The term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity” means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (i.e., an “algorithm”). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al., 1988, SIAM J. Applied Math. 48:1073.

In calculating percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences. One example of a computer program that can be used to determine percent identity is the GCG program package, which includes GAP (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI). The computer algorithm
GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the “matched span”, as determined by the algorithm). A gap opening penalty (which is calculated as 3x the average diagonal, wherein the “average diagonal” is the average of the diagonal of the comparison matrix being used; the “diagonal” is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOsum 62 are used in conjunction with the algorithm. In certain embodiments, a standard comparison matrix (see, Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for the BLOSum 62 comparison matrix) is also used by the algorithm.

Examples of parameters that can be employed in determining percent identity for polypeptides or nucleotide sequences using the GAP program are the following:

- Comparison matrix: BLOsum 62 from Henikoff et al., 1992, supra
- Gap Penalty: 12 (but with no penalty for end gaps)
- Gap Length Penalty: 4
- Threshold of Similarity: 0

Certain alignment schemes for aligning two amino acid sequences may result in matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, the selected alignment method (GAP program) can be adjusted if so desired to result in an alignment that spans at least 50 or other number of contiguous amino acids of the target polypeptide.

As used herein, the twenty conventional (e.g., naturally occurring) amino acids and their abbreviations follow conventional usage. See Immunology--A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference for any purpose. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as
α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids can also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline).

In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5’ end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5’ direction. The direction of 5’ to 3’ addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5’ to the 5’ end of the RNA transcript are referred to as “upstream sequences”; sequence regions on the DNA strand having the same sequence as the RNA and which are 3’ to the 3’ end of the RNA transcript are referred to as “downstream sequences.”

Conservative amino acid substitutions can encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues can be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions can involve the exchange of a member of one of these classes for a member from another class. Such substituted residues can be
introduced, for example, into regions of a human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

In making changes to the antigen binding protein or the PCSK9 protein, according to certain embodiments, the hydrophobic index of amino acids can be considered. Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157:105-131 (1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydrophobic index or score and still retain a similar biological activity. In making changes based upon the hydrophobic index, in certain embodiments, the substitution of amino acids whose hydrophobic indices are within ±2 is included. In certain embodiments, those which are within ±1 are included, and in certain embodiments, those within ±0.5 are included.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in
certain embodiments, the substitution of amino acids whose hydrophilicity values are within ±2 is included, in certain embodiments, those which are within ±1 are included, and in certain embodiments, those within ±0.5 are included. One can also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Exemplary amino acid substitutions are set forth in Table 1.

**TABLE 1**
Amino Acid Substitutions

<table>
<thead>
<tr>
<th>Original Residues</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Val, Leu, Ile</td>
<td>Val</td>
</tr>
<tr>
<td>Arg</td>
<td>Lys, Gln, Asn</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn</td>
<td>Gln</td>
<td>Gln</td>
</tr>
<tr>
<td>Asp</td>
<td>Glu</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys</td>
<td>Ser, Ala</td>
<td>Ser</td>
</tr>
<tr>
<td>Gln</td>
<td>Asn</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu</td>
<td>Asp</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly</td>
<td>Pro, Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>His</td>
<td>Asn, Gln, Lys, Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Ile</td>
<td>Leu, Val, Met, Ala,</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td>Phe, Norleucine</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Norleucine, Ile,</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td>Val, Met, Ala, Phe</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Arg, 1,4 Diamino-butyric</td>
<td>Arg</td>
</tr>
<tr>
<td></td>
<td>Acid, Gln, Asn</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>Leu, Phe, Ile</td>
<td>Leu</td>
</tr>
<tr>
<td>Phe</td>
<td>Leu, Val, Ile, Ala,</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td>Tyr</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Ala</td>
<td>Gly</td>
</tr>
<tr>
<td>Ser</td>
<td>Thr, Ala, Cys</td>
<td>Thr</td>
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<tr>
<td>Original Residues</td>
<td>Exemplary Substitutions</td>
<td>Preferred Substitutions</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Thr</td>
<td>Ser</td>
<td>Ser</td>
</tr>
<tr>
<td>Trp</td>
<td>Tyr, Phe</td>
<td>Tyr</td>
</tr>
<tr>
<td>Tyr</td>
<td>Trp, Phe, Thr, Ser</td>
<td>Phe</td>
</tr>
<tr>
<td>Val</td>
<td>Ile, Met, Leu, Phe, Ala, Norleucine</td>
<td>Leu</td>
</tr>
</tbody>
</table>

The term “derivative” refers to a molecule that includes a chemical modification other than an insertion, deletion, or substitution of amino acids (or nucleic acids). In certain embodiments, derivatives comprise covalent modifications, including, but not limited to, chemical bonding with polymers, lipids, or other organic or inorganic moieties. In certain embodiments, a chemically modified antigen binding protein can have a greater circulating half-life than an antigen binding protein that is not chemically modified. In certain embodiments, a chemically modified antigen binding protein can have improved targeting capacity for desired cells, tissues, and/or organs. In some embodiments, a derivative antigen binding protein is covalently modified to include one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. See, e.g., U.S. Patent Nos: 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. In certain embodiments, a derivative antigen binding protein comprises one or more polymer, including, but not limited to, 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, as well as mixtures of such polymers.

In certain embodiments, a derivative is covalently modified with polyethylene glycol (PEG) subunits. In certain embodiments, one or more water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a derivative. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains of a derivative. In certain embodiments, PEG is
used to improve the therapeutic capacity for an antigen binding protein. In certain embodiments, PEG is used to improve the therapeutic capacity for a humanized antibody. Certain such methods are discussed, for example, in U.S. Patent No. 6,133,426, which is hereby incorporated by reference for any purpose.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics." Fauchere, J., Adv. Drug Res., 15:29 (1986); Veber & Freidinger, TINS, p.392 (1985); and Evans et al., J. Med. Chem., 30:1229 (1987), which are incorporated herein by reference for any purpose. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce a similar therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from: --CH2 NH--, --CH2 S--, --CH2 -CH2 --, --CH=CH-(cis and trans), --COCH2 --, --CH(OH)CH2 --, and --CH2 SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used in certain embodiments to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation can be generated by methods known in the art (Rizo and Gierasch, Ann. Rev. Biochem., 61:387 (1992), incorporated herein by reference for any purpose); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The term "naturally occurring" as used throughout the specification in connection with biological materials such as polypeptides, nucleic acids, host cells, and the like, refers to materials which are found in nature or a form of the materials that is found in nature.

An "antigen binding protein" ("ABP") as used herein means any protein that binds a specified target antigen. In the instant application, the specified target antigen
is the PCSK9 protein or fragment thereof. “Antigen binding protein” includes but is not limited to antibodies and binding parts thereof, such as immunologically functional fragments. Peptodies are another example of antigen binding proteins. The term “immunologically functional fragment” (or simply “fragment”) of an antibody or immunoglobulin chain (heavy or light chain) antigen binding protein, as used herein, is a species of antigen binding protein comprising a portion (regardless of how that portion is obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which is still capable of specifically binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other antigen binding proteins, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. In some embodiments, the fragments can block or reduce the likelihood of the interaction between LDLR and PCSK9. In one aspect, such a fragment will retain at least one CDR present in the full-length light or heavy chain, and in some embodiments will comprise a single heavy chain and/or light chain or portion thereof. These biologically active fragments can be produced by recombinant DNA techniques, or can be produced by enzymatic or chemical cleavage of antigen binding proteins, including intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, a diabody (heavy chain variable domain on the same polypeptide as a light chain variable domain, connected via a short peptide linker that is too short to permit pairing between the two domains on the same chain), Fab’, F(ab’)2, Fv, domain antibodies and single-chain antibodies, and can be derived from any mammalian source, including but not limited to human, mouse, rat, camelid or rabbit. It is further contemplated that a functional portion of the antigen binding proteins disclosed herein, for example, one or more CDRs, could be covalently bound to a second protein or to a small molecule to create a therapeutic agent directed to a particular target in the body, possessing bifunctional therapeutic properties, or having a prolonged serum half-life. As will be appreciated by one of skill in the art, an antigen binding protein can include nonprotein components. In some sections of the present disclosure, examples of ABPs are described herein in terms of “number/letter/number” (e.g., 25A7). In these cases, the exact name denotes a specific
antibody (e.g., 25A7 versus 21B12). That is, an ABP named 25A7 is not necessarily
the same as an antibody named 25A7.1, (unless they are explicitly taught as the same in
the specification, e.g., 25A7 and 25A7.3). Unless otherwise stated, the ABP name is
understood to be a generic designation denoting an antibody.

Certain antigen binding proteins described herein are antibodies or are derived
from antibodies. In certain embodiments, the polypeptide structure of the antigen
binding proteins is based on antibodies, including, but not limited to, monoclonal
antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies
(sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized
antibodies, human antibodies, antibody fusions (sometimes referred to herein as
“antibody conjugates”), and fragments thereof, respectively. In some embodiments, the
ABP comprises or consists of avimers (tightly binding peptide). These various antigen
binding proteins are further described herein. Moreover, examples of antibodies are
provided in e.g., U.S.P. 8,030,457, U.S.P. 8,168,762. Further examples of antibodies
are provided in e.g., U.S.P. 8,188,233, U.S.P. 8,188,234, U.S.P. 8,080,243, U.S.P.

An “Fe” region comprises two heavy chain fragments comprising the C_{H1} and
C_{H2} domains of an antibody. The two heavy chain fragments are held together by two
or more disulfide bonds and by hydrophobic interactions of the C_{H3} domains.

A “Fab fragment” comprises one light chain and the C_{H1} and variable regions
of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond
with another heavy chain molecule.

A “Fab’ fragment” comprises one light chain and a portion of one heavy chain
that contains the VH domain and the C_{H1} domain and also the region between the C_{H1}
and C_{H2} domains, such that an interchain disulfide bond can be formed between the
two heavy chains of two Fab’ fragments to form an F(ab’)_{2} molecule.

A “F(ab’)_{2} fragment” contains two light chains and two heavy chains containing
a portion of the constant region between the C_{H1} and C_{H2} domains, such that an
interchain disulfide bond is formed between the two heavy chains. A F(ab’)_{2} fragment
thus is composed of two Fab’ fragments that are held together by a disulfide bond between the two heavy chains.

The “Fv region” comprises the variable regions from both the heavy and light chains, but lacks the constant regions.

“Single-chain antibodies” are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen binding region. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649 and United States Patent Nos. 4,946,778 and No. 5,260,203, the disclosures of which are incorporated by reference.

A “domain antibody” is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_{H} regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_{H} regions of a bivalent domain antibody can target the same or different antigens.

A “bivalent antigen binding protein” or “bivalent antibody” comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. Bivalent antigen binding proteins and bivalent antibodies can be bispecific, see, infra. A bivalent antibody other than a “multispecific” or “multifunctional” antibody, in certain embodiments, typically is understood to have each of its binding sites identical.

A “multispecific antigen binding protein” or “multispecific antibody” is one that targets more than one antigen or epitope.

A “bispecific,” “dual-specific” or “bifunctional” antigen binding protein or antibody is a hybrid antigen binding protein or antibody, respectively, having two different antigen binding sites. Bispecific antigen binding proteins and antibodies are a species of multispecific antigen binding protein antibody and can be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab’ fragments. See, e.g., Songsivilai and Lachmann, 1990, Clin. Exp. Immunol. 79:315-321; Kostelny et al., 1992, J. Immunol. 148:1547-1553. The two binding sites
of a bispecific antigen binding protein or antibody will bind to two different epitopes, which can reside on the same or different protein targets.

An antigen binding protein is said to “specifically bind” its target antigen when the dissociation constant (K_d) is ≤10^{-7} M. The ABP specifically binds antigen with “high affinity” when the K_d is ≤5 x 10^{-9} M, and with “very high affinity” when the K_d is ≤5 x 10^{-10} M. In one embodiment, the ABP has a K_d of ≤10^{-9} M. In one embodiment, the off-rate is <1 x 10^{-5}. In other embodiments, the ABPs will bind to human PCSK9 with a K_d of between about 10^{-9} M and 10^{-13} M, and in yet another embodiment the ABPs will bind with a K_d ≤5 x 10^{-10}. As will be appreciated by one of skill in the art, in some embodiments, any or all of the antigen binding fragments can specifically bind to PCSK9.

An antigen binding protein is “selective” when it binds to one target more tightly than it binds to a second target.

“Antigen binding region” means a protein, or a portion of a protein, that specifically binds a specified antigen (e.g., a paratope). For example, that portion of an antigen binding protein that contains the amino acid residues that interact with an antigen and confer on the antigen binding protein its specificity and affinity for the antigen is referred to as “antigen binding region.” An antigen binding region typically includes one or more “complementary binding regions” (“CDRs”). Certain antigen binding regions also include one or more “framework” regions. A “CDR” is an amino acid sequence that contributes to antigen binding specificity and affinity. “Framework” regions can aid in maintaining the proper conformation of the CDRs to promote binding between the antigen binding region and an antigen. Structurally, framework regions can be located in antibodies between CDRs. Examples of framework and CDR regions are shown in FIGs. 2A-3D, 3CCC-3JJJ. In some embodiments, the sequences for CDRs for the light chain of antibody 3B6 are as follows: CDR1 TLLSGYSSYEVD (SEQ ID NO: 279); CDR2 VDTGGIVGSKGE (SEQ ID NO: 280); CDR3 GADHGSNTFVVEV (SEQ ID NO: 281), and the FRs are as follows: FR1 QPVLTQPLFASASLGASVTLTC (SEQ ID NO: 282); FR2 WYQQRPGKPRFVRMR (SEQ ID NO: 283);
FR3 GIPDRFSVLGSGLNRNYLTIKNQIEDESDEYHC (SEQ ID NO: 284); and FR4 FGGGTKLTVL (SEQ ID NO: 285).

In certain aspects, recombinant antigen binding proteins that bind PCSK9, for example human PCSK9, are provided. In this context, a “recombinant antigen binding protein” is a protein made using recombinant techniques, i.e., through the expression of a recombinant nucleic acid as described herein. Methods and techniques for the production of recombinant proteins are well known in the art.

The term “antibody” refers to an intact immunoglobulin of any isotype, or a fragment thereof that can compete with the intact antibody for specific binding to the target antigen, and includes, for instance, chimeric, humanized, fully human, and bispecific antibodies. An “antibody” is a species of an antigen binding protein. An intact antibody will generally comprise at least two full-length heavy chains and two full-length light chains, but in some instances can include fewer chains such as antibodies naturally occurring in camelids which can comprise only heavy chains. Antibodies can be derived solely from a single source, or can be “chimeric,” that is, different portions of the antibody can be derived from two different antibodies as described further below. The antigen binding proteins, antibodies, or binding fragments can be produced in hybridomas, by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Unless otherwise indicated, the term “antibody” includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below. Furthermore, unless explicitly excluded, antibodies include monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (sometimes referred to herein as “antibody conjugates”), and fragments thereof, respectively. In some embodiments, the term also encompasses peptibodies.

Naturally occurring antibody structural units typically comprise a tetramer. Each such tetramer typically is composed of two identical pairs of polypeptide chains, each pair having one full-length “light” (in certain embodiments, about 25 kDa) and one full-length “heavy” chain (in certain embodiments, about 50-70 kDa). The amino-
terminal portion of each chain typically includes a variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant region that can be responsible for effector function. Human light chains are typically classified as kappa and lambda light chains. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA is similarly subdivided into subclasses including, but not limited to, IgA1 and IgA2. Within full-length light and heavy chains, typically, the variable and constant regions are joined by a “J” region of about 12 or more amino acids, with the heavy chain also including a “D” region of about 10 more amino acids. See, e.g., *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair typically form the antigen binding site.

The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which can enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, J. Mol. Biol., 196:901-917 (1987); Chothia et al., Nature, 342:878-883 (1989).

In certain embodiments, an antibody heavy chain binds to an antigen in the absence of an antibody light chain. In certain embodiments, an antibody light chain binds to an antigen in the absence of an antibody heavy chain. In certain embodiments, an antibody binding region binds to an antigen in the absence of an antibody light chain. In certain embodiments, an antibody binding region binds to an antigen in the
absence of an antibody heavy chain. In certain embodiments, an individual variable region specifically binds to an antigen in the absence of other variable regions.

In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition and the contact definition.


By convention, the CDR regions in the heavy chain are typically referred to as H1, H2, and H3 and are numbered sequentially in the direction from the amino terminus to the carboxy terminus. The CDR regions in the light chain are typically referred to as L1, L2, and L3 and are numbered sequentially in the direction from the amino terminus to the carboxy terminus.
The term “light chain” includes a full-length light chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length light chain includes a variable region domain, $V_L$, and a constant region domain, $C_L$. The variable region domain of the light chain is at the amino-terminus of the polypeptide. Light chains include kappa chains and lambda chains.

The term “heavy chain” includes a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length heavy chain includes a variable region domain, $V_H$, and three constant region domains, $C_{H1}$, $C_{H2}$, and $C_{H3}$. The $V_H$ domain is at the amino-terminus of the polypeptide, and the $C_H$ domains are at the carboxyl-terminus, with the $C_{H3}$ being closest to the carboxy-terminus of the polypeptide. Heavy chains can be of any isotype, including IgG (including IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM and IgE.

A bispecific or bifunctional antibody typically is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai et al., Clin. Exp. Immunol., 79: 315-321 (1990); Kostelny et al., J. Immunol., 148:1547-1553 (1992).

Some species of mammals also produce antibodies having only a single heavy chain.

Each individual immunoglobulin chain is typically composed of several “immunoglobulin domains,” each consisting of roughly 90 to 110 amino acids and having a characteristic folding pattern. These domains are the basic units of which antibody polypeptides are composed. In humans, the IgA and IgD isotypes contain four heavy chains and four light chains; the IgG and IgE isotypes contain two heavy chains and two light chains; and the IgM isotype contains five heavy chains and five light chains. The heavy chain C region typically comprises one or more domains that can be responsible for effector function. The number of heavy chain constant region domains will depend on the isotype. IgG heavy chains, for example, contain three C region domains known as $C_{H1}$, $C_{H2}$ and $C_{H3}$. The antibodies that are provided can
have any of these isotypes and subtypes. In certain embodiments of the present invention, an anti-PCSK9 antibody is of the IgG2 or IgG4 subtype.

The term “variable region” or “variable domain” refers to a portion of the light and/or heavy chains of an antibody, typically including approximately the amino-terminal 120 to 130 amino acids in the heavy chain and about 100 to 110 amino terminal amino acids in the light chain. In certain embodiments, variable regions of different antibodies differ extensively in amino acid sequence even among antibodies of the same species. The variable region of an antibody typically determines specificity of a particular antibody for its target.

The term “neutralizing antigen binding protein” or “neutralizing antibody” refers to an antigen binding protein or antibody, respectively, that binds to a ligand and prevents or reduces the biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand and altering the ligand’s ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, the term can also denote an antigen binding protein that prevents the protein to which it is bound from performing a biological function. In assessing the binding and/or specificity of an antigen binding protein, e.g., an antibody or immunologically functional fragment thereof, an antibody or fragment can substantially inhibit binding of a ligand to its binding partner when an excess of antibody reduces the quantity of binding partner bound to the ligand by at least about 1-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-85%, 85-90%, 90-95%, 95-97%, 97-98%, 98-99% or more (as measured in an in vitro competitive binding assay). In some embodiments, in the case of PCSK9 antigen binding proteins, such a neutralizing molecule can diminish the ability of PCSK9 to bind the LDLR. In some embodiments, the neutralizing ability is characterized and/or described via a competition assay. In some embodiments, the neutralizing ability is described in terms of an IC$_{50}$ or EC$_{50}$ value. In some embodiments, ABPs 27B2, 13H1, 13B5 and 3C4 are non-neutralizing ABPs, 3B6, 9C9 and 31A4 are weak neutralizers, and the remaining ABPs in Table 2 are strong neutralizers. In some embodiments, the antibodies or antigen binding proteins neutralize by binding to PCSK9 and preventing PCSK9 from binding to LDLR (or reducing the ability of PCSK9 to bind to LDLR).
some embodiments, the antibodies or ABPs neutralize by binding to PCSK9, and while still allowing PCSK9 to bind to LDLR, preventing or reducing the PCSK9 mediated degradation of LDLR. Thus, in some embodiments, a neutralizing ABP or antibody can still permit PCSK9/LDLR binding, but will prevent (or reduce) subsequent PCSK9 involved degradation of LDLR.

The term “target” refers to a molecule or a portion of a molecule capable of being bound by an antigen binding protein. In certain embodiments, a target can have one or more epitopes. In certain embodiments, a target is an antigen. The use of “antigen” in the phrase “antigen binding protein” simply denotes that the protein sequence that comprises the antigen can be bound by an antibody. In this context, it does not require that the protein be foreign or that it be capable of inducing an immune response.

The term “compete” when used in the context of antigen binding proteins (e.g., neutralizing antigen binding proteins or neutralizing antibodies) that compete for the same epitope means competition between antigen binding proteins as determined by an assay in which the antigen binding protein (e.g., antibody or immunologically functional fragment thereof) being tested prevents or inhibits (e.g., reduces) specific binding of a reference antigen binding protein (e.g., a ligand, or a reference antibody) to a common antigen (e.g., PCSK9 or a fragment thereof). Numerous types of competitive binding assays can be used to determine if one antigen binding protein competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see, e.g., Stahli et al., 1983, Methods in Enzymology 9:242-253); solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., 1986, J. Immunol. 137:3614-3619) solid phase direct labeled assay, solid phase direct labeled sandwich assay (see, e.g., Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (see, e.g., Morel et al., 1988, Molec. Immunol. 25:7-15); solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., 1990, Virology 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, Scand. J. Immunol. 32:77-82). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabelled
test antigen binding protein and a labeled reference antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur. Additional details regarding methods for determining competitive binding are provided in the examples herein. Usually, when a competing antigen binding protein is present in excess, it will inhibit (e.g., reduce) specific binding of a reference antigen binding protein to a common antigen by at least 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or 75% or more. In some instances, binding is inhibited by at least 80-85%, 85-90%, 90-95%, 95-97%, or 97% or more.

The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antigen binding protein (including, e.g., an antibody or immunological functional fragment thereof). In some embodiments, the antigen is capable of being used in an animal to produce antibodies capable of binding to that antigen. An antigen can possess one or more epitopes that are capable of interacting with different antigen binding proteins, e.g., antibodies.

The term “epitope” includes any determinant capable being bound by an antigen binding protein, such as an antibody or to a T-cell receptor. An epitope is a region of an antigen that is bound by an antigen binding protein that targets that antigen, and when the antigen is a protein, includes specific amino acids that directly contact the antigen binding protein. Most often, epitopes reside on proteins, but in some instances can reside on other kinds of molecules, such as nucleic acids. Epitope determinants can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl groups, and can have specific three dimensional structural characteristics, and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen in a complex mixture of proteins and/or macromolecules.
As used herein, “substantially pure” means that the described species of molecule is the predominant species present, that is, on a molar basis it is more abundant than any other individual species in the same mixture. In certain embodiments, a substantially pure molecule is a composition wherein the object species comprises at least 50% (on a molar basis) of all macromolecular species present. In other embodiments, a substantially pure composition will comprise at least 80%, 85%, 90%, 95%, or 99% of all macromolecular species present in the composition. In other embodiments, the object species is purified to essential homogeneity wherein contaminating species cannot be detected in the composition by conventional detection methods and thus the composition consists of a single detectable macromolecular species.

The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotin moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain embodiments, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and can be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., $^3$H, $^{14}$C, $^{15}$N, $^{35}$S, $^{90}$Y, $^{99}$Tc, $^{111}$In, $^{125}$I, $^{131}$I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In certain embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term “biological sample”, as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things
include, but are not limited to, humans, mice, monkeys, rats, rabbits, and other animals. Such substances include, but are not limited to, blood, serum, urine, cells, organs, tissues, bone, bone marrow, lymph nodes, and skin.

The term “pharmaceutical agent composition” (or agent or drug) as used herein refers to a chemical compound, composition, agent or drug capable of inducing a desired therapeutic effect when properly administered to a patient. It does not necessarily require more than one type of ingredient.

The term “therapeutically effective amount” refers to the amount of a PCSK9 antigen binding protein determined to produce a therapeutic response in a mammal. Such therapeutically effective amounts are readily ascertained by one of ordinary skill in the art.

The term “modulator,” as used herein, is a compound that changes or alters the activity or function of a molecule. For example, a modulator can cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Certain exemplary activities and functions of a molecule include, but are not limited to, binding affinity, enzymatic activity, and signal transduction. Certain exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptidodies, carbohydrates or small organic molecules. Peptidodies are described in, e.g., U.S. Patent No. 6,660,843 (corresponding to PCT Application No. WO 01/83525).

The terms “patient” and “subject” are used interchangeably and include human and non-human animal subjects as well as those with formally diagnosed disorders, those without formally recognized disorders, those receiving medical attention, those at risk of developing the disorders, etc.

The term “treat” and “treatment” includes therapeutic treatments, prophylactic treatments, and applications in which one reduces the risk that a subject will develop a disorder or other risk factor. Treatment does not require the complete curing of a disorder and encompasses embodiments in which one reduces symptoms or underlying risk factors.
The term “prevent” does not require the 100% elimination of the possibility of an event. Rather, it denotes that the likelihood of the occurrence of the event has been reduced in the presence of the compound or method.

Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures can be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Antigen Binding Proteins to PCSK9

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a serine protease involved in regulating the levels of the low density lipoprotein receptor (LDLR) protein (Horton et al., 2007; Seidah and Prat, 2007). PCSK9 is a prohormone-proprotein convertase in the subtilisin (S8) family of serine proteases (Seidah et al., 2003). An exemplary human PCSK9 amino acid sequence is presented as SEQ ID NO: 1 in FIG. 1A (depicting the “pro” domain of the protein as underlined) and SEQ ID NO:3 in FIG. 1B (depicting the signal sequence in bold and the pro domain underlined). An exemplary human PCSK9 coding sequence is presented as SEQ ID NO: 2 (FIG. 1B). As described herein, PCSK9 proteins can also include fragments of the full length PCSK9 protein. The structure of the PCSK9 protein was solved by two groups (Cunningham et al., Nature Structural & Molecular Biology, 2007, and Piper et al.,
Structure, 15:1-8, 2007), the entirities of both of which are herein incorporated by reference. PCSK9 includes a signal sequence, a N-terminal prodomain, a subtilisin-like catalytic domain and a C-terminal domain.

Antigen binding proteins (ABPs) that bind PCSK9, including human PCSK9, are used in the methods provided herein. In some embodiments, the antigen binding proteins are polypeptides which comprise one or more complementary determining regions (CDRs), as described herein. In some antigen binding proteins, the CDRs are embedded into a “framework” region, which orients the CDR(s) such that the proper antigen binding properties of the CDR(s) is achieved. In some embodiments, antigen binding proteins that can be used in the methods provided herein can interfere with, block, reduce or modulate the interaction between PCSK9 and LDLR. Such antigen binding proteins are denoted as “neutralizing.” In some embodiments, binding between PCSK9 and LDLR can still occur, even though the antigen binding protein is neutralizing and bound to PCSK9. For example, in some embodiments, the ABP useful in the methods provided herein prevents or reduces the adverse influence of PCSK9 on LDLR without blocking the LDLR binding site on PCSK9. Thus, in some embodiments, the ABP modulates or alters PCSK9’s ability to result in the degradation of LDLR, without having to prevent the binding interaction between PCSK9 and LDLR. Such ABPs can be specifically described as “non-competitively neutralizing” ABPs. In some embodiments, the neutralizing ABP binds to PCSK9 in a location and/or manner that prevents PCSK9 from binding to LDLR. Such ABPs can be specifically described as “competitively neutralizing” ABPs. Both of the above neutralizers can result in a greater amount of free LDLR being present in a subject, which results in more LDLR binding to LDL (thereby reducing the amount of LDL in the subject). In turn, this results in a reduction in the amount of serum cholesterol present in a subject.

In some embodiments, the antigen binding proteins provided herein are capable of inhibiting PCSK9-mediated activity (including binding). In some embodiments, antigen binding proteins binding to these epitopes inhibit, inter alia, interactions between PCSK9 and LDLR and other physiological effects mediated by PCSK9. In
some embodiments, the antigen binding proteins are human, such as fully human antibodies to PCSK9.

In some embodiments, the ABP binds to the catalytic domain of PCSK9. In some embodiments, the ABP binds to the mature form of PCSK9. In some embodiments the ABP binds in the prodomain of PCSK9. In some embodiments, the ABP selectively binds to the mature form of PCSK9. In some embodiments, the ABP binds to the catalytic domain in a manner such that PCSK9 cannot bind or bind as efficiently to LDLR. In some embodiments, the antigen binding protein does not bind to the c-terminus of the catalytic domain. In some embodiments, the antigen binding protein does not bind to the n-terminus of the catalytic domain. In some embodiments, the ABP does not bind to the n- or c-terminus of the PCSK9 protein. In some embodiments, the ABP binds to any one of the epitopes bound by the antibodies discussed herein. In some embodiments, this can be determined by competition assays between the antibodies disclosed herein and other antibodies. In some embodiments, the ABP binds to an epitope bound by one of the antibodies described in Table 2. In some embodiments, the antigen binding proteins bind to a specific conformational state of PCSK9 so as to prevent PCSK9 from interacting with LDLR. In some embodiments, the ABP binds to the V domain of PCSK9. In some embodiments, the ABP binds to the V domain of PCSK9 and prevents (or reduces) PCSK9 from binding to LDLR. In some embodiments, the ABP binds to the V domain of PCSK9, and while it does not prevent (or reduce) the binding of PCSK9 to LDLR, the ABP prevents or reduces the adverse activities mediated through PCSK9 on LDLR.

The disclosed antigen binding proteins that are useful in the methods provided herein have a variety of utilities. Some of the antigen binding proteins, for instance, are useful in specific binding assays, affinity purification of PCSK9, in particular human PCSK9 or its ligands and in screening assays to identify other antagonists of PCSK9 activity. Some of the antigen binding proteins are useful for inhibiting binding of PCSK9 to LDLR, or inhibiting PCSK9-mediated activities.

In some embodiments, the antigen binding proteins that are useful in the methods provided herein comprise one or more CDRs (e.g., 1, 2, 3, 4, 5 or 6 CDRs). In some embodiments, the antigen binding protein comprises (a) a polypeptide structure
and (b) one or more CDRs that are inserted into and/or joined to the polypeptide structure. The polypeptide structure can take a variety of different forms. For example, it can be, or comprise, the framework of a naturally occurring antibody, or fragment or variant thereof, or can be completely synthetic in nature. Examples of various polypeptide structures are further described below.

In certain embodiments, the polypeptide structure of the antigen binding proteins is an antibody or is derived from an antibody, including, but not limited to, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as “antibody conjugates”), and portions or fragments of each, respectively. In some instances, the antigen binding protein is an immunological fragment of an antibody (e.g., a Fab, a Fab’, a F(ab’)2, or a scFv). The various structures are further described and defined herein.

Certain of the antigen binding proteins that are useful in the methods provided herein specifically and/or selectively bind to human PCSK9. In some embodiments, the antigen binding protein specifically and/or selectively binds to human PCSK9 protein having and/or consisting of residues 153-692 of SEQ ID NO: 3. In some embodiments the ABP specifically and/or selectively binds to human PCSK9 having and/or consisting of residues 31-152 of SEQ ID NO: 3. In some embodiments, the ABP selectively binds to a human PCSK9 protein as depicted in FIG. 1A (SEQ ID NO: 1). In some embodiments, the antigen binding protein specifically binds to at least a fragment of the PCSK9 protein and/or a full length PCSK9 protein, with or without a signal sequence.

In embodiments where the antigen binding protein is used for therapeutic applications, an antigen binding protein can inhibit, interfere with or modulate one or more biological activities of PCSK9. In one embodiment, an antigen binding protein binds specifically to human PCSK9 and/or substantially inhibits binding of human PCSK9 to LDLR by at least about 20%-40%, 40-60%, 60-80%, 80-85%, or more (for example, by measuring binding in an in vitro competitive binding assay). Some of the antigen binding proteins that are provided herein are antibodies. In some embodiments,
the ABP has a $K_d$ of less (binding more tightly) than $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$, $10^{-11}$, $10^{-12}$, $10^{-13}$ M. In some embodiments, the ABP has an IC$_{50}$ for blocking the binding of LDLR to PCSK9 (D374Y, high affinity variant) of less than 1 microM, 1000 nM to 100 nM, 100 nM to 10 nM, 10 nM to 1 nM, 1000 pM to 500 pM, 500 pM to 200 pM, less than 200 pM, 200 pM to 150 pM, 200 pM to 100 pM, 100 pM to 10 pM, 10 pM to 1 pM.

One example of an IgG2 heavy chain constant domain of an anti-PCSK9 antibody of the present invention has the amino acid sequence as shown in SEQ ID NO: 154, FIG. 3KK.

One example of an IgG4 heavy chain constant domain of an anti-PCSK9 antibody of the present invention has the amino acid sequence as shown in SEQ ID NO: 155, FIG. 3KK.

One example of a kappa light chain constant domain of an anti-PCSK9 antibody has the amino acid sequence as shown in SEQ ID NO: 157, FIG. 3KK.

One example of a lambda light chain constant domain of an anti-PCSK9 antibody has the amino acid sequence as shown in SEQ ID NO: 156, FIG. 3KK.

Variable regions of immunoglobulin chains generally exhibit the same overall structure, comprising relatively conserved framework regions (FR) joined by three hypervariable regions, more often called “complementarity determining regions” or CDRs. The CDRs from the two chains of each heavy chain/light chain pair mentioned above typically are aligned by the framework regions to form a structure that binds specifically with a specific epitope on the target protein (e.g., PCSK9). From N-terminal to C-terminal, naturally-occurring light and heavy chain variable regions both typically conform with the following order of these elements: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. A numbering system has been devised for assigning numbers to amino acids that occupy positions in each of these domains. This numbering system is defined in Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, MD), or Chothia & Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342:878-883.

Various heavy chain and light chain variable regions are provided herein and are depicted in FIGs. 2A-3JJ and 3LL-3JJJ and 3LLL. In some embodiments, each of these variable regions can be attached to the above heavy and light chain constant
regions to form a complete antibody heavy and light chain, respectively. Further, each of the so generated heavy and light chain sequences can be combined to form a complete antibody structure.

Specific examples of some of the variable regions of the light and heavy chains of the antibodies that are provided and their corresponding amino acid sequences are summarized in TABLE 2.

**TABLE 2: Exemplary Heavy and Light Chain Variable Regions**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Light/Heavy SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>30A4</td>
<td>5/74</td>
</tr>
<tr>
<td>3C4</td>
<td>7/85</td>
</tr>
<tr>
<td>23B5</td>
<td>9/71</td>
</tr>
<tr>
<td>25G4</td>
<td>10/72</td>
</tr>
<tr>
<td>31H4</td>
<td>12/67</td>
</tr>
<tr>
<td>27B2</td>
<td>13/67</td>
</tr>
<tr>
<td>25A7</td>
<td>15/58</td>
</tr>
<tr>
<td>27H5</td>
<td>16/52</td>
</tr>
<tr>
<td>26H5</td>
<td>17/51</td>
</tr>
<tr>
<td>31D1</td>
<td>18/53</td>
</tr>
<tr>
<td>20D10</td>
<td>19/48</td>
</tr>
<tr>
<td>27E7</td>
<td>20/54</td>
</tr>
<tr>
<td>30B9</td>
<td>21/55</td>
</tr>
<tr>
<td>19H9</td>
<td>22/56</td>
</tr>
<tr>
<td>26E10</td>
<td>23/49</td>
</tr>
<tr>
<td>21B12</td>
<td>23/49</td>
</tr>
<tr>
<td>21B12v1</td>
<td>591/590</td>
</tr>
<tr>
<td>17C2</td>
<td>24/57</td>
</tr>
<tr>
<td>23G1</td>
<td>26/50</td>
</tr>
<tr>
<td>13H1</td>
<td>28/91</td>
</tr>
<tr>
<td>9C9</td>
<td>30/64</td>
</tr>
<tr>
<td>9H6</td>
<td>31/62</td>
</tr>
<tr>
<td>31A4</td>
<td>32/89</td>
</tr>
<tr>
<td>1A12</td>
<td>33/65</td>
</tr>
<tr>
<td>16F12</td>
<td>35/79</td>
</tr>
<tr>
<td>22E2</td>
<td>36/80</td>
</tr>
<tr>
<td>27A6</td>
<td>37/76</td>
</tr>
<tr>
<td>28B12</td>
<td>38/77</td>
</tr>
<tr>
<td>28D6</td>
<td>39/78</td>
</tr>
<tr>
<td>31G11</td>
<td>40/83</td>
</tr>
<tr>
<td>13B5</td>
<td>42/69</td>
</tr>
<tr>
<td>31B12</td>
<td>44/81</td>
</tr>
<tr>
<td>3B6</td>
<td>46/60</td>
</tr>
<tr>
<td>5H5</td>
<td>421/419</td>
</tr>
<tr>
<td>24F7</td>
<td>425/423</td>
</tr>
<tr>
<td>22B11</td>
<td>429/427</td>
</tr>
<tr>
<td>30F1</td>
<td>433/431</td>
</tr>
<tr>
<td>24B9.1</td>
<td>437/435</td>
</tr>
<tr>
<td>24B9.2</td>
<td>441/439</td>
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<tr>
<td>20A5.1</td>
<td>445/443</td>
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<tr>
<td>20A5.2</td>
<td>449/447</td>
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<tr>
<td>20E5.1</td>
<td>453/451</td>
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<tr>
<td>20E5.2</td>
<td>457/455</td>
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<tr>
<td>8A3</td>
<td>461/459</td>
</tr>
<tr>
<td>11F1</td>
<td>465/463</td>
</tr>
<tr>
<td>12H11</td>
<td>469/467</td>
</tr>
<tr>
<td>11H4</td>
<td>473/471</td>
</tr>
<tr>
<td>11H8</td>
<td>477/475</td>
</tr>
<tr>
<td>11G1</td>
<td>481/479</td>
</tr>
<tr>
<td>8A1</td>
<td>485/483</td>
</tr>
</tbody>
</table>

Again, each of the exemplary variable heavy chains listed in Table 2 can be combined with any of the exemplary variable light chains shown in Table 2 to form an antibody. Table 2 shows exemplary light and heavy chain pairings found in several of the antibodies disclosed herein. In some instances, the antibodies include at least one variable heavy chain and one variable light chain from those listed in Table 2. In other instances, the antibodies contain two identical light chains and two identical heavy chains. As an example, an antibody or antigen binding protein can include a heavy chain and a light chain, two heavy chains, or two light chains. In some embodiments the antigen binding protein comprises (and/or consists of) 1, 2, and/or 3 heavy and/or
light CDRs from at least one of the sequences listed in Table 2 (CDRs for the sequences are outlined in FIGs. 2A-3D, and other embodiments in FIGs. 3CCC-3JJJ and 3KKK and 13A-13J). In some embodiments, all 6 CDRs (CDR1-3 from the light (CDRL1, CDRL2, CDRL3) and CDR1-3 from the heavy (CDRH1, CDRH2, and CDRH3)) are part of the ABP. In some embodiments, 1, 2, 3, 4, 5, or more CDRs are included in the ABP. In some embodiments, one heavy and one light CDR from the CDRs in the sequences in Table 2 is included in the ABP (CDRs for the sequences in Table 2 are outlined in FIGs. 2A-3D). In some embodiments, additional sections (e.g., as depicted in FIG. 2A-2D, 3A-3D, and other embodiments in 3CCC-3JJJ and 3LLL and 13A-13J) are also included in the ABP. Examples of CDRs and FRs for the heavy and light chains noted in Table 2 are outlined in FIGs. 2A-3D (and other embodiments in FIGs. 3CCC-3JJJ and 3LLL and 13A-J). Optional light chain variable sequences (including CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4) can be selected from the following: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, 46, 421, 425, 429, 432, 433, 437, 441, 445, 449, 453, 457, 461, 465, 469, 473, 477, 481, and 485. Optional heavy chain variable sequences (including CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4) can be selected from the following: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483. In some of the entries in FIG. 2A-3D and FIG. 3CCC-3JJJ and 3LLL variations of the sequences or alternative boundaries of the CDRs and FRs are identified. These alternatives are identified with a “v1” following the ABP name. As most of these alternatives are minor in nature, only sections with differences are displayed in the table. It is understood that the remaining section of the light or heavy chain is the same as shown for the base ABP in the other panels. Thus, for example, 19H9v1 in FIG. 2C has the same FR1, CDR1, and FR2 as 19H9 in FIG. 2A as the only difference is noted in FIG. 2C. For three of the nucleic acid sequences (ABPs 26E10, 30B9, and 31B12), additional alternative nucleic acid sequences are provided in the figures. As will be appreciated by one of skill in the art, no more than one such sequence need actually be used in the creation of an antibody or ABP.
Indeed, in some embodiments, only one or neither of the specific heavy or light chain nucleic acids need be present.


In some embodiments, the ABP is encoded by a nucleic acid sequence that can encode any of the protein sequences in Table 2.

In some embodiments, the ABP binds selectively to the form of PCSK9 that binds to LDLR (e.g., the autocatalyzed form of the molecule). In some embodiments, the antigen binding protein does not bind to the c-terminus of the catalytic domain (e.g., the 5. 5-10, 10-15, 15-20, 20-25, 25-30, 30-40 most amino acids in the c-terminus). In some embodiments, the antigen binding protein does not bind to the n-terminus of the catalytic domain (e.g., the 5. 5-10, 10-15, 15-20, 20-25, 25-30, 30-40 most amino acids in the n-terminus). In some embodiments, the ABP binds to amino acids within amino acids 1-100 of the mature form of PCSK9. In some embodiments, the ABP binds to amino acids within (and/or amino acid sequences consisting of) amino acids 31-100, 100-200, 31-152, 153-692, 200-300, 300-400, 452-683, 400-500, 500-600, 31-692, 31-449, and/or 600-692. In some embodiments, the ABP binds to the catalytic domain. In some embodiments, the neutralizing and/or non-neutralizing ABP binds to the prodomain. In some embodiments, the ABP binds to both the catalytic and pro domains. In some embodiments, the ABP binds to the catalytic domain so as to obstruct an area on the catalytic domain that interacts with the pro domain. In some embodiments, the ABP binds to the catalytic domain at a location or surface that the pro-domain interacts with as outlined in Piper et al. (Structure 15:1-8 (2007), the entirety of which is hereby incorporated by reference, including the structural representations therein). In some embodiments, the ABP binds to the catalytic domain and restricts the mobility of the prodomain. In some embodiments, the ABP binds to the catalytic domain without binding to the pro-domain. In some embodiments, the
ABP binds to the catalytic domain, without binding to the pro-domain, while preventing the pro-domain from reorienting to allow PCSK9 to bind to LDLR. In some embodiments, the ABP binds in the same epitope as those surrounding residues 149-152 of the pro-domain in Piper et al. In some embodiments, the ABPs bind to the groove (as outlined in Piper et al.) on the V domain. In some embodiments, the ABPs bind to the histidine-rich patch proximal to the groove on the V domain. In some embodiments, such antibodies (that bind to the V domain) are not neutralizing. In some embodiments, antibodies that bind to the V domain are neutralizing. In some embodiments, the neutralizing ABPs prevent the binding of PCSK9 to LDLR. In some embodiments, the neutralizing ABPs, while preventing the PCSK9 degradation of LDLR, do not prevent the binding of PCSK9 to LDLR (for example ABP 31A4). In some embodiments, the ABP binds to or blocks at least one of the histidines depicted in Figure 4 of the Piper et al. paper. In some embodiments, the ABP blocks the catalytic triad in PCSK9.

In some embodiments, the antibody binds selectively to variant PCSK9 proteins, e.g., D374Y over wild type PCSK9. In some embodiments, these antibodies bind to the variant at least twice as strongly as the wild type, and preferably 2-5, 5-10, 10-100, 100-1000, 1000-10,000 fold or more to the mutant than the wild type (as measured via a K_d). In some embodiments, the antibody selectively inhibits variant D374Y PCSK9 from interacting with LDLR over wild type PCSK9’s ability to interact with LDLR. In some embodiments, these antibodies block the variant’s ability to bind to LDLR more strongly than the wild type’s ability, e.g., at least twice as strongly as the wild type, and preferably 2-5, 5-10, 10-100, 100-1000 fold or more to the mutant than the wild type (as measured via an IC_{50}). In some embodiments, the antibody binds to and neutralizes both wild type PCSK9 and variant forms of PCSK9, such as D374Y at similar levels. In some embodiments, the antibody binds to PCSK9 to prevent variants of LDLR from binding to PCSK9. In some embodiments, the variants of LDLR are at least 50% identical to human LDLR. It is noted that variants of LDLR are known to those of skill in the art (e.g., Brown MS et al, “Calcium cages, acid baths and recycling receptors” Nature 388: 629-630, 1997). In some embodiments, the ABP can
raise the level of effective LDLR in heterozygote familial hypercholesterolemia (where a loss-of-function variant of LDLR is present).

In some embodiments, the ABP binds to (but does not block) variants of PCSK9 that are at least 50%, 50-60, 60-70, 70-80, 80-90, 90-95, 95-99, or greater percent identity to the form of PCSK9 depicted in FIG. 1A and/or FIG. 1B. In some embodiments, the ABP binds to (but does not block) variants of PCSK9 that are at least 50%, 50-60, 60-70, 70-80, 80-90, 90-95, 95-99, or greater percent identity to the mature form of PCSK9 depicted in FIG. 1A and/or FIG. 1B. In some embodiments, the ABP binds to and prevents variants of PCSK9 that are at least 50%, 50-60, 60-70, 70-80, 80-90, 90-95, 95-99, or greater percent identity to the form of PCSK9 depicted in FIG. 1A and/or FIG. 1B from interacting with LDLR. In some embodiments, the ABP binds to and prevents variants of PCSK9 that are at least 50, 50-60, 60-70, 70-80, 80-90, 90-95, 95-99, or greater percent identity to the mature form of PCSK9 depicted in FIG. 1B from interacting with LDLR. In some embodiments, the variant of PCSK9 is a human variant, such as variants at position 474, E620G, and/or E670G. In some embodiments, the amino acid at position 474 is valine (as in other humans) or threonine (as in cyno and mouse). Given the cross-reactivity data presented herein, it is believed that the present antibodies will readily bind to the above variants.

In some embodiments, the ABP binds to an epitope bound by one of the antibodies described in Table 2. In some embodiments, the antigen binding proteins bind to a specific conformational state of PCSK9 so as to prevent PCSK9 from interacting with LDLR.

**Humanized Antigen Binding Proteins (e.g., Antibodies)**

As described herein, an antigen binding protein to PCSK9 can comprise a humanized antibody and/or part thereof. An important practical application of such a strategy is the “humanization” of the mouse humoral immune system.

In certain embodiments, a humanized antibody is substantially non-immunogenic in humans. In certain embodiments, a humanized antibody has substantially the same affinity for a target as an antibody from another species from

In certain embodiments, amino acids of an antibody variable domain that can be modified without diminishing the native affinity of the antigen binding domain while reducing its immunogenicity are identified. See, e.g., U.S. Patent Nos. 5,766,886 and 5,869,619.

In certain embodiments, modification of an antibody by methods known in the art is typically designed to achieve increased binding affinity for a target and/or to reduce immunogenicity of the antibody in the recipient. In certain embodiments, humanized antibodies are modified to eliminate glycosylation sites in order to increase affinity of the antibody for its cognate antigen. See, e.g., Co et al., Mol. Immunol., 30:1361-1367 (1993). In certain embodiments, techniques such as “reshaping,” “hyperchimerization,” or “veneering/resurfacing” are used to produce humanized antibodies. See, e.g., Vaswami et al., Annals of Allergy, Asthma, & Immunol. 81:105 (1998); Roguska et al., Prot. Engineer., 9:895-904 (1996); and U.S. Patent No. 6,072,035. In certain such embodiments, such techniques typically reduce antibody immunogenicity by reducing the number of foreign residues, but do not prevent anti-idiotypic and anti-allotypic responses following repeated administration of the antibodies. Certain other methods for reducing immunogenicity are described, e.g., in Gilliland et al., J. Immunol., 62(6): 3663-71 (1999).

In certain instances, humanizing antibodies results in a loss of antigen binding capacity. In certain embodiments, humanized antibodies are “back mutated.” In certain such embodiments, the humanized antibody is mutated to include one or more of the amino acid residues found in the donor antibody. See, e.g., Saldanha et al., Mol Immunol 36:709-19 (1999).

In certain embodiments the complementarity determining regions (CDRs) of the light and heavy chain variable regions of an antibody to PCSK9 can be grafted to framework regions (FRs) from the same, or another, species. In certain embodiments, the CDRs of the light and heavy chain variable regions of an antibody to PCSK9 can be grafted to consensus human FRs. To create consensus human FRs, in certain embodiments, FRs from several human heavy chain or light chain amino acid
sequences are aligned to identify a consensus amino acid sequence. In certain embodiments, the FRs of an antibody to PCSK9 heavy chain or light chain are replaced with the FRs from a different heavy chain or light chain. In certain embodiments, rare amino acids in the FRs of the heavy and light chains of an antibody to PCSK9 are not replaced, while the rest of the FR amino acids are replaced. Rare amino acids are specific amino acids that are in positions in which they are not usually found in FRs. In certain embodiments, the grafted variable regions from an antibody to PCSK9 can be used with a constant region that is different from the constant region of an antibody to PCSK9. In certain embodiments, the grafted variable regions are part of a single chain Fv antibody. CDR grafting is described, e.g., in U.S. Patent Nos. 6,180,370, 6,054,297, 5,693,762, 5,859,205, 5,693,761, 5,565,332, 5,585,089, and 5,530,101, and in Jones et al., Nature, 321: 522-525 (1986); Riechmann et al., Nature, 332: 323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988), Winter, FEBS Letts., 430:92-94 (1998), which are hereby incorporated by reference for any purpose.

Human Antigen Binding Proteins (e.g., Antibodies)

As described herein, an antigen binding protein that binds to PCSK9 can comprise a human (i.e., fully human) antibody and/or part thereof. In certain embodiments, nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to the variable regions are provided. In certain embodiments, sequences corresponding to complementarity determining regions (CDR's), specifically from CDR1 through CDR3, are provided. According to certain embodiments, a hybridoma cell line expressing such an immunoglobulin molecule is provided. According to certain embodiments, a hybridoma cell line expressing such a monoclonal antibody is provided. In certain embodiments a hybridoma cell line is selected from at least one of the cell lines described in Table 2, e.g., 21B12, 16F12 and 31H4. In certain embodiments, a purified human monoclonal antibody to human PCSK9 is provided.

One can engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce human antibodies in the absence of mouse antibodies. Large human Ig fragments can
preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains can yield high affinity fully human antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity can be produced and selected. Certain exemplary methods are described in WO 98/24893, U.S. Patent No. 5,545,807, EP 546073, and EP 546073.

In certain embodiments, one can use constant regions from species other than human along with the human variable region(s).

The ability to clone and reconstruct megabase sized human loci in yeast artificial chromosomes (YACs) and to introduce them into the mouse germline provides an approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

Human antibodies avoid some of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, fully human antibodies can be generated through the introduction of functional human antibody loci into a rodent, other mammal or animal so that the rodent, other mammal or animal produces fully human antibodies.

Humanized antibodies are those antibodies that, while initially starting off containing antibody amino acid sequences that are not human, have had at least some of these nonhuman antibody amino acid sequences replaced with human antibody sequences. This is in contrast with human antibodies, in which the antibody is encoded (or capable of being encoded) by genes possessed a human.
Antigen Binding Protein Variants

Other antibodies that are provided are variants of the ABPs listed above formed by combination or subparts of the variable heavy and variable light chains shown in Table 2 and FIGs 2A-3JJ and 3LL-JJJ and 3LLL and comprise variable light and/or variable heavy chains that each have at least 50%, 50-60, 60-70, 70-80%, 80-85%, 85-90%, 90-95%, 95-97%, 97-99%, or above 99% identity to the amino acid sequences of the sequences in Table 2 (either the entire sequence or a subpart of the sequence, e.g., one or more CDR) and FIGs 2A-3JJ and 3LL-JJJ and 3LLL. In some instances, such antibodies include at least one heavy chain and one light chain, whereas in other instances the variant forms contain two identical light chains and two identical heavy chains (or subparts thereof). In some embodiments, the sequence comparison in FIG. 2A-3D (and 13A-13J, other embodiments in FIGs. 31A and 31B) can be used in order to identify sections of the antibodies that can be modified by observing those variations that impact binding and those variations that do not appear to impact binding. For example, by comparing similar sequences, one can identify those sections (e.g., particular amino acids) that can be modified and how they can be modified while still retaining (or improving) the functionality of the ABP. In some embodiments, variants of ABPs include those consensus groups and sequences depicted in FIGs. 13A, 13C, 13F, 13G, 13H, 13I, 13J, and/or 31A and 31B and variations are allowed in the positions identified as variable in the figures. The CDRs shown in FIGs. 13A, 13C, 13F, 13G, 31A and 31B were defined based upon a hybrid combination of the Chothia method (based on the location of the structural loop regions, see, e.g., “Standard conformations for the canonical structures of immunoglobulins,” Bissam Al-Lazikani, Arthur M. Lesk and Cyrus Chothia, Journal of Molecular Biology, 273(4): 927-948, 7 November (1997)) and the Kabat method (based on sequence variability, see, e.g., Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242, Kabat et al., (1991)). Each residue determined by either method, was included in the final list of CDR residues (and is presented in FIGs. 13A, 13C, 13F, 13G, and 31A and 31B). The CDRs in FIGs. 13H, 13I, and 13J were obtained by the Kabat method alone. Unless specified otherwise, the defined consensus sequences,
CDRs, and FRs in FIGs. 13H-13J will define and control the noted CDRs and FRs for the referenced ABPs in FIG. 13.

In certain embodiments, an antigen binding protein comprises a heavy chain comprising a variable region comprising an amino acid sequence at least 90% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483. In certain embodiments, an antigen binding protein comprises a heavy chain comprising a variable region comprising an amino acid sequence at least 95% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483. In certain embodiments, an antigen binding protein comprises a heavy chain comprising a variable region comprising an amino acid sequence at least 99% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483.

In some embodiments, the antigen binding protein comprises a sequence that is at least 90%, 90-95%, and/or 95-99% identical to one or more CDRs from the CDRs in at least one of sequences of SEQ ID NO: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483. In some embodiments, 1, 2, 3, 4, 5, or 6 CDR (each being at least 90%, 90-95%, and/or 95-99% identical to the above sequences) is present.

In some embodiments, the antigen binding protein comprises a sequence that is at least 90%, 90-95%, and/or 95-99% identical to one or more FRs from the FRs in at least one of sequences of SEQ ID NO: 74, 85, 71, 72, 67, 87, 58, 52, 51, 53, 48, 54, 55, 56, 49, 57, 50, 91, 64, 62, 89, 65, 79, 80, 76, 77, 78, 83, 69, 81, 60, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, 463, 467, 471, 475, 479, and 483. In some
embodiments, 1, 2, 3, or 4 FR (each being at least 90%, 90-95%, and/or 95-99% identical to the above sequences) is present.

In certain embodiments, an antigen binding protein comprises a light chain comprising a variable region comprising an amino acid sequence at least 90% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, 46, 421, 425, 429, 433, 437, 441, 445, 49, 453, 457, 461, 465, 469, 473, 477, 481, and 485. In certain embodiments, an antigen binding protein comprises a light chain comprising a variable region comprising an amino acid sequence at least 95% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, and 46. In certain embodiments, an antigen binding protein comprises a light chain comprising a variable region comprising an amino acid sequence at least 99% identical to an amino acid sequence selected from at least one of the sequences of SEQ ID NO: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, 46, 421, 425, 429, 433, 437, 441, 445, 49, 453, 457, 461, 465, 469, 473, 477, 481, and 485.

In some embodiments, the antigen binding protein comprises a sequence that is at least 90%, 90-95%, and/or 95-99% identical to one or more CDRs from the CDRs in at least one of sequences of SEQ ID NO: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, 46, 421, 425, 429, 433, 437, 441, 445, 49, 453, 457, 461, 465, 469, 473, 477, 481, and 485. In some embodiments, 1, 2, 3, 4, 5, or 6 CDR (each being at least 90%, 90-95%, and/or 95-99% identical to the above sequences) is present.

In some embodiments, the antigen binding protein comprises a sequence that is at least 90%, 90-95%, and/or 95-99% identical to one or more FRs from the FRs in at least one of sequences of SEQ ID NO: 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 42, 44, 46, 421, 425, 429, 433, 437, 441, 445, 49, 453, 457, 461, 465, 469, 473, 477, 481, and 485. In some embodiments, 1, 2, 3, or 4 FR (each being at least 90%, 90-95%, and/or 95-99% identical to the above sequences) is present.
In light of the present disclosure, a skilled artisan will be able to determine suitable variants of the ABPs as set forth herein using well-known techniques. In certain embodiments, one skilled in the art can identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that can be important for biological activity or for structure can be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art can opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar ABPs. In view of such information, one skilled in the art can predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art can choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues can be involved in important interactions with other molecules. Moreover, one skilled in the art can generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants can be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide’s or protein’s structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.


In certain embodiments, antigen binding protein variants include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, protein variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X can be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which
eliminate this sequence will remove an existing N-linked carbohydrate chain. Also
provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-
linked glycosylation sites (typically those that are naturally occurring) are eliminated
and one or more new N-linked sites are created. Additional preferred antibody variants
include cysteine variants wherein one or more cysteine residues are deleted from or
substituted for another amino acid (e.g., serine) as compared to the parent amino acid
sequence. Cysteine variants can be useful when antibodies must be refolded into a
biologically active conformation such as after the isolation of insoluble inclusion
bodies. Cysteine variants generally have fewer cysteine residues than the native
protein, and typically have an even number to minimize interactions resulting from
unpaired cysteines.

According to certain embodiments, amino acid substitutions are those which:
(1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter
binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4)
confer or modify other physicochemical or functional properties on such polypeptides.
According to certain embodiments, single or multiple amino acid substitutions (in
certain embodiments, conservative amino acid substitutions) can be made in the
naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide
outside the domain(s) forming intermolecular contacts). In certain embodiments, a
conservative amino acid substitution typically may not substantially change the
structural characteristics of the parent sequence (e.g., a replacement amino acid should
not tend to break a helix that occurs in the parent sequence, or disrupt other types of
secondary structure that characterizes the parent sequence). Examples of art-
recognized polypeptide secondary and tertiary structures are described in Proteins,
Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company,
New York (1984)); Introduction to Protein Structure (C. Branden & J. Tooze, eds.,
(1991), which are each incorporated herein by reference.

In some embodiments, the variants are variants of the nucleic acid sequences of
the ABPs disclosed herein. One of skill in the art will appreciate that the above
discussion can be used for identifying, evaluating, and/creating ABP protein variants
and also for nucleic acid sequences that can encode for those protein variants. Thus, nucleic acid sequences encoding for those protein variants (as well as nucleic acid sequences that encode for the ABPs in Table 2, but are different from those explicitly disclosed herein) are contemplated. For example, an ABP variant can have at least 80, 80-85, 85-90, 90-95, 95-97, 97-99 or greater identity to at least one nucleic acid sequence described in SEQ ID NOs: 152, 153, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 296, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, and 484 or at least one to six (and various combinations thereof) of the CDR(s) encoded by the nucleic acid sequences in SEQ ID NOs: 152, 153, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, and 151, 296, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, and 484.

In some embodiments, the antibody (or nucleic acid sequence encoding it) is a variant if the nucleic acid sequence that encodes the particular ABP (or the nucleic acid sequence itself) can selectively hybridize to any of the nucleic acid sequences that encode the proteins in Table 2 (such as, but not limited to SEQ ID NO: 152, 153, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 296, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, and 484) under stringent conditions. In one embodiment, suitable moderately stringent conditions include prewashing in a solution of 5XSSC; 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50° C, -65° C, 5xSSC, overnight or,
in the event of cross-species homology, at 45° C with 0.5xSSC; followed by washing
twice at 65° C for 20 minutes with each of 2x, 0.5x and 0.2xSSC containing 0.1% SDS.
Such hybridizing DNA sequences are also within the scope of this invention, as are
nucleotide sequences that, due to code degeneracy, encode an antibody polypeptide that
is encoded by a hybridizing DNA sequence and the amino acid sequences that are
encoded by these nucleic acid sequences. In some embodiments, variants of CDRs
include nucleic acid sequences and the amino acid sequences encoded by those
sequences, that hybridize to one or more of the CDRs within the sequences noted above
(individual CDRs can readily be determined in light of FIGs. 2A-3D, and other
embodiments in FIGs. 3CCC-3JJJ and 15A-15D). The phrase "selectively hybridize"
referred to in this context means to detectably and selectively bind. Polynucleotides,
oligonucleotides and fragments thereof in accordance with the invention selectively
hybridize to nucleic acid strands under hybridization and wash conditions that
minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High
stringency conditions can be used to achieve selective hybridization conditions as
known in the art and discussed herein. Generally, the nucleic acid sequence homology
between the polynucleotides, oligonucleotides, and fragments of the invention and a
nucleic acid sequence of interest will be at least 80%, and more typically with
preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two
amino acid sequences are homologous if there is a partial or complete identity between
their sequences. For example, 85% homology means that 85% of the amino acids are
identical when the two sequences are aligned for maximum matching. Gaps (in either
of the two sequences being matched) are allowed in maximizing matching; gap lengths
of 5 or less are preferred with 2 or less being more preferred. Alternatively and
preferably, two protein sequences (or polypeptide sequences derived from them of at
least 30 amino acids in length) are homologous, as this term is used herein, if they have
an alignment score of at more than 5 (in standard deviation units) using the program
ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff,
M. O., in Atlas of Protein Sequence and Structure, pp. 101-110 (Volume 5, National
Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10.
The two sequences or parts thereof are more preferably homologous if their amino
acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATA" corresponds to a reference sequence "TATA" and is complementary to a reference sequence "GTATA".

Preparation of Antigen Binding Proteins (e.g., Antibodies)

In certain embodiments, antigen binding proteins (such as antibodies) are produced by immunization with an antigen (e.g., PCSK9). In certain embodiments, antibodies can be produced by immunization with full-length PCSK9, a soluble form of PCSK9, the catalytic domain alone, the mature form of PCSK9 shown in FIG. 1A, a splice variant form of PCSK9, or a fragment thereof. In certain embodiments, the antibodies of the invention can be polyclonal or monoclonal, and/or can be recombinant antibodies. In certain embodiments, antibodies of the invention are human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, PCT Published Application No. WO 93/12227).

In certain embodiments, certain strategies can be employed to manipulate inherent properties of an antibody, such as the affinity of an antibody for its target. Such strategies include, but are not limited to, the use of site-specific or random mutagenesis of the polynucleotide molecule encoding an antibody to generate an antibody variant. In certain embodiments, such generation is followed by screening for antibody variants that exhibit the desired change, e.g. increased or decreased affinity.

In certain embodiments, the amino acid residues targeted in mutagenic strategies are those in the CDRs. In certain embodiments, amino acids in the framework regions of the variable domains are targeted. In certain embodiments, such framework regions have been shown to contribute to the target binding properties of

In certain embodiments, smaller and more effectively screened libraries of antibody variants are produced by restricting random or site-directed mutagenesis to hyper-mutation sites in the CDRs, which are sites that correspond to areas prone to mutation during the somatic affinity maturation process. See, e.g., Chowdhury & Pastan, Nature Biotechnol., 17: 568-572 (1999) and references therein. In certain embodiments, certain types of DNA elements can be used to identify hyper-mutation sites including, but not limited to, certain direct and inverted repeats, certain consensus sequences, certain secondary structures, and certain palindromes. For example, such DNA elements that can be used to identify hyper-mutation sites include, but are not limited to, a tetra-base sequence comprising a purine (A or G), followed by guanine (G), followed by a pyrimidine (C or T), followed by either adenosine or thymidine (A or T) (i.e., A/G-G-C/T-A/T). Another example of a DNA element that can be used to identify hyper-mutation sites is the serine codon, A-G-C/T.

Preparation of Fully Human ABPs (e.g., Antibodies)

In certain embodiments, a phage display technique is used to generate monoclonal antibodies. In certain embodiments, such techniques produce fully human monoclonal antibodies. In certain embodiments, a polynucleotide encoding a single Fab or Fv antibody fragment is expressed on the surface of a phage particle. See, e.g., Hoogenboom et al., J. Mol. Biol., 227: 381 (1991); Marks et al., J Mol Biol 222: 581 (1991); U.S. Patent No. 5,885,793. In certain embodiments, phage are “screened” to identify those antibody fragments having affinity for target. Thus, certain such processes mimic immune selection through the display of antibody fragment repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to target. In certain such procedures, high affinity functional neutralizing antibody fragments are isolated. In certain such embodiments (discussed in more detail below), a complete repertoire of human antibody genes is created by cloning naturally rearranged human V genes from peripheral blood lymphocytes. See, e.g., Mullinax et al., Proc Natl Acad Sci (USA), 87: 8095-8099 (1990).
According to certain embodiments, antibodies of the invention are prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving this result are disclosed in the patents, applications and references disclosed in the specification, herein. In certain embodiments, one can employ methods such as those disclosed in PCT Published Application No. WO 98/24893 or in Mendez et al., Nature Genetics, 15:146-156 (1997), which are hereby incorporated by reference for any purpose.

Generally, fully human monoclonal ABPs (e.g., antibodies) specific for PCSK9 can be produced as follows. Transgenic mice containing human immunoglobulin genes are immunized with the antigen of interest, e.g. PCSK9, lymphatic cells (such as B-cells) from the mice that express antibodies are obtained. Such recovered cells are fused with a myeloid-type cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. In certain embodiments, the production of a hybridoma cell line that produces antibodies specific to PCSK9 is provided.

In certain embodiments, fully human antibodies are produced by exposing human splenocytes (B or T cells) to an antigen in vitro, and then reconstituting the exposed cells in an immunocompromised mouse, e.g. SCID or nod/SCID. See, e.g., Brams et al., J.Immunol. 160: 2051-2058 (1998); Carballido et al., Nat. Med., 6: 103-106 (2000). In certain such approaches, engraftment of human fetal tissue into SCID mice (SCID-hu) results in long-term hematopoiesis and human T-cell development. See, e.g., McCune et al., Science, 241:1532-1639 (1988); Ifversen et al., Sem. Immunol., 8:243-248 (1996). In certain instances, humoral immune response in such chimeric mice is dependent on co-development of human T-cells in the animals. See, e.g., Martensson et al., Immunol., 83:1271-179 (1994). In certain approaches, human peripheral blood lymphocytes are transplanted into SCID mice. See, e.g., Mosier et al.,
Nature, 335:256-259 (1988). In certain such embodiments, when such transplanted cells are treated either with a priming agent, such as Staphylococcal Enterotoxin A (SEA), or with anti-human CD40 monoclonal antibodies, higher levels of B cell production is detected. See, e.g., Martensson et al., Immunol., 84: 224-230 (1995); Murphy et al., Blood, 86:1946-1953 (1995).

Thus, in certain embodiments, fully human antibodies can be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells. In other embodiments, antibodies can be produced using the phage display techniques described herein.

The antibodies described herein were prepared through the utilization of the XenoMouse® technology, as described herein. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the background section herein. In particular, however, a preferred embodiment of transgenic production of mice and antibodies therefrom is disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996 and International Patent Application Nos. WO 98/24893, published June 11, 1998 and WO 00/76310, published December 21, 2000, the disclosures of which are hereby incorporated by reference. See also Mendez et al., Nature Genetics, 15:146-156 (1997), the disclosure of which is hereby incorporated by reference.

Through the use of such technology, fully human monoclonal antibodies to a variety of antigens have been produced. Essentially, XenoMouse® lines of mice are immunized with an antigen of interest (e.g. PCSK9), lymphatic cells (such as B-cells) are recovered from the hyper-immunized mice, and the recovered lymphocytes are fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. These hybridoma cell lines are screened and selected to identify hybridoma cell lines that produced antibodies specific to the antigen of interest. Provided herein are methods for the production of multiple hybridoma cell lines that produce antibodies specific to PCSK9. Further, provided herein are characterization of the antibodies produced by
such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.


In an alternative approach, others, including GenPharm International, Inc., have utilized a “minilocus” approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and usually a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Patent No. 5,545,807 to Surani et al. and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,877,397, 5,874,299, and 6,255,458 each to Lonberg & Kay, U.S. Patent No. 5,591,669 and 6,023,010 to Krimpenfort & Berns, U.S. Patent Nos. 5,612,205, 5,721,367, and 5,789,215 to Berns et al., and U.S. Patent No. 5,643,763 to Choi & Dunn, and
Kirin has also demonstrated the generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. See European Patent Application Nos. 773 288 and 843 961, the disclosures of which are hereby incorporated by reference. Additionally, KM™ mice, which are the result of cross-breeding of Kirin’s Tc mice with Medarex’s minilocus (Humab) mice have been generated. These mice possess the human IgH transchromosome of the Kirin mice and the kappa chain transgene of the Genpharm mice (Ishida et al., Cloning Stem Cells, (2002) 4:91-102).

Human antibodies can also be derived by in vitro methods. Suitable examples include but are not limited to phage display (CAT, Morphosys, Dyax, Biosite/Medarex, Xoma, Symphogen, Alexion (formerly Proliferon), Affimmed) ribosome display (CAT), yeast display, and the like.

In some embodiments, the antibodies described herein possess human IgG4 heavy chains as well as IgG2 heavy chains. Antibodies can also be of other human isotypes, including IgG1. The antibodies possessed high affinities, typically possessing a Kd of from about $10^{-6}$ through about $10^{-13}$ M or below, when measured by various techniques.
As will be appreciated, antibodies can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used to transform a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), human epithelial kidney 293 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce antibodies with constitutive PCSK9 binding properties.

In certain embodiments, antibodies and/or ABP are produced by at least one of the following hybridomas: 21B12, 31H4, 16F12, any of the other hybridomas listed in Table 2 or disclosed in the examples. In certain embodiments, antigen binding proteins bind to PCSK9 with a dissociation constant (K\textsubscript{D}) of less than approximately 1 nM, e.g., 1000pM to 100 pM, 100 pM to 10 pM, 10 pM to 1 pM, and/or 1 pM to 0.1 pM or less.

In certain embodiments, antigen binding proteins comprise an immunoglobulin molecule of at least one of the IgG1, IgG2, IgG3, IgG4, Ig E, IgA, IgD, and IgM isotype. In certain embodiments, antigen binding proteins comprise a human kappa light chain and/or a human heavy chain. In certain embodiments, the heavy chain is of the IgG1, IgG2, IgG3, IgG4, IgE, IgA, IgD, or IgM isotype. In certain embodiments,
antigen binding proteins have been cloned for expression in mammalian cells. In certain embodiments, antigen binding proteins comprise a constant region other than any of the constant regions of the IgG1, IgG2, IgG3, IgG4, IgE, IgA, IgD, and IgM isotype.

In certain embodiments, antigen binding proteins comprise a human lambda light chain and a human IgG2 heavy chain. In certain embodiments, antigen binding proteins comprise a human lambda light chain and a human IgG4 heavy chain. In certain embodiments, antigen binding proteins comprise a human lambda light chain and a human IgG1, IgG3, IgE, IgA, IgD or IgM heavy chain. In other embodiments, antigen binding proteins comprise a human kappa light chain and a human IgG2 heavy chain. In certain embodiments, antigen binding proteins comprise a human kappa light chain and a human IgG4 heavy chain. In certain embodiments, antigen binding proteins comprise a human kappa light chain and a human IgG1, IgG3, IgE, IgA, IgD or IgM heavy chain. In certain embodiments, antigen binding proteins comprise variable regions of antibodies ligated to a constant region that is neither the constant region for the IgG2 isotype, nor the constant region for the IgG4 isotype. In certain embodiments, antigen binding proteins have been cloned for expression in mammalian cells.

In certain embodiments, conservative modifications to the heavy and light chains of antibodies from at least one of the hybridoma lines: 21B12, 31H4 and 16F12 (and corresponding modifications to the encoding nucleotides) will produce antibodies to PCSK9 having functional and chemical characteristics similar to those of the antibodies from the hybridoma lines: 21B12, 31H4 and 16F12. In addition, certain other modifications to the heavy and light chains of the antibodies from at least one of the hybridoma lines: 21B12, 31H4 and 16F12 (and corresponding modifications to the encoding nucleotides) will produce antibodies to PCSK9 having functional and chemical characteristics similar to those of the antibodies from the hybridoma lines: 21B12, 31H4 and 16F12 (such as, for example, SEQ ID NOS:592-593). In contrast, in certain embodiments, substantial modifications in the functional and/or chemical characteristics of antibodies to PCSK9 can be accomplished by selecting substitutions in the amino acid sequence of the heavy and light chains that differ significantly in their
effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a “conservative amino acid substitution” can involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide can also be substituted with alanine, as has been previously described for “alanine scanning mutagenesis.”

Desired amino acid insertions or substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such insertions or substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to PCSK9, or to increase or decrease the affinity of the antibodies to PCSK9 as described herein.

In certain embodiments, antibodies of the present invention can be expressed in cell lines other than hybridoma cell lines. In certain embodiments, sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. According to certain embodiments, transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference for any purpose). In certain embodiments, the transformation procedure used can depend upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to, many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese
hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. In certain embodiments, cell lines can be selected through determining which cell lines have high expression levels and produce antibodies with constitutive HGF binding properties. Appropriate expression vectors for mammalian host cells are well known.

In certain embodiments, antigen binding proteins comprise one or more polypeptides. In certain embodiments, any of a variety of expression vector/host systems can be utilized to express polynucleotide molecules encoding polypeptides comprising one or more ABP components or the ABP itself. Such systems include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV, tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems.

In certain embodiments, a polypeptide comprising one or more ABP components or the ABP itself is recombinantly expressed in yeast. Certain such embodiments use commercially available expression systems, e.g., the Pichia Expression System (Invitrogen, San Diego, CA), following the manufacturer’s instructions. In certain embodiments, such a system relies on the pre-pro-alpha sequence to direct secretion. In certain embodiments, transcription of the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol.

In certain embodiments, a secreted polypeptide comprising one or more ABP components or the ABP itself is purified from yeast growth medium. In certain embodiments, the methods used to purify a polypeptide from yeast growth medium is the same as those used to purify the polypeptide from bacterial and mammalian cell supernatants.

In certain embodiments, a nucleic acid encoding a polypeptide comprising one or more ABP components or the ABP itself is cloned into a baculovirus expression vector, such as pVL1393 (PharMingen, San Diego, CA). In certain embodiments, such
a vector can be used according to the manufacturer's directions (PharMingen) to infect *Spodoptera frugiperda* cells in sF9 protein-free media and to produce recombinant polypeptide. In certain embodiments, a polypeptide is purified and concentrated from such media using a heparin-Sepharose column (Pharmacia).

In certain embodiments, a polypeptide comprising one or more ABP components or the ABP itself is expressed in an insect system. Certain insect systems for polypeptide expression are well known to those of skill in the art. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. In certain embodiments, a nucleic acid molecule encoding a polypeptide can be inserted into a nonessential gene of the virus, for example, within the polyhedrin gene, and placed under control of the promoter for that gene. In certain embodiments, successful insertion of a nucleic acid molecule will render the nonessential gene inactive. In certain embodiments, that inactivation results in a detectable characteristic. For example, inactivation of the polyhedrin gene results in the production of virus lacking coat protein.

In certain embodiments, recombinant viruses can be used to infect *S. frugiperda* cells or *Trichoplusia* larvae. See, e.g., Smith *et al.*, J. Virol., 46: 584 (1983); Engelhard *et al.*, Proc. Nat. Acad. Sci. (USA), 91: 3224-7 (1994).

In certain embodiments, polypeptides comprising one or more ABP components or the ABP itself made in bacterial cells are produced as insoluble inclusion bodies in the bacteria. In certain embodiments, host cells comprising such inclusion bodies are collected by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA; and treated with 0.1 mg/ml lysozyme (Sigma, St. Louis, MO) for 15 minutes at room temperature. In certain embodiments, the lysate is cleared by sonication, and cell debris is pelleted by centrifugation for 10 minutes at 12,000 X g. In certain embodiments, the polypeptide-containing pellet is resuspended in 50 mM Tris, pH 8, and 10 mM EDTA; layered over 50% glycerol; and centrifuged for 30 minutes at 6000 X g. In certain embodiments, that pellet can be resuspended in standard phosphate buffered saline solution (PBS) free of Mg$^{++}$ and Ca$^{++}$. In certain embodiments, the polypeptide is further purified by fractionating the resuspended pellet in a denaturing
SDS polyacrylamide gel (See, e.g., Sambrook et al., supra). In certain embodiments, such a gel can be soaked in 0.4 M KCl to visualize the protein, which can be excised and electroeluted in gel-running buffer lacking SDS. According to certain embodiments, a Glutathione-S-Transferase (GST) fusion protein is produced in bacteria as a soluble protein. In certain embodiments, such GST fusion protein is purified using a GST Purification Module (Pharmacia).

In certain embodiments, it is desirable to “refold” certain polypeptides, e.g., polypeptides comprising one or more ABP components or the ABP itself. In certain embodiments, such polypeptides are produced using certain recombinant systems discussed herein. In certain embodiments, polypeptides are “refolded” and/or oxidized to form desired tertiary structure and/or to generate disulfide linkages. In certain embodiments, such structure and/or linkages are related to certain biological activity of a polypeptide. In certain embodiments, refolding is accomplished using any of a number of procedures known in the art. Exemplary methods include, but are not limited to, exposing the solubilized polypeptide agent to a pH typically above 7 in the presence of a chaotrope agent. An exemplary chaotrope agent is guanidine. In certain embodiments, the refolding/oxidation solution also contains a reducing agent and the oxidized form of that reducing agent. In certain embodiments, the reducing agent and its oxidized form are present in a ratio that will generate a particular redox potential that allows disulfide shuffling to occur. In certain embodiments, such shuffling allows the formation of cysteine bridges. Exemplary redox couples include, but are not limited to, cysteine/cystamine, glutathione/dithiobisGSH, cupric chloride, dithiothreitol DTT/dithiane DTT, and 2-mercaptopethanol (bME)/dithio-bME. In certain embodiments, a co-solvent is used to increase the efficiency of refolding. Exemplary cosolvents include, but are not limited to, glycerol, polyethylene glycol of various molecular weights, and arginine.

In certain embodiments, one substantially purifies a polypeptide comprising one or more ABP components or the ABP itself. Certain protein purification techniques are known to those of skill in the art. In certain embodiments, protein purification involves crude fractionation of polypeptide fractionations from non-polypeptide fractions. In certain embodiments, polypeptides are purified using chromatographic and/or
electrophoretic techniques. Exemplary purification methods include, but are not limited to, precipitation with ammonium sulphate; precipitation with PEG; immunoprecipitation; heat denaturation followed by centrifugation; chromatography, including, but not limited to, affinity chromatography (e.g., Protein-A-Sepharose), ion exchange chromatography, exclusion chromatography, and reverse phase chromatography; gel filtration; hydroxyapatite chromatography; isoelectric focusing; polyacrylamide gel electrophoresis; and combinations of such and other techniques. In certain embodiments, a polypeptide is purified by fast protein liquid chromatography or by high pressure liquid chromatography (HPLC). In certain embodiments, purification steps can be changed or certain steps can be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide.

In certain embodiments, one quantitates the degree of purification of a polypeptide preparation. Certain methods for quantifying the degree of purification are known to those of skill in the art. Certain exemplary methods include, but are not limited to, determining the specific binding activity of the preparation and assessing the amount of a polypeptide within a preparation by SDS/PAGE analysis. Certain exemplary methods for assessing the amount of purification of a polypeptide preparation comprise calculating the binding activity of a preparation and comparing it to the binding activity of an initial extract. In certain embodiments, the results of such a calculation are expressed as “fold purification.” The units used to represent the amount of binding activity depend upon the particular assay performed.

In certain embodiments, a polypeptide comprising one or more ABP components or the ABP itself is partially purified. In certain embodiments, partial purification can be accomplished by using fewer purification steps or by utilizing different forms of the same general purification scheme. For example, in certain embodiments, cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater “fold purification” than the same technique utilizing a low-pressure chromatography system. In certain embodiments, methods resulting in a lower degree of purification can have advantages in total recovery of polypeptide, or in maintaining binding activity of a polypeptide.
In certain instances, the electrophoretic migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE. See, e.g., Capaldi et al., Biochem. Biophys. Res. Comm., 76: 425 (1977). It will be appreciated that under different electrophoresis conditions, the apparent molecular weights of purified or partially purified polypeptide can be different.

Exemplary Epitopes

Epitopes to which anti-PCSK9 antibodies useful in the methods provided herein bind are described. In some embodiments, epitopes that are bound by the presently disclosed antibodies are particularly useful. In some embodiments, antigen binding proteins that bind to any of the epitopes that are bound by the antibodies described herein are useful. In some embodiments, the epitopes bound by any of the antibodies listed in Table 2 and FIGs. 2 and 3 are especially useful. In some embodiments, the epitope is on the catalytic domain PCSK9.

In some embodiments, antigen binding proteins disclosed herein bind specifically to N-terminal prodomain, a subtilisin-like catalytic domain and/or a C-terminal domain. In some embodiments, the antigen binding protein binds to the substrate-binding groove of PCSK-9 (described in Cunningham et al., incorporated herein in its entirety by reference).

In some embodiments, the domain(s)/region(s) containing residues that are in contact with or are buried by an antibody can be identified by mutating specific residues in PCSK9 (e.g., a wild-type antigen) and determining whether the antigen binding protein can bind the mutated or variant PCSK9 protein. By making a number of individual mutations, residues that play a direct role in binding or that are in sufficiently close proximity to the antibody such that a mutation can affect binding between the antigen binding protein and antigen can be identified. From knowledge of these amino acids, the domain(s) or region(s) of the antigen that contain residues in contact with the antigen binding protein or covered by the antibody can be elucidated. Such a domain can include the binding epitope of an antigen binding protein. One specific example of this general approach utilizes an arginine/glutamic acid scanning protocol (see, e.g., Nanevicz, T., et al., 1995, J. Biol. Chem., 270:37, 21619-21625 and
Zupnick, A., et al., 2006, J. Biol. Chem., 281:29, 20464-20473). In general, arginine and glutamic acids are substituted (typically individually) for an amino acid in the wild-type polypeptide because these amino acids are charged and bulky and thus have the potential to disrupt binding between an antigen binding protein and an antigen in the region of the antigen where the mutation is introduced. Arginines that exist in the wild-type antigen are replaced with glutamic acid. A variety of such individual mutants are obtained and the collected binding results analyzed to determine what residues affect binding.

An alteration (for example a reduction or increase) in binding between an antigen binding protein and a variant PCSK9 as used herein means that there is a change in binding affinity (e.g., as measured by known methods such as Biacore testing or the bead based assay described below in the examples), EC50, and/or a change (for example a reduction) in the total binding capacity of the antigen binding protein (for example, as evidenced by a decrease in Bmax in a plot of antigen binding protein concentration versus antigen concentration). A significant alteration in binding indicates that the mutated residue is directly involved in binding to the antigen binding protein or is in close proximity to the binding protein when the binding protein is bound to antigen.

In some embodiments, a significant reduction in binding means that the binding affinity, EC50, and/or capacity between an antigen binding protein and a mutant PCSK9 antigen is reduced by greater than 10%, greater than 20%, greater than 40%, greater than 50 %, greater than 55 %, greater than 60 %, greater than 65 %, greater than 70 %, greater than 75 %, greater than 80 %, greater than 85 %, greater than 90% or greater than 95% relative to binding between the antigen binding protein and a wild type PCSK9 (e.g., shown in SEQ ID NO: 1 and/or SEQ ID NO: (303). In certain embodiments, binding is reduced below detectable limits. In some embodiments, a significant reduction in binding is evidenced when binding of an antigen binding protein to a variant PCSK9 protein is less than 50% (for example, less than 40%, 35%, 30%, 25%, 20%, 15% or 10%) of the binding observed between the antigen binding protein and a wild-type PCSK9 protein (for example, the protein of SEQ ID NO: 1
and/or SEQ ID NO: (303). Such binding measurements can be made using a variety of binding assays known in the art.

In some embodiments, antigen binding proteins are provided that exhibit significantly lower binding for a variant PCSK9 protein in which a residue in a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303 is substituted with arginine or glutamic acid. In some embodiments, binding of an antigen binding protein is significantly reduced or increased for a variant PCSK9 protein having any one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 244) of the following mutations: R207E, D208R, R185E, R439E, E513R, V538R, E539R, T132R, S351R, A390R, A413R, E582R, D162R, R164E, E167R, S123R, E129R, A311R, D313R, D337R, R519E, H521R, and Q554R as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303). In the shorthand notation used here, the format is: Wild type residue: Position in polypeptide: Mutant residue, with the numbering of the residues as indicated in SEQ ID NO: 1 or SEQ ID NO: 303.

In some embodiments, binding of an antigen binding protein is significantly reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, or more) mutations at the following positions: 207, 208, 185, 181, 439, 513, 538, 539, 132, 351, 390, 413, 582, 162, 164, 167, 123, 129, 311, 313, 337, 519, 521, and 554, as shown in SEQ ID NO: 1 as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303). In some embodiments, binding of an antigen binding protein is reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, or more) mutations at the following positions: 207, 208, 185, 181, 439, 513, 538, 539, 132, 351, 390, 413, 582, 162, 164, 167, 123, 129, 311, 313, 337, 519, 521, and 554, as shown in SEQ ID NO: 1 as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303). In some embodiments, binding of an antigen binding protein is substantially reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, or more) mutations at the following positions: 207, 208, 185, 181, 439, 513, 538, 539, 132, 351, 390, 413, 582, 162, 164, 167, 123, 129, 311, 313, 337, 519, 521, and 554, within SEQ ID NO: 1 as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303).
In some embodiments, binding of an ABP is significantly reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, etc.) of the following mutations: R207E, D208R, R185E, R439E, E513R, V538R, E539R, T132R, S351R, A390R, A413R, E582R, D162R, R164E, E167R, S123R, E129R, A311R, D313R, D337R, R519E, H521R, and Q554R within SEQ ID NO: 1 or SEQ ID NO: 303, as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303).

In some embodiments, binding of an ABP is significantly reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, etc.) of the following mutations: R207E, D208R, R185E, R439E, E513R, V538R, E539R, T132R, S351R, A390R, A413R, and E582R within SEQ ID NO: 1 or SEQ ID NO: 303, as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303). In some embodiments, the binding is reduced. In some embodiments, the reduction in binding is observed as a change in EC50. In some embodiments, the change in EC50 is an increase in the numerical value of the EC50 (and thus is a decrease in binding).

In some embodiments, binding of an ABP is significantly reduced or increased for a mutant PCSK9 protein having one or more (e.g., 1, 2, 3, 4, 5, etc.) of the following mutations: D162R, R164E, E167R, S123R, E129R, A311R, D313R, D337R, R519E, H521R, and Q554R within SEQ ID NO: 1, as compared to a wild-type PCSK9 protein (e.g., SEQ ID NO: 1 or SEQ ID NO: 303). In some embodiments, the binding is reduced. In some embodiments, the reduction in binding is observed as a change in Bmax. In some embodiments, the shift in Bmax is a reduction of the maximum signal generated by the ABP. In some embodiments, for an amino acid to be part of an epitope, the Bmax is reduced by at least 10%, for example, reductions of at least any of the following amounts: 20, 30, 40, 50, 60, 70, 80, 90, 95, 98, 99, or 100 percent can, in some embodiments, indicate that the residue is part of the epitope.

Although the variant forms just listed are referenced with respect to the wild-type sequence shown in SEQ ID NO: 1 or SEQ ID NO: 303, it will be appreciated that in an allelic variant of PCSK9 the amino acid at the indicated position could differ. Antigen binding proteins showing significantly lower binding for such allelic forms of PCSK9 are also contemplated. Accordingly, in some embodiments, any of the above
embodiments can be compared to an allelic sequence, rather than purely the wild-type sequence shown in FIG. 1A.

In some embodiments, binding of an antigen binding protein is significantly reduced for a variant PCSK9 protein in which the residue at a selected position in the wild-type PCSK9 protein is mutated to any other residue. In some embodiments, the herein described arginine/glutamic acid replacements are used for the identified positions. In some embodiments, alanine is used for the identified positions.

As noted above, residues directly involved in binding or covered by an antigen binding protein can be identified from scanning results. These residues can thus provide an indication of the domains or regions of SEQ ID NO: 1 (or SEQ ID NO: 303 or SEQ ID NO: 3) that contain the binding region(s) to which antigen binding proteins bind. In some embodiments an antigen binding protein binds to a domain containing at least one of amino acids: 207, 208, 185, 181, 439, 513, 538, 539, 132, 351, 390, 413, 582, 162, 164, 167, 123, 129, 311, 313, 337, 519, 521, and 554 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, the antigen binding protein binds to a region containing at least one of amino acids 207, 208, 185, 181, 439, 513, 538, 539, 132, 351, 390, 413, 582, 162, 164, 167, 123, 129, 311, 313, 337, 519, 521, and 554 of SEQ ID NO: 1 or SEQ ID NO: 303.

In some embodiments, the antigen binding protein binds to a region containing at least one of amino acids 162, 164, 167, 207 and/or 208 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, more than one (e.g., 2, 3, 4, or 5) of the identified residues are part of the region that is bound by the ABP. In some embodiments, the ABP competes with ABP 21B12.

In some embodiments, the antigen binding protein binds to a region containing at least one of amino acid 185 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, the ABP competes with ABP 31H4.

In some embodiments, the antigen binding protein binds to a region containing at least one of amino acids 439, 513, 538, and/or 539 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, more than one (e.g., 2, 3, or 4) of the identified residues are part of the region that is bound by the ABP. In some embodiments, the ABP competes with ABP 31A4.
In some embodiments, the antigen binding protein binds to a region containing at least one of amino acids 123, 129, 311, 313, 337, 132, 351, 390, and/or 413 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, more than one (e.g., 2, 3, 4, 5, 6, 7, 8, or 9) of the identified residues are part of the region that is bound by the ABP. In some embodiments, the ABP competes with ABP 12H11.

In some embodiments, the antigen binding protein binds to a region containing at least one of amino acid 582, 519, 521, and/or 554 of SEQ ID NO: 1 or SEQ ID NO: 303. In some embodiments, more than one (e.g., 2, 3, or 4) of the identified residues are part of the region that is bound by the ABP. In some embodiments, the ABP competes with ABP 3C4.

In some embodiments, the antigen binding proteins binds to the foregoing regions within a fragment or the full length sequence of SEQ ID NO: 1 or SEQ ID NO: 303. In other embodiments, antigen binding proteins bind to polypeptides consisting of these regions. The reference to “SEQ ID NO: 1 or SEQ ID NO: 303” denotes that one or both of these sequences can be employed or relevant. The phrase does not denote that only one should be employed.

As noted above, the above description references specific amino acid positions with reference to SEQ ID NO: 1. However, throughout the specification generally, reference is made to a Pro/Cat domain that commences at position 31, which is provided in SEQ ID NO: 3. As noted below, SEQ ID NO: 1 and SEQ ID NO: 303 lack the signal sequence of PCSK9. As such, any comparison between these various disclosures should take this difference in numbering into account. In particular, any amino acid position in SEQ ID NO: 1, will correspond to an amino acid position 30 amino acids further into the protein in SEQ ID NO: 3. For example, position 207 of SEQ ID NO: 1, corresponds to position 237 of SEQ ID NO: 3 (the full length sequence, and the numbering system used in the present specification generally). Table 39.6 outlines how the above noted positions, which reference SEQ ID NO: 1 (and/or SEQ ID NO: 303) correspond to SEQ ID NO: 3 (which includes the signal sequence). Thus, any of the above noted embodiments that are described in regard to SEQ ID NO: 1 (and/or SEQ ID NO: 303), are described in reference to SEQ ID NO: 3, by the noted corresponding positions.
In some embodiments, ABP 21B12 binds to an epitope including residues 162-167 (e.g., residues D162-E167 of SEQ ID NO: 1). In some embodiments, ABP 12H11 binds to an epitope that includes residues 123-132 (e.g., S123-T132 of SEQ ID NO: 1). In some embodiments, ABP 12H11 binds to an epitope that includes residues 311-313 (e.g., A311-D313 of SEQ ID NO: 1). In some embodiments, ABPs can bind to an epitope that includes any one of these strands of sequences.

Competing Antigen Binding Proteins

In another aspect, antigen binding proteins are provided that compete with one of the exemplified antibodies or functional fragments binding to the epitope described herein for specific binding to PCSK9. Such antigen binding proteins can also bind to the same epitope as one of the herein exemplified antigen binding proteins, or an overlapping epitope. Antigen binding proteins and fragments that compete with or bind to the same epitope as the exemplified antigen binding proteins are expected to show similar functional properties. The exemplified antigen binding proteins and fragments include those described above, including those with the heavy and light chains, variable region domains and CDRs included in TABLE 2 and/or FIGs. 2-3. Thus, as a specific example, the antigen binding proteins that are provided include those that compete with an antibody or antigen binding protein having:

(a) all 6 of the CDRs listed for an antibody listed in FIGs. 2-3;
(b) a VH and a VL listed for an antibody listed in Table 2; or
(c) two light chains and two heavy chains as specified for an antibody listed in Table 2.

Therapeutic Pharmaceutical Formulations and Administration

Provided herein are pharmaceutical formulations containing antigen binding proteins to PCSK9 that are useful in the described methods. As used herein, “pharmaceutical formulation” is a sterile composition of a pharmaceutically active drug, namely, at least one antigen binding protein to PCSK9, that is suitable for parenteral administration (including but not limited to intravenous, intramuscular, subcutaneous, aerosolized, intrapulmonary, intranasal, or intrathecal) to a patient in
need thereof and includes only pharmaceutically acceptable excipients, diluents, and other additives deemed safe by the Federal Drug Administration or other foreign national authorities. Pharmaceutical formulations include liquid, e.g., aqueous, solutions that may be directly administered, and lyophilized powders which may be reconstituted into solutions by adding a diluent before administration. Specifically excluded from the scope of the term “pharmaceutical formulation” are compositions for topical administration to patients, compositions for oral ingestion, and compositions for parenteral feeding.

In certain embodiments, the pharmaceutical formulation is a stable pharmaceutical formulation. As used herein, the phrases, “stable pharmaceutical formulation,” “stable formulation” or “a pharmaceutical formulation is stable” refers to a pharmaceutical formulation of biologically active proteins that exhibit increased aggregation and/or reduced loss of biological activity of not more than 5% when stored at 2-8°C for at least 1 month, or 2 months, or 3 months, or 6 months, or 1 year or 2 years compared with a control formula sample. Formulation stability can be easily determined by a person of skill in the art using any number of standard assays, including but not limited to size exclusion HPLC (“SEC-HPLC”), cation-exchange HPLC (CEX-HPLC), Subvisible Particle Detection by Light Obscuration (“HIAC”) and/or visual inspection.

In certain embodiments, the pharmaceutical formulation comprises any of the antigen binding proteins to PCSK9 comprising: one or more heavy chain complementary determining regions (CDRHs) and one or more light chain complementary determining regions (CDRLs) depicted in Table 2 and FIGs. 2 and/or 3 and FIGS. 31A and 31B. In certain other embodiments, the pharmaceutical formulation comprises an antigen binding protein to PCSK9 comprising: a light chain variable region that comprises an amino acid sequence that is at least 90% identical the antigen binding proteins to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and FIGs 31A and 31B, and a heavy chain variable region that comprises and amino acid sequence that is at least 90% identical to that of any of the antigen binding proteins to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and FIGs 31A and 31B. In still other embodiments, the pharmaceutical formulation comprises any of the antigen binding
proteins to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and FIGs 31A and 31B. In certain other embodiments, the pharmaceutical formulation may comprise other antigen binding proteins to PCSK9; namely an antibody comprised of a light chain variable domain, SEQ ID NO:588 and a heavy chain variable domain, SEQ ID NO:589. In some embodiments the pharmaceutical formulation comprises any one of 21B12, 26H5, 31H4, 8A3, 11F1 or 8A1.

In some embodiments, the pharmaceutical formulation comprises more than one different antigen binding protein to PCSK9. In certain embodiments, pharmaceutical formulations comprise more than one antigen binding protein to PCSK9 wherein the antigen binding proteins to PCSK9 bind more than one epitope. In some embodiments, the various antigen binding proteins will not compete with one another for binding to PCSK9. In some embodiments, any of the antigen binding proteins depicted in Table 2 and FIGs. 2 and/or 3 can be combined together in a pharmaceutical formulation.

In certain embodiments, an antigen binding protein to PCSK9 and/or a therapeutic molecule is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, polyethylene glycol, glycogen (e.g., glycosylation of the ABP), and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082, now US Patent No. 6,660,843 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

In certain embodiments, acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, the formulation material(s) are for s.c. and/or I.V. administration. In certain embodiments, the pharmaceutical formulation comprises formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition.

In certain embodiments, suitable formulation materials include, but are not limited to, amino acids (such as proline, arginine, lysine, methionine, taurine, glycine, glutamine, or asparagine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, sodium
phosphate ("NaOAc"), Tris-HCl, Tris buffer, citrates, phosphate buffer, phosphate-buffered saline (i.e., PBS buffer) or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, sucrose, fructose, lactose, mannose, trehalose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counter ions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapol); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol or sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company (1995).

In certain embodiments, the optimal pharmaceutical formulation will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, Remington's Pharmaceutical Sciences, supra. In certain embodiments, such formulations may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the antibodies of the invention.

In one aspect, the pharmaceutical formulation comprises high concentrations of antigen binding protein to PCSK9. In certain embodiments, ABP concentration ranges from about 70 mg/ml to about 250 mg/ml, e.g., about 70 mg/ml, about 80 mg/ml, about 90 mg/ml, about 100 mg/ml, about 100 mg/ml, about 120 mg/ml, about 130 mg/ml, about 140 mg/ml, about 150 mg/ml, about 160 mg/ml, about 170 mg/ml, about 180
mg/ml, about 190 mg/ml, about 200 mg/ml, about 210 mg/ml, about 220 mg/ml, about 230 mg/ml, about 240 mg/ml, or about 250 mg/ml, and including all values in between. In some embodiments, the concentration of 21B12, 26H5, or 31H4 ranges from about 100 mg/ml to about 150 mg/ml, e.g., 100 mg/ml, about 100 mg/ml, about 120 mg/ml, about 130 mg/ml, about 140 mg/ml, or about 150 mg/ml. In some embodiments, the concentration of 8A3, 11F1 or 8A1 ranges from about 140 mg/ml to about 220 mg/ml, e.g., 140 mg/ml, about 150 mg/ml, about 160 mg/ml, about 170 mg/ml, about 180 mg/ml, about 190 mg/ml, about 200 mg/ml, about 210 mg/ml, about 220 mg/ml, or about 250 mg/ml.

In another aspect, the pharmaceutical formulation comprises at least one buffering agent such as, for example, sodium acetate, sodium chloride, phosphates, phosphate buffered saline ("PBS"), and/or Tris buffer of about pH 7.0-8.5. The buffer serves to maintain a physiologically suitable pH. In addition, the buffer can serve to enhance isotonicity and chemical stability of the pharmaceutical formulation. In certain embodiments, the buffering agent ranges from about 0.05 mM to about 40 mM, e.g., about 0.05 mM, about 0.1 mM, about 0.5 mM, about 1.0 mM, about 5.0 mM, about 10 mM, about 15 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, or about 100 mM buffering agent, inclusive of all values in between. In certain embodiments, the buffering agent is NaOAc. Exemplary pHs of the pharmaceutical formulation include from about 4 to about 6, or from about 4.8 to about 5.8, or from about 5.0 to about 5.2, or about 5, or about 5.2.

In certain embodiments, the pharmaceutical formulation is isotonic with an osmolality ranging from between about 250 to about 350 miliosmol/kg, e.g., about 250 mOsm/kg, about 260 mOsm/kg, about 270 mOsm/kg, about 280 mOsm/kg, about 290 mOsm/kg, about 300 mOsm/kg, about 310 mOsm/kg, about 320 mOsm/kg, about 330 mOsm/kg, about 340 mOsm/kg, or about 350 mOsm/kg, and including all values in between. As used herein, "osmolality" is the measure of the ratio of solutes to volume fluid. In other words, it is the number of molecules and ions (or molecules) per kilogram of a solution. Osmolality may be measured on an analytical instrument called an osmometer, such as Advanced Instruments 2020 Multi-sample Osmometer,
Norwood, MA. The Advanced Instruments 2020 Multi-sample Osmometer measures osmolality by using the Freezing Point Depression method. The higher the osmolytes in a solution, the temperature in which it will freeze drops. Osmolality may also be measured using any other methods and in any other units known in the art such as linear extrapolation.

In still another aspect, the pharmaceutical formulation comprises at least one surfactant including but not limited to Polysorbate-80, Polysorbate-60, Polysorbate-40, and Polysorbate-20. In certain embodiments, the pharmaceutical formulation comprises a surfactant at a concentration that ranges from about 0.004% to about 10% weight per volume ("w/v") of the formulation, e.g., about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.05%, about 0.1%, about 0.5%, about 1%, about 5%, or about 10% surfactant w/v of the formulation. In certain embodiments, the pharmaceutical formulation comprises polysorbate 80 at a concentration that ranges from about 0.004% to about 0.1% w/v of the formulation. In certain embodiments, the pharmaceutical formulation comprises polysorbate 20 at a concentration that ranges from about 0.004% to about 0.1% w/v of the formulation.

In certain embodiments, the pharmaceutical formulation comprises at least one stabilizing agent, such as a polyhydroxy hydrocarbon (including but not limited to sorbitol, mannitol, glycerol and dulcitol) and/or a disaccharide (including but not limited to sucrose, lactose, maltose and threhalose) and/or an amino acid (including but not limited to proline, arginine, lysine, methionine, and taurine) and/or benzyl alcohol; the total of said polyhydroxy hydrocarbon and/or disaccharide and/or amino acid and/or benzyl alcohol being about 0.5% to about 10% w/v of the formulation. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10% sucrose. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of about 5% sucrose. In certain embodiments, the pharmaceutical formulation comprises a a stabilizing agent at a concentration of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10% sorbitol. In certain
embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of about 9% sorbitol. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of about 1%, about 2%, about 3%, about 4%, about 5% proline, arginine, lysine, methionine, and/or taurine. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of between about 2-3% proline. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of about 1%, about 2%, about 3%, about 4%, about 5% benzyl alcohol. In certain embodiments, the pharmaceutical formulation comprises a stabilizing agent at a concentration of between about 1-2% benzyl alcohol.

In one aspect, the pharmaceutical formulation has a viscosity level of less than about 30 centipoise (cP) as measured at room temperature (i.e., 25°C). As used herein, “viscosity” is a fluid’s resistance to flow, and may be measured in units of centipoise (cP) or milliPascal-second (mPa-s), where 1 cP = 1 mPa-s, at a given shear rate. Viscosity may be measured by using a viscometer, e.g., Brookfield Engineering Dial Reading Viscometer, model LVT. Viscosity may also be measured using any other methods and in any other units known in the art (e.g., absolute, kinematic or dynamic viscosity or absolute viscosity). In certain embodiments, the pharmaceutical formulation has a viscosity level of less than about 25 cP, about 20 cP, about 18 cP, about 15 cP, about 12 cP, about 10 cP; about 8 cP, about 6 cP, about 4 cP; about 2 cP; or about 1 cP.

In one aspect, the pharmaceutical formulation is stable as measured by at least one stability assay known to one of skill in the art, such as assays that examine the biophysical or biochemical characteristics of biologically active proteins over time. As mentioned above, a stable pharmaceutical formulation of the present invention is a pharmaceutical formulation of biologically active proteins that exhibits increased aggregation and/or reduced loss of biological activity of not more than 5% when stored at 2-8°C for at least 1 month, or 2 months, or 3 months, or 6 months, or 1 year or 2 years compared with a control formula sample. In certain embodiments, the pharmaceutical formulation stability is measured using size exclusion HPLC (“SEC-HPLC”). SEC-HPLC separates proteins based on differences in their hydrodynamic
volumes. Molecules with larger hydrodynamic proteins volumes elute earlier than molecules with smaller volumes. In the case of SEC-HPLC, a stable pharmaceutical formulation should exhibit no more than about a 5% increase in high molecular weight species as compared to a control sample. In certain other embodiments, the pharmaceutical formulation should exhibit no more than about a 4%, no more than about a 3%, no more than about a 2%, no more than about a 1%, no more than about a 0.5% increase in high molecular weight species as compared to a control sample.

In certain embodiments, the pharmaceutical formulation stability is measured using cation-exchange HPLC (CEX-HPLC). CEX-HPLC separates proteins based on differences in their surface charge. At a set pH, charged isoforms of an anti-PCSK9 ABP are separated on a cation-exchange column and eluted using a salt gradient. The eluent is monitored by UV absorbance. The charged isoform distribution is evaluated by determining the peak area of each isoform as a percent of the total peak area. In the case of CEX-HPLC, a stable pharmaceutical formulation should exhibit no more than about a 5% decrease in the main isoform peak as compared to a control sample. In certain other embodiments, a stable pharmaceutical formulation should exhibit no more than about a 3% to about a 5% decrease in the main isoform peak as compared to a control sample. In certain embodiments, the pharmaceutical formulation should exhibit no more than about a 4% decrease, no more than about a 3% decrease, no more than about a 2% decrease, no more than about a 1% decrease, no more than about a 0.5% decrease in the main isoform peak as compared to a control sample.

In certain embodiments, the pharmaceutical formulation stability is measured using Subvisible Particle Detection by Light Obscuration ("HIAC"). An electronic, liquid-borne particle-counting system (HIAC/Royco 9703 or equivalent) containing a light-obscuration sensor (HIAC/Royco HRLD-150 or equivalent) with a liquid sampler quantifies the number of particles and their size range in a given test sample. When particles in a liquid pass between the light source and the detector they diminish or "obscure" the beam of light that falls on the detector. When the concentration of particles lies within the normal range of the sensor, these particles are detected one-by-one. The passage of each particle through the detection zone reduces the incident light on the photo-detector and the voltage output of the photo-detector is momentarily
reduced. The changes in the voltage register as electrical pulses that are converted by the instrument into the number of particles present. The method is non-specific and measures particles regardless of their origin. Particle sizes monitored are generally 10 μm, and 25 μm. In the case of HIAC, a stable pharmaceutical formulation should exhibit no more than 6000 10μm particles per container (or unit), as compared to a control sample. In certain embodiments, a stable pharmaceutical formulation should exhibit no more than 5000, no more than 4000, no more than 3000, no more than 2000, no more than 1000, 10μm particles per container (or unit) as compared to a control sample. In still other embodiments, a stable pharmaceutical formulation should exhibit no more than 600 25μm particles per container (or unit) as compared to a control sample. In certain embodiments, a stable pharmaceutical formulation should exhibit no more than 500, no more than 400, no more than 300, no more than 200, no more than 100, no more than 50 25μm particles per container (or unit) as compared to a control sample.

In certain embodiments, the pharmaceutical formulation stability is measured using visual assessment. Visual assessment is a qualitative method used to describe the visible physical characteristics of a sample. The sample is viewed against a black and/or white background of an inspection booth, depending on the characteristic being evaluated (e.g., color, clarity, presence of particles or foreign matter). Samples are also viewed against an opalescent reference standard and color reference standards. In the case of visual assessment, a stable pharmaceutical formulation should exhibit no significant change in color, clarity, presence of particles or foreign matter as compared to a control sample.

One aspect of the present invention is a pharmaceutical formulation which comprises: (i) about 70 mg/ml to about 250 mg/ml of antigen binding protein to PCSK9; (ii) about 0.05 mM to about 40 mM of a buffer such as sodium acetate ("NaOAC") serves as a buffering agent; (iii) about 1% to about 5% proline, arginine, lysine, methionine, or taurine (also know as 2-aminoethanesulfonic acid) and/or 0.5% to about 5% benzyl alcohol which serves as a stabilizing agent; and (iv) about 0.004% to about 10% w/v of the formulation of a non-ionic surfactant (including but not limited to Polysorbate-80, Polysorbate-60, Polysorbate-40, and Polysorbate-20); wherein said
formulation has a pH in the range of about 4.0 to 6.0. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 70 mg/ml, about 100 mg/ml, about 120 mg/ml, about 140 mg/ml, about 150 mg/ml, about 160 mg/ml, about 170 mg/ml, about 180 mg/ml, about 190 mg/ml, about 200 mg/ml of an anti-PCSK9 antibody; (ii) about 10 mM NAOAC; (iii) about 0.01% polysorbate 80; and (iv) between about 2%-3% proline (or about 250 mM to about 270 mM proline), wherein the formulation has a pH of about 5. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 70 mg/ml, about 100 mg/ml, about 120 mg/ml, about 140 mg/ml of the anti-PCSK9 antibody, 21B12, 26H5 and/or 31H4; (ii) about 10 mM NAOAC; (iii) about 0.01% polysorbate 80; and (iv) between about 2%-3% proline (or about 250 mM to about 270 mM proline), wherein the formulation has a pH of about 5. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 150 mg/ml, about 160 mg/ml, about 170 mg/ml, about 180 mg/ml, about 190 mg/ml, about 200 mg/ml of the anti-PCSK9 antibody, 8A3, 11F1 and/or 8A1; (ii) about 10 mM NAOAC; (iii) about 0.01% polysorbate 80; and (iv) between about 2%-3% proline (or about 250 mM to about 270 mM proline), wherein the formulation has a pH of about 5.

One aspect of the present invention is a pharmaceutical formulation which comprises (i) at least about 70 mg/ml to about 250 mg/ml of an anti-PCSK9 antibody; (ii) about 5 mM to about 20 mM of a buffer, such as NAOAC; (iii) about 1% to about 10% w/v of the formulation comprises a polyhydroxy hydrocarbon such as sorbitol, or a disaccharide such as sucrose; and (iv) about 0.004% to about 10% w/v of the formulation of a surfactant, such as polysorbate 20 or polysorbate 80; wherein said formulation has a pH in the range of about 4.8 to 5.8; and wherein the pharmaceutical formulation optionally comprises about 80 mM to about 300 mM proline, arginine, lysine, methionine, or taurine and/or 0.5% to about 5% benzyl alcohol which serves to reduce viscosity. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 70 mg/ml to about 250 mg/ml of the anti-PCSK9 antibody; (ii) about 10 mM NAOAC; (iii) about 9% sucrose; and (iv) about 0.004% polysorbate 20, wherein the formulation has a pH of about 5.2. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about
70 mg/ml, about 100 mg/ml, about 120 mg/ml, about 140 mg/ml, about 160 mg/ml, about 180 mg/ml, about 200 mg/ml of an anti-PCSK9 antibody; (ii) about 15 mM NAOAC; (iii) about 9% sucrose; and (iv) about 0.01% polysorbate 20, wherein the formulation has a pH of about 5.2. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 70 mg/ml, about 100 mg/ml, about 120 mg/ml, about 140 mg/ml, about 160 mg/ml, about 180 mg/ml, about 200 mg/ml of an anti-PCSK9 antibody; (ii) about 20 mM NAOAC; (iii) about 9% sucrose; and (iv) about 0.01% polysorbate 20, wherein the formulation has a pH of about 5.2. In certain other embodiments, pharmaceutical formulations of this invention comprise (i) at least about 70 mg/ml, about 100 mg/ml, about 120 mg/ml, about 140 mg/ml, about 160 mg/ml, about 180 mg/ml, about 200 mg/ml of an anti-PCSK9 antibody; (ii) about 10 mM NAOAC; (iii) about 9% sucrose; (iv) about 0.01% polysorbate 80; and (v) about 250 mM proline, wherein the formulation has a pH of about 5.

Pharmaceutical formulations of the invention can be administered in combination therapy, i.e., combined with other agents. In certain embodiments, the combination therapy comprises an antigen binding protein capable of binding PCSK9, in combination with at least one anti-cholesterol agent. Agents include, but are not limited to, in vitro synthetically prepared chemical formulations, antibodies, antigen binding regions, and combinations and conjugates thereof. In certain embodiments, an agent can act as an agonist, antagonist, allosteric modulator, or toxin. In certain embodiments, an agent can act to inhibit or stimulate its target (e.g., receptor or enzyme activation or inhibition), and thereby promote increased expression of LDLR or decrease serum cholesterol levels.

In certain embodiments, an antigen binding protein to PCSK9 can be administered prior to, concurrent with, and subsequent to treatment with a cholesterol-lowering (serum and/or total cholesterol) agent. In certain embodiments, an antigen binding protein to PCSK9 can be administered prophylactically to prevent or mitigate the onset of hypercholesterolemia, heart disease, diabetes, and/or any of the cholesterol related disorder. In certain embodiments, an antigen binding protein to PCSK9 can be administered for the treatment of an existing hypercholesterolemia condition. In some embodiments, the ABP delays the onset of the disorder and/or symptoms associated
with the disorder. In some embodiments, the ABP is provided to a subject lacking any symptoms of any one of the cholesterol related disorders or a subset thereof.

In certain embodiments, an antigen binding protein to PCSK9 is used with particular therapeutic agents to treat homozygous familial hypercholesterolemia. In certain embodiments, two, three, or more agents can be administered. In certain embodiments, such agents can be provided together by inclusion in the same formulation. In certain embodiments, such agent(s) and an antigen binding protein to PCSK9 can be provided together by inclusion in the same formulation. In certain embodiments, such agents can be formulated separately and provided together by inclusion in a treatment kit. In certain embodiments, such agents and an antigen binding protein to PCSK9 can be formulated separately and provided together by inclusion in a treatment kit. In certain embodiments, such agents can be provided separately.

In certain embodiments, a formulation comprising an antigen binding protein to PCSK9, with or without at least one additional therapeutic agents, can be prepared for storage by mixing the selected formulation having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, supra) in the form of a lyophilized cake or an aqueous solution. Further, in certain embodiments, a formulation comprising an antigen binding protein to PCSK9, with or without at least one additional therapeutic agent, can be formulated as a lyophilizate using appropriate excipients.

In certain embodiments, when parenteral administration is contemplated, a therapeutic formulation can be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising a desired antigen binding protein to PCSK9, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, a vehicle for parenteral injection is sterile distilled water in which an antigen binding protein to PCSK9, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that can provide for
the controlled or sustained release of the product which can then be delivered via a
depot injection. In certain embodiments, hyaluronic acid can also be used, and can
have the effect of promoting sustained duration in the circulation. In certain
embodiments, implantable drug delivery devices can be used to introduce the desired
molecule.

In certain embodiments, a pharmaceutical formulation can be formulated for
inhalation. In certain embodiments, an antigen binding protein to PCSK9, with or
without at least one additional therapeutic agent, can be formulated as a dry powder for
inhalation. In certain embodiments, an inhalation solution comprising an antigen
binding protein to PCSK9, with or without at least one additional therapeutic agent, can
be formulated with a propellant for aerosol delivery. In certain embodiments, solutions
can be nebulized. Pulmonary administration is further described in PCT application no.
PCT/US94/001875, which describes pulmonary delivery of chemically modified
proteins.

In certain embodiments, a pharmaceutical formulation can involve an effective
quantity of an antigen binding protein to PCSK9, with or without at least one additional
therapeutic agent, in a mixture with non-toxic excipients which are suitable for the
manufacture of tablets. In certain embodiments, by dissolving the tablets in sterile
water, or another appropriate vehicle, solutions can be prepared in unit-dose form. In
certain embodiments, suitable excipients include, but are not limited to, inert diluents,
such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium
phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents
such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical formulations will be evident to those skilled in the
art, including formulations involving antigen binding proteins to PCSK9, with or
without at least one additional therapeutic agent(s), in sustained- or controlled-delivery
formulations. In certain embodiments, techniques for formulating a variety of other
sustained- or controlled-delivery means, such as liposome carriers, bio-erodible
microparticles or porous beads and depot injections, are also known to those skilled in
the art. See for example, PCT Application No. PCT/US93/00829 which describes the
controlled release of porous polymeric microparticles for the delivery of

The pharmaceutical formulation to be used for in vivo administration typically is sterile. In certain embodiments, this can be accomplished by filtration through sterile filtration membranes. In certain embodiments, where the formulation is lyophilized, sterilization using this method can be conducted either prior to or following lyophilization and reconstitution. In certain embodiments, the formulation for parenteral administration can be stored in lyophilized form or in a solution. In certain embodiments, parenteral formulations generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

In certain embodiments, once the pharmaceutical formulation has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. In certain embodiments, such formulations can be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

In certain embodiments, once the pharmaceutical formulation has been formulated, it can be stored in pre-filled syringes as a solution or suspension in a ready-to-use form.

In certain embodiments, kits are provided for producing a single-dose administration unit. In certain embodiments, the kit can contain both a first container having a dried protein and a second container having an aqueous formulation. In
certain embodiments, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are included.

In certain embodiments, the effective amount of a pharmaceutical formulation comprising an antigen binding protein to PCSK9, with or without at least one additional therapeutic agent, to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment, according to certain embodiments, will thus vary depending, in part, upon the molecule delivered, the indication for which an antigen binding protein to PCSK9, with or without at least one additional therapeutic agent, is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In certain embodiments, the clinician can titrate the dosage and modify the route of administration to obtain the optimal therapeutic effect.

In certain embodiments, the formulation can be administered locally via implantation of a membrane, sponge or another appropriate material onto which the desired molecule has been absorbed or encapsulated. In certain embodiments, where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired molecule can be via diffusion, timed-release bolus, or continuous administration.

**Dosage and Dosing Regimens**

Any of the antigen binding proteins to PCSK9 comprising: one or more heavy chain complementary determining regions (CDRHs) and one or more light chain complementary determining regions (CDRLs) depicted in Table 2 and FIGS. 2 and/or 3 and FIGS. 31A and 31B can be administered to a patient diagnosed with homozygous familial hypercholesterolemia according to the methods of the present invention. In certain other embodiments, antigen binding protein to PCSK9 comprising: a light chain variable region that comprises an amino acid sequence that is at least 90% identical the antigen binding proteins to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and FIGs 31A and 31B, and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of any of the antigen binding proteins
to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and FIGs 31A and 31B can be
administered to a patient diagnosed with homozygous familial hypercholesterolemia
according to the methods of the present invention. In still other embodiments, any of
the antigen binding proteins to PCSK9 depicted in Table 2 and FIGs. 2 and/or 3 and/or
13 and/or FIG.31A and 31B can be administered to a patient diagnosed with
homozygous familial hypercholesterolemia according to the methods of the present
invention. In certain other embodiments, other antigen binding proteins to PCSK9;
namely an antibody comprised of a light chain variable domain, SEQ ID NO:588 and a
heavy chain variable domain, SEQ ID NO:589 can be administered to a patient
diagnosed with homozygous familial hypercholesterolemia. In some embodiments any
one of 21B12, 26H5, 31H4, 8A3, 11F1 or 8A1 can be administered to a patient
diagnosed with homozygous familial hypercholesterolemia.

The amount of an antigen binding protein to PCSK9 (e.g., an anti-PCS K9
antibody) administered to a patient according to the methods of the present invention is,
generally, a therapeutically effective amount. The amount of ABP may be expressed in
terms of milligrams of antibody (i.e., mg) or milligrams of antibody per kilogram of
patient body weight (i.e., mg/kg). In certain embodiments, a typical dosage of a
PCSK9 antigen binding protein can range from about 0.1 μg/kg to up to about 100
mg/kg or more of antigen binding protein to PCSK9. In certain embodiments, the
dosage can range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100
mg/kg; or 5 μg/kg up to about 100 mg/kg of antigen binding protein to PCSK9; or 1
mg/kg to about 50 mg/kg of antigen binding protein to PCSK9; or 2 mg/kg to about 20
mg/kg of antigen binding protein to PCSK9; or 2 mg/kg to about 10 mg/kg of antigen
binding protein to PCSK9.

In certain embodiments, the amount (or dose) of antigen binding protein to
PCSK9 can range from at least about 120 mg to about 3000 mg, of about 140 mg to
about 2800 mg, of about 140 mg to about 2500 mg, of about 140 mg to about 2000 mg,
of about 140 mg to about 1800 mg, of about 140 mg to about 1400 mg, of about 120
mg to about 1200 mg, of about 120 mg to about 1000 mg, of about 120 mg to about
700 mg, of about 140 mg to about 700 mg, of about 140 mg to about 600 mg, of about
140 mg to about 450 mg, of about 120 mg to about 450 mg, of about 120 mg to about
450 mg, of about 140 mg to about 450 mg, of about 210 mg to about 450 mg, or of about 280 mg to about 450 mg, of about 210 mg to about 420 mg, of about 280 mg to about 420 mg, of about 420 mg to about 3000 mg, of about 700 mg to about 3000 mg, of about 1000 mg to about 3000 mg, of about 1200 to about 3000 mg, of about 1400 mg to about 3000 mg, of about 1800 mg to about 3000 mg, of about 2000 mg to about 3000 mg, of about 2400 mg to about 3000 mg, or about 2800 mg to about 3000 mg. In some embodiments of this aspect, the anti-PCSK9 antibody is administered to a patient at a dose of about 35 mg, of about 45 mg, of about 70 mg, of about 105 mg, of about 120 mg of about 140 mg, of about 150 mg, of about 160 mg, of about 170 mg, of about 180 mg, of about 190 mg, of about 200 mg, of about 210 mg, of about 280 mg, of about 360 mg, of about 420 mg, of about 450 mg, of about 600 mg, of about 700 mg, of about 1200 mg, of about 1400 mg, of about 1800 mg, of about 2000 mg, of about 2500 mg, of about 2800 mg, or about 3000 mg.

In certain embodiments, the frequency of dosing will take into account the pharmacokinetic parameters of an antigen binding protein to PCSK9 and/or any additional therapeutic agents in the formulation used. In certain embodiments, a clinician will administer the formulation until a dosage is reached that achieves the desired effect. In certain embodiments, the formulation can therefore be administered as a single dose, or as two, three, four or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. The formulation can also be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, pen delivery devices, as well as autoinjector delivery devices, have applications in delivering a pharmaceutical formulation of the present invention.

Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. In certain embodiments, appropriate dosages can be ascertained through use of appropriate dose-response data. In some embodiments, the amount and frequency of administration can take into account the desired cholesterol level (serum and/or total) to be obtained and the subject's present cholesterol level, LDL level, and/or LDLR levels, all of which can be obtained by methods that are well known to those of skill in the art.
In certain embodiments, a dose of at least about 120 mg; or up to about 140 mg; or up to about 210 mg; or up to about 280 mg; or up to about 350 mg, or up to about 420 mg, or up to about 450 mg of an antigen binding protein to PCSK9 is administered once a week (QW) to a patient in need thereof.

In some other embodiments, a dose of at least about 120 mg, or up to about 140 mg; or up to about 150 mg, or up to about 210 mg, or up to about 280 mg; or up to about 350 mg, or up to about 420 mg; or up to about 450 mg of an antigen binding protein to PCSK9 is administered once every other week, (or every two weeks)(Q2W), to a patient in need thereof.

In certain other embodiments, a dose of at least about 250 mg; or up to about 280 mg; or up to about 300 mg; or up to about 350 mg; or up to about 400 mg; or up to about 420 mg; or up to about 450 mg; or up to about 600 mg; or up to about 700 mg; or up to about 1000 mg; or up to about 2000 mg; or up to about 3000 mg every four weeks of a an antigen binding protein to PCSK9 is administered once every four weeks, (or once a month)(Q4W), to a patient in need thereof.

In certain other embodiments, a dose of at least about 400 mg; or up to about 420 mg; or up to about 450 mg; or up to about 600 mg; or up to about 700 mg; or up to about 1000 mg; or up to about 2000 mg; or up to about 3000 mg every other month of a an antigen binding protein to PCSK9 is administered once every 8 weeks, (or once every other month), to a patient in need thereof.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 10%, as compared to a predose serum LDL cholesterol level. In some embodiments, the serum LDL cholesterol level is reduced by at least about 15%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 20%.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 25%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 30%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 40%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 50%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 55%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%. In some embodiments, the serum LDL cholesterol
level is reduced by at least about 65%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 10%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 15%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 20%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 25%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 30%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.
In some embodiments, the serum LDL cholesterol level is reduced by at least about 35%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 40%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 45%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 50%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 55%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.
In some embodiments, the serum LDL cholesterol level is reduced by at least about 65%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.

In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%, as compared to a predose serum LDL cholesterol level, and the reduction is sustained for a period of at least about 3 days, at least about 5 days, at least about 7 days, at least about 10 days, at least about 14 days, at least about 21 days, at least about 25 days, at least about 28 days, or at least about 31 days relative to a predose level.
Certain Therapeutic Applications

As will be appreciated by one of skill in the art, the methods provided herein are for treatment of patients diagnosed with homozygous familial hypercholesterolemia using antigen binding proteins, including antibodies, against proprotein convertase

subtilisin/kexin type 9 (PCSK9).

In one aspect, an antigen binding protein to PCSK9 is used to modulate serum LDL cholesterol levels in a patient diagnosed with homozygous familial hypercholesterolemia. In some embodiments, the antigen binding protein to PCSK9 is used to decrease the amount of serum LDL cholesterol from an abnormally high level or even a normal level. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 10% as compared to a predose level. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 15%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 20%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 25%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 30%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 35%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 40%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 45%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 50%. In certain embodiments, the serum LDL cholesterol level is reduced by at least about 55%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 60%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 65%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 70%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 75%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 80%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 85%. In some embodiments, the serum LDL cholesterol level is reduced by at least about 90%.

In one aspect, an antigen binding protein to PCSK9 is used to modulate serum PCSK9 values in a patient diagnosed with homozygous familial hypercholesterolemia.
In certain embodiments, the antigen binding protein to PCSK9 is neutralizing. In some embodiments, the antigen binding protein to PCSK9 is used to decrease PCSK9 values from an abnormally high level or even a normal level. In some embodiments, the serum PCSK9 value is reduced by at least about 20% as compared to a predose level. In some embodiments, the serum PCSK9 value is reduced by at least about 25%. In some embodiments, the serum PCSK9 value is reduced by at least about 30%. In some embodiments, the serum PCSK9 value is reduced by at least about 35%. In some embodiments, the serum PCSK9 value is reduced by at least about 40%. In some embodiments, the serum PCSK9 value is reduced by at least about 45%. In some embodiments, the serum PCSK9 value is reduced by at least about 50%. In some embodiments, the serum PCSK9 value is reduced by at least about 55%. In some embodiments, the serum PCSK9 value is reduced by at least about 60%. In some embodiments, the serum PCSK9 value is reduced by at least about 65%. In some embodiments, the serum PCSK9 value is reduced by at least about 70%. In some embodiments, the serum PCSK9 value is reduced by at least about 75%. In some embodiments, the serum PCSK9 value is reduced by at least about 80%. In some embodiments, the serum PCSK9 value is reduced by at least about 85%. In some embodiments, the serum PCSK9 value is reduced by at least about 90%.

In one aspect, an antigen binding protein to PCSK9 is used to modulate total cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia. In certain embodiments, the antigen binding protein to PCSK9 is neutralizing. In some embodiments, the antigen binding protein to PCSK9 is used to decrease the amount of total cholesterol from an abnormally high level or even a normal level. In some embodiments, the total cholesterol level is reduced by at least about 20% as compared to a predose level. In some embodiments, the total cholesterol level is reduced by at least about 25%. In some embodiments, the total cholesterol level is reduced by at least about 30%. In some embodiments, the total cholesterol level is reduced by at least about 35%. In some embodiments, the total cholesterol level is reduced by at least about 40%. In some embodiments, the total cholesterol level is reduced by at least about 45%. In some embodiments, the total cholesterol level is reduced by at least about 50%. In some embodiments, the total cholesterol
level is reduced by at least about 55%. In some embodiments, the total cholesterol level is reduced by at least about 60%.

In one aspect, an antigen binding protein to PCSK9 is used to modulate the non-HDL cholesterol level in a patient diagnosed with homozygous familial hypercholesterolemia. In certain embodiments, the antigen binding protein to PCSK9 is neutralizing. In some embodiments, the antigen binding protein to PCSK9 is used to decrease the non-HDL cholesterol from an abnormally high level or even a normal level. In some embodiments, the non-HDL cholesterol level is reduced by at least about 30%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 35%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 40%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 50%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 55%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 60%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 65%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 70%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 75%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 80%. In some embodiments, the non-HDL cholesterol level is reduced by at least about 85%.

In one aspect, an antigen binding protein to PCSK9 is used to modulate the ApoB levels in a patient diagnosed with homozygous familial hypercholesterolemia. In certain embodiments, the antigen binding protein to PCSK9 is neutralizing. In some embodiments, the antigen binding protein to PCSK9 is used to decrease the amount of ApoB from an abnormally high level or even a normal level. In some embodiments, the ApoB level is reduced by at least about 10% as compared to a predose level. In some embodiments, the ApoB level is reduced by at least about 15%. In some embodiments, the ApoB level is reduced by at least about 20%. In some embodiments, the ApoB level is reduced by at least about 25%. In some embodiments, the ApoB level is reduced by at least about 30%. In some embodiments, the ApoB level is reduced by at least about 35%. In some embodiments, the ApoB level is reduced by at least about 40%. In some embodiments, the ApoB level is reduced by at least about 45%. In some embodiments, the ApoB level is reduced by at least about 50%. In some embodiments, the ApoB level is reduced by at least about 55%. In some embodiments, the ApoB level is reduced by at least about 60%. In some embodiments, the ApoB level is reduced by at least about 65%. In some embodiments, the ApoB level is reduced by at least about 70%. In some embodiments, the ApoB level is reduced by at least about 75%. In some embodiments, the ApoB level is reduced by at least about 80%. In some embodiments, the ApoB level is reduced by at least about 85%.
least about 45%. In some embodiments, the ApoB level is reduced by at least about 50%. In some embodiments, the ApoB level is reduced by at least about 55%. In some embodiments, the ApoB level is reduced by at least about 60%. In some embodiments, the ApoB level is reduced by at least about 65%. In some embodiments, the ApoB level is reduced by at least about 70%. In some embodiments, the ApoB level is reduced by at least about 75%

In one aspect, an antigen binding protein to PCSK9 is used to modulate the Lp(a) levels in a patient diagnosed with homozygous familial hypercholesterolemia. In certain embodiments, the antigen binding protein to PCSK9 is neutralizing. In some embodiments, the antigen binding protein to PCSK9 is used to decrease the amount of Lp(a) from an abnormally high level or even a normal level. In some embodiments, the Lp(a) level is reduced by at least about 10% as compared to a predose level. In some embodiments, the Lp(a) level is reduced by at least about 15%. In some embodiments, the Lp(a) level is reduced by at least about 20%. In some embodiments, the Lp(a) level is reduced by at least about 25%. In some embodiments, the Lp(a) level is reduced by at least about 30%. In some embodiments, the Lp(a) level is reduced by at least about 35%. In some embodiments, the Lp(a) level is reduced by at least about 40%. In some embodiments, the Lp(a) level is reduced by at least about 45%. In some embodiments, the Lp(a) level is reduced by at least about 50%. In some embodiments, the Lp(a) level is reduced by at least about 55%. In some embodiments, the Lp(a) level is reduced by at least about 60%. In some embodiments, the Lp(a) level is reduced by at least about 65%

Combination Therapies

In certain embodiments, methods are provided of treating homozygous familial hypercholesterolemia, comprising administering a therapeutically effective amount of one or more antigen binding proteins to PCSK9 and another therapeutic agent. In certain embodiments, an antigen binding protein to PCSK9 is administered prior to the administration of at least one other therapeutic agent. In certain embodiments, an antigen binding protein to PCSK9 is administered concurrent with the administration of at least one other therapeutic agent. In certain embodiments,
an antigen binding protein to PCSK9 is administered subsequent to the administration of at least one other therapeutic agent.

Therapeutic agents (apart from the antigen binding protein), include, but are not limited to, at least one other cholesterol-lowering (serum and/or total body cholesterol) agent. In some embodiments, the agent increases the expression of LDLR, have been observed to increase serum HDL levels, lower LDL levels or lower triglyceride levels. Exemplary agents include, but are not limited to, statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin), Nicotinic acid (Niacin) (NIACOR, NIASPAN (slow release niacin), SLO-NIACIN (slow release niacin), CORDAPTIVE (laropiprant)), Fibric acid (LOPID (Gemfibrozil), TRICOR (fenofibrate)), Bile acid sequestrants (QUESTRAN (cholestyramine), colestevam (WELCHOL), COLESTID (colestipol)), Cholesterol absorption inhibitors (ZETIA (ezetimibe)), Combining nicotinic acid with statin (ADVICOR (LOVASTATIN and NIASPAN), Combining a statin with an absorption inhibitor (VYTORIN (ZOCOR and ZETIA) and/or lipid modifying agents. In some embodiments, the ABP is combined with PPAR gamma agonists, PPAR alpha/gamma agonists, squalene synthase inhibitors, CETP inhibitors, anti-hypertensives, anti-diabetic agents (such as sulphonyl ureas, insulin, GLP-1 analogs, DDPIV inhibitors, e.g., metaformin), ApoB modulators, such as mipomersan, MTP inhibitoris and/or arteriosclerosis obliterans treatments. In some embodiments, the ABP is combined with an agent that increases the level of LDLR protein in a subject, such as statins, certain cytokines like oncostatin M, estrogen, and/or certain herbal ingredients such as berberine. In some embodiments, the ABP is combined with an agent that increases serum cholesterol levels in a subject (such as certain anti-psycotic agents, certain HIV protease inhibitors, dietary factors such as high fructose, sucrose, cholesterol or certain fatty acids and certain nuclear receptor agonists and antagonists for RXR, RAR, LXR, FXR). In some embodiments, the ABP is combined with an agent that increases the level of PCSK9 in a subject, such as statins and/or insulin. The combination of the two can allow for the undesirable side-effects of other agents to be mitigated by the ABP.
EXAMPLES

The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention. It is noted that the ABP names are used generically herein (e.g., “21B12” can be used as outlined in Example 7, as in Table 2, or as in Figures 2 and/or 3 and/or 13A, 13C, 13F-13J), unless explicitly denoted otherwise.

EXAMPLE 1

Immunization and Titering

Generation of Anti-PCSK9 Antibodies and Hybridomas

Antibodies to the mature form of PCSK9 (depicted as the sequence in FIG. 1A, with the pro-domain underlined), were raised in XenoMouse® mice (Abgenix, Fremont, CA), which are mice containing human immunoglobulin genes. Two groups of XenoMouse® mice, group 1 and 2, were used to produce antibodies to PCSK9. Group 1 included mice of the XenoMouse® strain XMG2-KL, which produces fully human IgG2κ and IgG2α antibodies. Group 1 mice were immunized with human PCSK9. PCSK9 was prepared using standard recombinant techniques using the GenBank sequence as reference (NM_174936). Group 2 involved mice of the XenoMouse® strain XMG4-KL, which produce fully human IgG4κ and IgG4α antibodies. Group 2 mice were also immunized with human PCSK9.

The mice of both groups were injected with antigen eleven times, according to the schedule in Table 3. In the initial immunizations, each mouse was injected with a total of 10 µg of antigen delivered intraperitoneally into the abdomen. Subsequent boosts are 5µg doses and injection method is staggered between intraperitoneal injections into the abdomen and sub-cutaneous injections at the base of the tail. For intraperitoneal injections antigen is prepared as an emulsion with TiterMax® Gold (Sigma, Cat # T2684) and for subcutaneous injections antigen is mixed with Alum (aluminum phosphate) and CpG oligos. In injections 2 through 8 and 10, each mouse was injected with a total of 5 µg of antigen in the adjuvant alum gel. A final injection
of 5 μg of antigen per mouse is delivered in Phospho buffered saline and delivered into 2 sites 50% IP into the abdomen and 50% SQ at the base of tail. The immunization programs are summarized in Table 1.1, shown below.
<table>
<thead>
<tr>
<th>mouse strain</th>
<th>XMG2/kl</th>
<th>XMG4/kl</th>
</tr>
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<tbody>
<tr>
<td># of animals</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>immunogen</td>
<td>PCSK9-V5/His</td>
<td>PCSK9-V5/His</td>
</tr>
<tr>
<td>1st boost</td>
<td>IP injection, 10ug each</td>
<td>IP injection, 10ug each</td>
</tr>
<tr>
<td></td>
<td>Titermax Gold</td>
<td>Titermax Gold</td>
</tr>
<tr>
<td>2nd boost</td>
<td>tail injection, 5ug each</td>
<td>tail injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Alum/CpG ODN</td>
<td>Alum/CpG ODN</td>
</tr>
<tr>
<td>3rd boost</td>
<td>IP injection, 5ug each</td>
<td>IP injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Titermax Gold</td>
<td>Titermax Gold</td>
</tr>
<tr>
<td>4th boost</td>
<td>tail injection, 5ug each</td>
<td>tail injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Alum/CpG ODN</td>
<td>Alum/CpG ODN</td>
</tr>
<tr>
<td>5th boost</td>
<td>IP injection, 5ug each</td>
<td>IP injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Titermax Gold</td>
<td>Titermax Gold</td>
</tr>
<tr>
<td>6th boost</td>
<td>tail injection, 5ug each</td>
<td>tail injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Alum/CpG ODN</td>
<td>Alum/CpG ODN</td>
</tr>
<tr>
<td>7th boost</td>
<td>IP injection, 5ug each</td>
<td>IP injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Titermax Gold</td>
<td>Titermax Gold</td>
</tr>
<tr>
<td>8th boost</td>
<td>tail injection, 5ug each</td>
<td>tail injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Alum/CpG ODN</td>
<td>Alum/CpG ODN</td>
</tr>
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<td>IP injection, 5ug each</td>
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<td>9th boost</td>
<td>Titermax Gold</td>
<td>Titermax Gold</td>
</tr>
<tr>
<td>10th boost</td>
<td>tail injection, 5ug each</td>
<td>tail injection, 5ug each</td>
</tr>
<tr>
<td></td>
<td>Alum/CpG ODN</td>
<td>Alum/CpG ODN</td>
</tr>
<tr>
<td>11th boost</td>
<td>BIP, 5ug each</td>
<td>BIP, 5ug each</td>
</tr>
<tr>
<td>harvest</td>
<td>PBS</td>
<td>PBS</td>
</tr>
</tbody>
</table>

The protocol used to titer the XenoMouse animals was as follows: Costar 3368 medium binding plates were coated with neutravidin @ 8ug/ml (50ul/well) and incubated at 4°C in 1XPBS/0.05% azide overnight. They were washed using TiterTek 3-cycle wash with RO water. Plates were blocked using 250ul of 1XPBS/1%milk and
incubated for at least 30 minutes at RT. Block was washed off using TiterTek 3-cycle wash with RO water. One then captured b-human PCSK9 @ 2μg/ml in 1XPBS/1%milk/10mM Ca²⁺ (assay diluent) 50μl/well and incubated for 1hr at RT. One then washed using TiterTek 3-cycle wash with RO water. For the primary antibody, sera were titrated 1:3 in duplicate from 1:100. This was done in assay diluent 50μl/well and incubated for 1hr at RT. One then washed using TiterTek 3-cycle wash with RO water. The secondary antibody was goat anti Human IgG Fc HRP @ 400 ng/ml in assay diluent at 50μl/well. This was incubated for 1hr at RT. This was then washed using TiterTek 3-cycle wash with RO water and patted dry on paper towels. For the substrate, one-step TMB solution (Neogen, Lexington, Kentucky) was used (50μl/well) and it was allowed to develop for 30 min at RT.

The protocols followed in the ELISA assays were as follows: For samples comprising b-PCSK9 with no V5His tag the following protocol was employed: Costar 3368 medium binding plates (Corning Life Sciences) were employed. The plates were coated with neutravidin at 8 μg/ml in 1XPBS/0.05%Azide, (50 μl/well). The plates were incubated at 4°C overnight. The plates were then washed using a TiterTek M384 plate washer (TiterTek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 250 μl of 1XPBS/1% milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the M384 plate washer. A 3-cycle wash was performed. The capture was b-hu PCSK9, without a V5 tag, and was added at 2 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ (40 μl/well). The plates were then incubated for 1 hour at room temperature. A 3-cycle wash was performed. Sera were titrated 1:3 in duplicate from 1:100, and row H was blank for sera. The titration was done in assay diluent, at a volume of 50 μl/well. The plates were incubated for 1 hour at room temperature. Next, a 3-cycle wash was performed. Goat anti Human IgG Fc HRP at 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca²⁺ (50 μl/well) was added to the plate and was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. The plates were then patted dry with paper towel. Finally, 1 step TMB (Neogen, Lexington, Kentucky) (50 μl/well) was added to the plate and was quenched with 1N hydrochloric acid (50 μl/well) after 30 minutes at room temperature. OD's were read immediately at 450 nm using a TiterTek plate reader.
Positive controls to detect plate bound PCSK9 were soluble LDL receptor (R&D Systems, Cat #2148LD/CF) and a polyclonal rabbit anti-PCSK9 antibody (Caymen Chemical #10007185) titrated 1:3 in duplicate from 3 μg/ml in assay diluent. LDLR was detected with goat anti LDLR (R&D Systems, Cat #AF2148) and rabbit anti goat IgGFc HRP at a concentration of 400 ng/ml; the rabbit polyclonal was detected with goat anti-rabbit IgG Fc at a concentration of 400 ng/ml in assay diluent. Negative control was naive XMG2-KL and XMG4-KL sera titrated 1:3 in duplicate from 1:100 in assay diluent.

For samples comprising b-PCSK9 with a V5His tag the following protocol was employed: Costar 3368 medium binding plates (Corning Life Sciences) were employed. The plates were coated with neutravidin at 8 μg/ml in 1XPBS/0.05%Azide, (50 μl/well). The plates were incubated at 4°C overnight. The plates were then washed using a Titertek M384 plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 250 μl of 1XPBS/1% milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the M384 plate washer. A 3-cycle wash was performed. The capture was b-hu PCSK9, with a V5 tag, and was added at 2 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ (40 μl/well). The plates were then incubated for 1 hour at room temperature. A 3-cycle wash was performed. Sera were titrated 1:3 in duplicate from 1:100, and row H was blank for sera. The titration was done in assay diluent, at a volume of 50 μl/well. The plates were incubated for 1 hour at room temperature. Next, the plates were washed using the M384 plate washer operated using a 3-cycle wash. Goat anti Human IgG Fc HRP at 400 ng/ml in 1XPBS/1%milk/10mM Ca²⁺ was added at 50 μl/well to the plate and the plate was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. The plates were then patted dry with paper towel. Finally, 1 step TMB (Neogen, Lexington, Kentucky) (50 μl/well) was added to the plate and the plate was quenched with 1N hydrochloric acid (50 μl/well) after 30 minutes at room temperature. OD's were read immediately at 450 nm using a Titertek plate reader.

Positive control was LDLR, rabbit anti-PCSK9 titrated 1:3 in duplicate from 3 μg/ml in assay diluent. LDLR detect with goat anti-LDLR (R&D Systems, Cat #AF2148) and rabbit anti-goat IgG Fc HRP at a concentration of 400 ng/ml; rabbit poly
detected with goat anti-rabbit IgG Fc at a concentration of 400 ng/ml in assay diluent. Human anti-His 1.2.3 and anti-V5 1.7.1 titrated 1:3 in duplicate from 1 μg/ml in assay diluent; both detected with goat anti-human IgG Fc HRP at a concentration of 400 ng/ml in assay diluent. Negative control was naive XMG2-KL and XMG4-KL sera titrated 1:3 in duplicate from 1:100 in assay diluent.

Titers of the antibody against human PCSK9 were tested by ELISA assay for mice immunized with soluble antigen as described. Table 4 summarizes the ELISA data and indicates that there were some mice which appeared to be specific for PCSK9. See, e.g., Table 4. Therefore, at the end of the immunization program, 10 mice (in bold in Table 1.2) were selected for harvest, and splenocytes and lymphocytes were isolated from the spleens and lymph nodes respectively, as described herein.

**TABLE 1.2**

Summary of ELISA Results

<table>
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<th>Animal ID</th>
<th>Titer</th>
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<tbody>
<tr>
<td></td>
<td>b-hu PCSK9 (V5His) @ 2μg/ml</td>
<td>b-hu PCSK9 @ 2μg/ml</td>
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<tr>
<td><strong>Group 1 - IgG2k/l</strong></td>
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<td>P175807</td>
<td>&gt;72900 @ OD 2.2</td>
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</table>
EXAMPLE 2

Recovery of Lymphocytes, B-cell Isolations, Fusions
and Generation of Hybridomas

This example outlines how the immune cells were recovered and the hybridomas were generated. Selected immunized mice were sacrificed by cervical dislocation and the draining lymph nodes were harvested and pooled from each cohort. The B cells were dissociated from lymphoid tissue by grinding in DMEM to release the cells from the tissues, and the cells were suspended in DMEM. The cells were counted, and 0.9 ml DMEM per 100 million lymphocytes was added to the cell pellet to resuspend the cells gently but completely.

Lymphocytes were mixed with nonsecretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat.# CRL 1580 (Kearney et al., (1979) J. Immunol. 123, 1548-1550) at a ratio of 1:4. The cell mixture was gently pelleted by centrifugation at 400 x g 4 min. After decanting of the supernatant, the cells were gently mixed using a 1 ml pipette. Preheated PEG/DMSO solution from Sigma (cat# P7306) (1 ml per million of B-cells) was slowly added with gentle agitation over 1 min followed by 1 min of mixing. Preheated IDMEM (2 ml per million of B cells) (DMEM without glutamine, L-glutamine, pen/strep, MEM non-essential amino acids (all from Invitrogen), was then added over 2 minutes with gentle agitation. Finally preheated IDMEM (8 ml per 10⁶ B-cells) was added over 3 minutes.

The fused cells were spun down 400 x g 6 min and resuspended in 20 ml selection media (DMEM (Invitrogen), 15 % FBS (HyClone), supplemented with L-glutamine, pen/strep, MEM Non-essential amino acids, Sodium Pyruvate, 2-Mercaptoethanol (all from Invitrogen), HA-Azaserine Hypoxanthine and OPI (oxaloacetate, pyruvate, bovine insulin) (both from Sigma) and IL-6 (Boehringer Mannheim)) per million B-cells. Cells were incubated for 20-30 min at 37C and then
resuspended in 200 ml selection media and cultured for 3-4 days in T175 flask prior to 96 well plating. Thus, hybridomas that produced antigen binding proteins to PCSK9 were produced.

EXAMPLE 3
Selection of PCSK9 Antibodies

The present example outlines how the various PCSK9 antigen binding proteins were characterized and selected. The binding of secreted antibodies (produced from the hybridomas produced in Examples 1 and 2) to PCSK9 was assessed. Selection of antibodies was based on binding data and inhibition of PCSK9 binding to LDLR and affinity. Binding to soluble PCSK9 was analyzed by ELISA, as described below. BIAcore® (surface plasmon resonance) was used to quantify binding affinity.

Primary Screen

A primary screen for antibodies which bind to wild-type PCSK9 was performed. The primary screen was performed on two harvests. The primary screen comprised an ELISA assay and was performed using the following protocol:

Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed. The plates were coated with neutravidin at a concentration of 4 μg/ml in 1XPBS/0.05%Azide, at a volume of 40 μl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1XPBS/1%milk and incubated approximately 30 minutes at room temperature. The plates were then washed. Again, a 3-cycle wash was performed. The capture sample was biotinylated-PCSK9, without a V5 tag, and was added at 0.9 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ at a volume of 40 μl/well. The plates were then incubated for 1 hour at room temperature. Next, the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 10 μl of supernatant was transferred into 40 μl of 1XPBS/1%milk/10mM Ca²⁺ and incubated 1.5 hours at room temperature. Again the plates were washed using the Titertek plate washer operated using a 3-cycle wash.
40 µl/well of Goat anti-Human IgG Fc POD at a concentration of 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca\(^{2+}\) was added to the plate and was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. Finally, 40 µl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and quenching with 40 µl/well of 1N hydrochloric acid was performed after 30 minutes at room temperature. OD’s were read immediately at 450 nm using a Titertek plate reader.

The primary screen resulted in a total of 3104 antigen specific hybridomas being identified from the two harvests. Based on highest ELISA OD, 1500 hybridomas per harvest were advanced for a total of 3000 positives.

Confirmatory Screen

The 3000 positives were then rescreened for binding to wild-type PCSK9 to confirm stable hybridomas were established. The screen was performed as follows: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed. The plates were coated with neutravidin at 3 µg/ml in 1XPBS/0.05%Azide at a volume of 40 µl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 µl of 1XPBS/1%milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the M384 plate washer. A 3-cycle wash was performed. The capture sample was b-PCSK9, without a V5 tag, and was added at 0.9 µg/ml in 1XPBS/1%milk/10mM Ca\(^{2+}\) at a volume of 40 µl/well. The plates were then incubated for 1 hour at room temperature. Next, the plates were washed using a 3-cycle wash. 10 µl of supernatant was transferred into 40 µl of 1XPBS/1%milk/10mM Ca\(^{2+}\) and incubated 1.5 hours at room temperature. Again the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 40 µl/well of Goat anti-Human IgG Fc POD at a concentration of 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca\(^{2+}\) was added to the plate, and the plate was incubated 1 hour at room temperature. The plates were washed once again, using the Titertek plate washer operated using a 3-cycle wash. Finally, 40
μl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and was quenched with 40 μl/well of 1N hydrochloric acid after 30 minutes at room temperature. OD’s were read immediately at 450 nm using a Titertek plate reader. A total of 2441 positives repeated in the second screen. These antibodies were then used in the subsequent screenings.

Mouse Cross-reactivity Screen

The panel of hybridomas was then screened for cross-reactivity to mouse PCSK9 to make certain that the antibodies could bind to both human and mouse PCSK9. The following protocol was employed in the cross-reactivity screen: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed. The plates were coated with neutravidin at 3 μg/ml in 1XPBS/0.05%Azide at a volume of 40 μl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1XPBS/1%milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the Titertek plate washer. A 3-cycle wash was performed. The capture sample was biotinylated-mouse PCSK9, and was added at 1 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ at a volume of 40 μl/well. The plates were then incubated for 1 hour at room temperature. Next, the plates were washed using the Titertek plate washer operated using a 3-cycle wash. 50 μl of supernatant was transferred to the plates and incubated 1 hour at room temperature. Again the plates were washed using a 3-cycle wash. 40 μl/well of Goat anti-Human IgG Fc POD at a concentration of 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca²⁺ was added to the plate and the plate was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. Finally, 40 μl/well One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and was quenched with 40 μl/well of 1N hydrochloric acid after 30 minutes at room temperature. OD’s were read immediately at 450 nm using a Titertek plate reader. 579 antibodies were observed to cross-react with mouse PCSK9. These antibodies were then used in the subsequent screenings.
D374Y Mutant Binding Screen

The D374Y mutation in PCSK9 has been documented in the human population (e.g., Timms KM et al, “A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree”, Hum. Genet. 114: 349-353, 2004). In order to determine if the antibodies were specific for the wild type or also bound to the D374Y form of PCSK9, the samples were then screened for binding to the mutant PCSK9 sequence comprising the mutation D374Y. The protocol for the screen was as follows: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed in the screen. The plates were coated with neutravidin at 4 μg/ml in 1XPBS/0.05% Azide at a volume of 40 μl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1XPBS/1%milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the Titertek plate washer. A 3-cycle wash was performed. The plates were coated with biotinylated human PCSK9 D374Y at a concentration of 1 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ and incubated for 1 hour at room temperature. The plates were then washed using a Titertek plate washer. A 3-cycle wash was performed. Late exhaust hybridoma culture supernatant was diluted 1:5 in PBS/milk/Ca²⁺ (10 ml plus 40 ml) and incubated for 1 hour at room temperature. Next, 40 μl/well of rabbit anti-human PCSK9 (Cayman Chemical) and human anti-His 1.2.3 1:2 at 1μg/ml in 1XPBS/1%milk/10mM Ca²⁺ was titrated onto the plates, which were then incubated for 1 hour at room temperature. The plates were then washed using a Titertek plate washer. A 3-cycle wash was performed. 40 μl/well of Goat anti-Human IgG Fc HRP at a concentration of 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca²⁺ was added to the plate and the plate was incubated 1 hour at room temperature. 40 μl/well of Goat anti-rabbit IgG Fc HRP at a concentration of 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca²⁺ was added to the plate and the plate was incubated 1 hour at room temperature. The plates were then washed using a Titertek plate washer. A 3-cycle wash was performed. Finally, 40 μl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and was quenched with 40
μl/well of 1N hydrochloric acid after 30 minutes at room temperature. OD’s were read immediately at 450 nm using a TiterTek plate reader. Over 96% of the positive hits on the wild-type PCSK9 also bound mutant PCSK9.

Large Scale Receptor Ligand Blocking Screen

To screen for the antibodies that block PCSK9 binding to LDLR an assay was developed using the D374Y PCSK9 mutant. The mutant was used for this assay because it has a higher binding affinity to LDLR allowing a more sensitive receptor ligand blocking assay to be developed. The following protocol was employed in the receptor ligand blocking screen: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed in the screen. The plates were coated with goat anti-LDLR (R&D Cat #AF2148) at 2 μg/ml in 1XPBS/0.05%Azide at a volume of 40 μl/well. The plates were incubated at 4°C overnight. The plates were then washed using a TiterTek plate washer (TiterTek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1XPBS/1% milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the TiterTek plate washer. A 3-cycle wash was performed. The capture sample was LDLR (R&D, Cat #2148LD/CF), and was added at 0.4 μg/ml in 1XPBS/1% milk/10mM Ca²⁺ at a volume of 40 μl/well. The plates were then incubated for 1 hour and 10 minutes at room temperature. Contemporaneously, 20 ng/ml of biotinylated human D374Y PCSK9 was incubated with 15 micro liters of hybridoma exhaust supernatant in Nunc polypropylene plates and the exhaust supernatant concentration was diluted 1:5. The plates were then pre-incubated for about 1 hour and 30 minutes at room temperature. Next, the plates were washed using the TiterTek plate washer operated using a 3-cycle wash. 50 μl/well of the pre-incubated mixture was transferred onto the LDLR coated ELISA plates and incubated for 1 hour at room temperature. To detect LDLR-bound b-PCSK9, 40 μl/well streptavidin HRP at 500 ng/ml in assay diluent was added to the plates. The plates were incubated for 1 hour at room temperature. The plates were again washed using a TiterTek plate washer. A 3-cycle wash was performed. Finally, 40 μl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and was quenched with 40 μl/well of 1N hydrochloric acid after 30 minutes at room
temperature. OD’s were read immediately at 450 nm using a Titertek plate reader. The screen identified 384 antibodies that blocked the interaction between PCSK9 and the LDLR well, 100 antibodies blocked the interaction strongly (OD < 0.3). These antibodies inhibited the binding interaction of PCSK9 and LDLR greater than 90% (greater than 90% inhibition).

**Receptor Ligand Binding Assay on Blocker Subset**

The receptor ligand assay was then repeated using the mutant enzyme on the 384 member subset of neutralizers identified in the first large scale receptor ligand inhibition assay. The same protocol was employed in the screen of the 384 member blocker subset assay as was done in the large scale receptor ligand blocking screen. This repeat screen confirmed the initial screening data.

This screen of the 384 member subset identified 85 antibodies that blocked interaction between the PCSK9 mutant enzyme and the LDLR greater than 90%.

**Receptor Ligand Binding Assay of Blockers that Bind the Wild Type PCSK9 but not the D374Y Mutant**

In the initial panel of 3000 sups there were 86 antibodies shown to specifically bind to the wild-type PCSK9 and not to the huPCSK9(D374Y) mutant. These 86 sups were tested for the ability to block wild-type PCSK9 binding to the LDLR receptor. The following protocol was employed: Costar 3702 medium binding 384 well plates (Corning Life Sciences) were employed in the screen. The plates were coated with anti-His 1.2.3 at 10 μg/ml in 1XPBS/0.05% Azide at a volume of 40 μl/well. The plates were incubated at 4°C overnight. The plates were then washed using a Titertek plate washer (Titertek, Huntsville, AL). A 3-cycle wash was performed. The plates were blocked with 90 μl of 1XPBS/1%milk and incubated approximately 30 minutes at room temperature. The plates were then washed using the Titertek plate washer. A 3-cycle wash was performed. LDLR (R&D Systems, #2148LD/CF or R&D Systems, #2148LD) was added at 5 μg/ml in 1XPBS/1%milk/10mM Ca²⁺ at a volume of 40 μl/well. The plates were then incubated for 1 hour at room temperature. Next, the plates were washed using the Titertek plate washer operated using a 3-cycle wash.
Contemporaneously, biotinylated human wild-type PCSK9 was pre-incubated with hybridoma exhaust supernatant in Nunc polypropylene plates. 22 µl of hybridoma sup was transferred into 33ul of b-PCSK9 at a concentration of 583 ng/ml in 1XPBS/1%milk/10mMCa2+, giving a final b-PCSK9 concentration = 350 ng/ml and the exhaust supernatant at a final dilution of 1:2.5. The plates were pre-incubated for approximately 1 hour and 30 minutes at room temperature. 50 µl/well of the preincubated mixture was transferred onto LDLR captured ELISA plates and incubated for 1 hour at room temperature. The plates were then washed using the Titertek plate washer. A 3-cycle wash was performed. 40 µl/well streptavidin HRP at 500 ng/ml in assay diluent was added to the plates. The plates were incubated for 1 hour at room temperature. The plates were then washed using a Titertek plate washer. A 3-cycle wash was performed. Finally, 40 µl/well of One-step TMB (Neogen, Lexington, Kentucky) was added to the plate and was quenched with 40 µl/well of 1N hydrochloric acid after 30 minutes at room temperature. OD’s were read immediately at 450 nm using a Titertek plate reader.

**Screening Results**

Based on the results of the assays described, several hybridoma lines were identified as producing antibodies with desired interactions with PCSK9. Limiting dilution was used to isolate a manageable number of clones from each line. The clones were designated by hybridoma line number (e.g. 21B12) and clone number (e.g. 21B12.1). In general, no difference among the different clones of a particular line was detected by the functional assays described herein. In a few cases, clones were identified from a particular line that behaved differently in the functional assays, for example, 25A7.1 was found not to block PCSK9/LDLR but 25A7.3 (referred to herein as 25A7) was neutralizing. The isolated clones were each expanded in 50-100 ml of hybridoma media and allowed to grow to exhaustion, (i.e., less than about 10% cell viability). The concentration and potency of the antibodies to PCSK9 in the supernatants of those cultures were determined by ELISA and by in vitro functional testing, as described herein. As a result of the screening described herein, the
hybridomas with the highest titer of antibodies to PCSK9 were identified. The selected hybridomas are shown in FIGS 2A-3D and Table 2.

EXAMPLE 4.1

Production of Human 31H4 IgG4 Antibodies from Hybridomas

This example generally describes how one of the antigen binding proteins was produced from a hybridoma line. The production work used 50ml exhaust supernatant generation followed by protein A purification. Integra production was for scale up and was performed later. Hybridoma line 31H4 was grown in T75 flasks in 20 ml of media (Integra Media, Table 5). When the hybridoma was nearly confluent in the T75 flasks, it was transferred to an Integra flask (Integra Biosciences, Integra CL1000, cat# 90005).

The Integra flask is a cell culture flask that is divided by a membrane into two chambers, a small chamber and a large chamber. A volume of 20-30 ml hybridoma cells at a minimum cell density of 1x10^6 cells per ml from the 31H4 hybridoma line was placed into the small chamber of an Integra flask in Integra media (see Table 4.1 for components of Integra media). Integra media alone (1L) was placed in the large chambers of the Integra flasks. The membrane separating the two chambers is permeable to small molecular weight nutrients but is impermeable to hybridoma cells and to antibodies produced by those cells. Thus, the hybridoma cells and the antibodies produced by those hybridoma cells were retained in the small chamber.

After one week, media was removed from both chambers of the Integra flask and was replaced with fresh Integra media. The collected media from the small chambers was separately retained. After a second week of growth, the media from the small chamber was again collected. The collected media from week 1 from the hybridoma line was combined with the collected media from week 2 from the hybridoma line. The resulting collected media sample from the hybridoma line was spun to remove cells and debris (15 minutes at 3000rpm) and the resulting supernatant was filtered (0.22μm). Clarified conditioned media was loaded onto a Protein A-Sepharose column. Optionally, the media can be first concentrated and then loaded onto a Protein A Sepharose column. Non-specific bindings were removed by an
extensive PBS wash. Bound antibody proteins on the Protein A column were recovered by standard acidic antibody elution from Protein A columns (such as 50 mM Citrate, pH 3.0). Aggregated antibody proteins in the Protein A Sepharose pool were removed by size exclusion chromatography or binding ion exchange chromatography on anion exchanger resin such as Q Sepharose resin. The specific IEX conditions for the 31H4 proteins are Q-Sepharose HP at pH 7.8-8.0. Antibody was eluted with a NaCl gradient of 10 mM-500 mM in 25 column volumes.

**TABLE 4.1**
Composition of Media

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<tr>
<td>HSFM</td>
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**EXAMPLE 4.2**
Production of Recombinant 31H4 Human IgG2
Antibodies From Transfected Cells

The present example outlines how 31H4 IgG2 antibodies were produced from transfected cells. 293 cells for transient expression and CHO cells for stable expression were transfected with plasmids that encode 31H4 heavy and light chains. Conditioned media from transfected cells was recovered by removing cells and cell debris. Clarified conditioned media was loaded onto a Protein A-Sepharose column. Optionally, the media can first be concentrated and then loaded onto a Protein A Sepharose column. Non-specific bindings were removed by extensive PBS wash. Bound antibody proteins on the Protein A column were recovered by standard acidic antibody elution from Protein A columns (such as 50 mM citrate, pH 3.0). Aggregated antibody proteins in the Protein A Sepharose pool were removed by size exclusion chromatography or
binding ion exchange chromatography on anion exchanger resin such as Q Sepharose resin. The specific IEX conditions for the 31H4 proteins are Q-Sepharose HP at pH 7.8-8.0. The antibody was eluted with a NaCl gradient of 10 mM-500 mM in 25 column volumes.
EXAMPLE 5

Production of Human 21B12 IgG4 Antibodies from Hybridomas

The present example outlines how antibody 21B12 IgG4 was produced from hybridomas. Hybridoma line 21B12 was grown in T75 flasks in media (Integra Media, Table 5). When the hybridomas were nearly confluent in the T75 flasks, they were transferred to Integra flasks (Integra Biosciences, Integra CL1000, cat# 90 005).

The Integra flask is a cell culture flask that is divided by a membrane into two chambers, a small chamber and a large chamber. A volume of 20-30 ml hybridoma cells at a minimum cell density of 1x10^6 cells per ml from the 31H4 hybridoma line was placed into the small chamber of an Integra flask in Integra media (see Table 5 for components of Integra media). Integra media alone (1L) was placed in the large chambers of the Integra flasks. The membrane separating the two chambers is permeable to small molecular weight nutrients but is impermeable to hybridoma cells and to antibodies produced by those cells. Thus, the hybridoma cells and the antibodies produced by those hybridoma cells were retained in the small chamber.

After one week, media was removed from both chambers of the Integra flask and was replaced with fresh Integra media. The collected media from the small chambers was separately retained. After a second week of growth, the media from the small chamber was again collected. The collected media from week 1 from the hybridoma line was combined with the collected media from week 2 from the hybridoma line. The resulting collected media sample from the hybridoma line was spun to remove cells and debris (15 minutes at 3000 rpm) and the resulting supernatant was filtered (0.22 μm). Clarified conditioned media were loaded onto a Protein A Sepharose column. Optionally, the media are first concentrated and then loaded onto a Protein A Sepharose column. Non-specific bindings were removed by an extensive PBS wash. Bound antibody proteins on the Protein A column were recovered by standard acidic antibody elution from Protein A columns (such as 50 mM Citrate, pH 3.0). Aggregated antibody proteins in the Protein A Sepharose pool were removed by size exclusion chromatography or binding ion exchange chromatography on anion exchanger resin such as Q Sepharose resin. The specific IEX conditions for the 21B12 proteins are Q-
Sepharose HP at pH 7.8-8.0. The antibody was eluted with a NaCl gradient of 10 mM-500 mM in 25 column volumes.

EXAMPLE 6
Production of Human 21B12 IgG2 Antibodies

From Transfected Cells

The present example outlines how 21B12 IgG2 antibodies were produced from transfected cells. Cells (293 cells for transient expression and CHO cells for stable expression) were transfected with plasmids that encode 21B12 heavy and light chains. Conditioned media from hybridoma cells were recovered by removing cells and cell debris. Clarified conditioned media were loaded onto a Protein A-Sepharose column. Optionally, the media can first be concentrated and then loaded onto a Protein A Sepharose column. Non-specific bindings were removed by extensive PBS wash. Bound antibody proteins on the Protein A column were recovered by standard acidic antibody elution from Protein A columns (50 mM Citrate, pH 3.0). Aggregated antibody proteins in the Protein A Sepharose pool were removed by size exclusion chromatography or binding ion exchange chromatography on cation exchanger resin such as SP-Sepharose resin. The specific IEX conditions for the 21B12 proteins were SP-Sepharose HP at pH 5.2. Antibodies were eluted with 25 column volumes of buffer that contains a NaCl gradient of 10 mM-500 mM in 20 mM sodium acetate buffer.

EXAMPLE 7
Sequence Analysis of Antibody Heavy and Light Chains

The nucleic acid and amino acid sequences for the light and heavy chains of the above antibodies were then determined by Sanger (dideoxy) nucleotide sequencing. Amino acid sequences were then deduced for the nucleic acid sequences. The nucleic acid sequences for the variable domains are depicted in FIG.s 3E-3JJ and 3LL-BBB.

The cDNA sequences for the lambda light chain variable regions of 31H4, 21B12, and 16F12 were determined and are disclosed as SEQ ID NOs: 153, 95, and 105 respectively.
The cDNA sequences for the heavy chain variable regions of 31H4, 21B12, and 16F12 were determined and are disclosed as SEQ ID NOs: 152, 94, and 104 respectively.

The lambda light chain constant region (SEQ ID NO: 156), and the IgG2 and IgG4 heavy chain constant regions (SEQ ID NOs: 154 and 155) are shown in FIG. 3KK.

The polypeptide sequences predicted from each of those cDNA sequences were determined. The predicted polypeptide sequences for the lambda light chain variable regions of 31H4, 21B12, and 16F12 were predicted and are disclosed as SEQ ID NOs: 12, 23, and 35 respectively, the lambda light chain constant region (SEQ ID NO: 156), the heavy chain variable regions of 31H4, 21B12, and 16F12 were predicted and are disclosed as (SEQ. ID NOs. 67, 49, and 79 respectively. The IgG2 and IgG4 heavy chain constant regions (SEQ ID NOs: 154 and 155).

The FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 divisions are shown in FIG 2A-3D and FIG. 3CCC-3JJJ.

Based on the sequence data, the germline genes from which each heavy chain or light chain variable region was derived was determined. The identity of the germline genes are indicated next to the corresponding hybridoma line in FIGs. 2A-3D and FIG. 3CCC-3JJJ and each is represented by a unique SEQ ID NO. FIGs. 2A-3D and FIG. 3CCC-3JJJ also depict the determined amino acid sequences for additional antibodies that were characterized.

EXAMPLE 8
Production of Antibodies in E. coli

Some of the antibodies were also raised in E. coli and possess some minor amino acid differences from antibodies produced as in, for example, Examples 4-6. The first residue in the variable region was a glutamic acid instead of a glutamine for the heavy and light chains of 21B12 and for the light chain for 31H4. In addition to the differences in the sequence of variable region, there were also some differences in the constant region of the antibodies described by the coordinates (due to the fact that the antibody was raised in E. coli). FIG. 3LLL highlights (via underlining shading, or
bold) the differences between the constant regions of the 21B12, 31H4, and 31A4 Fabs (raised in E. coli) when compared to SEQ ID NOs: 156, and 155. For 21B12 31H4, and 31A4, the light chain constant sequence is similar to human lambda (SEQ ID NO: 156). The underlined glycine residue is an insertion between where the 21B12 and 31H4 variable sequences stop and the lambda sequence starts.

For both 21B12 and 31H4, the heavy chain constant is similar to human IgG4 (SEQ ID NO: 155). The highlighted differences in FIG. 3LLL are shown in Table 8.1:

<table>
<thead>
<tr>
<th>Crystal SEQ</th>
<th>ID NO: 155</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>C</td>
</tr>
<tr>
<td>K</td>
<td>R</td>
</tr>
<tr>
<td>G</td>
<td>E</td>
</tr>
<tr>
<td>G</td>
<td>S</td>
</tr>
<tr>
<td>Q</td>
<td>K</td>
</tr>
<tr>
<td>I</td>
<td>T</td>
</tr>
<tr>
<td>N</td>
<td>D</td>
</tr>
<tr>
<td>K</td>
<td>R</td>
</tr>
<tr>
<td>P</td>
<td>S</td>
</tr>
</tbody>
</table>

In regard to 31A4, while it also has the same distinctions noted above, there are three additional differences. As shown in FIG. 3LLL, there are two additional amino acids at the start, which comes from incomplete processing of the signal peptide in E. coli expression. In addition, there is one additional substitution in the 31A4 heavy chain constant region when compared to SEQ ID NO: 155, which is the adjustment of a L (in SEQ ID NO: 155) to a H. Finally, 31A4 does have a glutamine as the initial amino acid of the Fab, rather than the the adjustment to glutamic acid noted above for 21B12 and 31H4.

For all three antibodies, the end of the heavy chain (boxed in dark grey) differs as well, but the amino acids are not ordered in the structure so they do not appear in the coordinates. As will be appreciated by one of skill in the art, his-tags are not a required part of the ABP and should not be considered as part of the ABP's sequence, unless explicitly called out by reference to a specific SEQ ID NO that includes a histidine tag and a statement that the ABP sequence “includes the Histidine tag.”
EXAMPLE 9

Characterization of Binding of Antibodies to PCSK9

Having identified a number of antibodies that bind to PCSK9, several approaches were employed to quantify and further characterize the nature of the binding. In one aspect of the study, a Biacore affinity analysis was performed. In another aspect of the study a KinExA® affinity analysis was performed. The samples and buffers employed in these studies are presented in Table 9.1 below.

<table>
<thead>
<tr>
<th>sample</th>
<th>[sample] mg/ml</th>
<th>Buffer</th>
<th>[sample] μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPCSK9</td>
<td>1.26</td>
<td>PBS</td>
<td>16.6</td>
</tr>
<tr>
<td>mPCSK9-8xHIS</td>
<td>1.44</td>
<td>PBS</td>
<td>18.9</td>
</tr>
<tr>
<td>ePCSK9-V5-6xHIS</td>
<td>0.22</td>
<td>PBS</td>
<td>2.9</td>
</tr>
<tr>
<td>16F12, anti-PCSK9 huIgG4</td>
<td>4.6</td>
<td>20mM NaOAC, pH 5.2, 50mM NaCl</td>
<td>31.9</td>
</tr>
<tr>
<td>21B12, anti-PCSK9 huIgG4</td>
<td>3.84</td>
<td>10mM NaOAC, pH 5.2, 9% Sucrose</td>
<td>27.0</td>
</tr>
<tr>
<td>31H4, anti-PCSK9 huIgG4</td>
<td>3.3</td>
<td>10mM NaOAC, pH 5.2, 9% Sucrose</td>
<td>22.9</td>
</tr>
</tbody>
</table>

10 Biacore® Affinity Measurements

A Biacore® (surface plasmon resonance device, Biacore, Inc., Piscataway, NJ) affinity analysis of the 21B12 antibodies to PCSK9 described in this Example was performed according to the manufacturer’s instructions.

Briefly, the surface plasmon resonance experiments were performed using Biacore 2000 optical biosensors (Biacore, GE Healthcare, Piscataway, NJ). Each individual anti-PCSK9 antibody was immobilized to a research-grade CM5 biosensor chip by amine-coupling at levels that gave a maximum analyte binding response (Rmax) of no more than 200 resonance units (RU). The concentration of PCSK9 protein was varied at 2 fold intervals (the analyte) and was injected over the
immobilized antibody surface (at a flow rate of 100 μl/min for 1.5 minutes). Fresh HBS-P buffer (pH 7.4, 0.01 M Hepes, 0.15 M NaCl, 0.005% surfactant P-20, Biacore) supplemented with 0.01% BSA was used as binding buffer. Binding affinities of each anti-PCSK9 antibody were measured in separate experiments against each of the human, mouse, and cynomolgus monkey PCSK9 proteins at pH 7.4 (the concentrations used were 100, 50, 25, 12.5, 6.25, 3.125, and 0 nM).

In addition, the binding affinities of antibody to human PCSK9 were also measured at pH 6.0 with the pH 6.0 HBS-P buffer (pH 6.0, 0.01 M Hepes, 0.15 M NaCl, 0.005% surfactant P-20, Biacore) supplemented with 0.01% BSA. The binding signal obtained was proportional to the free PCSK9 in solution. The dissociation equilibrium constant (K_D) was obtained from nonlinear regression analysis of the competition curves using a dual-curve one-site homogeneous binding model (KinExA® software, Sapidyne Instruments Inc., Boise, ID) (n=1 for the 6.0 pH runs). Interestingly, the antibodies appeared to display a tighter binding affinity at the lower pH (where the Kd was 12.5, 7.3, and 29 pM for 31H4, 21B12, and 16F12 respectively).

Antibody binding kinetic parameters including k_a (association rate constant), k_d (dissociation rate constant), and K_D (dissociation equilibrium constant) were determined using the BIA evaluation 3.1 computer program (BIAcore, Inc. Piscataway, NJ). Lower dissociation equilibrium constants indicate greater affinity of the antibody for PCSK9. The K_D values determined by the BIAcore® affinity analysis are presented in Table 9.2, shown below.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>hPCSK9</th>
<th>CynoPCSK9</th>
<th>mPCSK9</th>
</tr>
</thead>
<tbody>
<tr>
<td>31H4</td>
<td>210 pM</td>
<td>190 pM</td>
<td>6 nM</td>
</tr>
<tr>
<td>21B12</td>
<td>190 pM</td>
<td>360 pM</td>
<td>460 nM</td>
</tr>
<tr>
<td>16F12</td>
<td>470 pM</td>
<td>870 pM</td>
<td>6.4 nM</td>
</tr>
</tbody>
</table>
Table 9.3 depicts the $k_{on}$ and $k_{off}$ rates.

<table>
<thead>
<tr>
<th></th>
<th>$k_{on}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_{off}$ (s$^{-1}$)</th>
<th>$K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>31H4.1, pH 7.4</td>
<td>2.45 e+5</td>
<td>5.348 e-5</td>
<td>210 pM</td>
</tr>
<tr>
<td>31H4.1, pH 6</td>
<td>5.536 e+6</td>
<td>6.936 e-5</td>
<td>12.5 pM</td>
</tr>
<tr>
<td>21B12.1, pH 7.4</td>
<td>3.4918 e+4</td>
<td>6.634 e-6</td>
<td>190 pM</td>
</tr>
<tr>
<td>21B12.1, pH 6</td>
<td>2.291 e+6</td>
<td>1.676 e-5</td>
<td>7.3 pM</td>
</tr>
<tr>
<td>16F12.1, pH 7.4</td>
<td>1.064 e+5</td>
<td>4.983 e-5</td>
<td>470 pM</td>
</tr>
<tr>
<td>16F12.1, pH 6</td>
<td>2.392 e+6</td>
<td>7.007 e-5</td>
<td>29 pM</td>
</tr>
</tbody>
</table>

5 KinExA® Affinity Measurements

A KinExA® (Sapidyne Instruments, Inc., Boise, ID) affinity analysis of 16F12 and 31H4 was performed according to the manufacturer’s instructions. Briefly, Reacti-Gel™ (6x) (Pierce) was pre-coated with one of human, V5-tagged cyno or His-tagged mouse PCSK9 proteins and blocked with BSA. 10 or 100 pM of antibody 31H4 and one of the PCSK9 proteins was then incubated with various concentrations (0.1 pM – 25 nM) of PCSK9 proteins at room temperature for 8 hours before being passed through the PCSK9-coated beads. The amount of the bead-bound 31H4 was quantified by fluorescently (Cy5) labeled goat anti-human IgG (H+L) antibody (Jackson Immuno Research). The binding signal is proportional to the concentration of free 31H4 at binding equilibrium. Equilibrium dissociation constant ($K_D$) were obtained from nonlinear regression of the two sets of competition curves using a one-site homogeneous binding model. The KinExA® Pro software was employed in the analysis. Binding curves generated in this analysis are presented as FIGs. 4A-4F.

Both the 16F12 and 31H4 antibodies showed similar affinity to human and cyno PCSK9, but approximately 10-250 fold lower affinity to mouse PCSK9. Of the two
antibodies tested using the KinExA® system, antibody 31H4 showed higher affinity to both human and cyno PCSK9 with 3 and 2 pM K_D, respectively. 16F12 showed slightly weaker affinity at 15pM K_D to human PCSK9 and 16 pM K_D to cyno PCSK9.

The results of the KinExA® affinity analysis are summarized in Table 9.4, shown below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>hPCSK</th>
<th>cPCSK</th>
<th>mPCSK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_D (pM)</td>
<td>95% CI</td>
<td>K_D (pM)</td>
</tr>
<tr>
<td>31H4.1</td>
<td>3</td>
<td>1-5</td>
<td>2</td>
</tr>
</tbody>
</table>

In addition, a SDS PAGE was run to check the quality and quantity of the samples and is shown in FIG. 5A. cPCSK9 showed around 50% less on the gel and also from the active binding concentration calculated from KinExA® assay. Therefore, the K_D of the mAbs to cPCSK9 was adjusted as 50% of the active cPCSK9 in the present.

A BIAcore solution equilibrium binding assay was used to measure the Kd values for ABP 21B12. 21B12.1 showed little signal using KinExA assay, therefore, biacore solution equilibrium assay was applied. Since no significant binding was observed on binding of antibodies to immobilized PCSK9 surface, 21B12 antibody was immobilized on the flow cell 4 of a CM5 chip using amine coupling with density around 7000 RU. Flow cell 3 was used as a background control. 0.3, 1, and 3 nM of human PCSK9 or cyno PCSK9 were mixed with a serial dilutions of 21B12.1 antibody samples (ranged from 0.001 ~ 25 nM) in PBS plus 0.1mg/ml BSA, 0.005% P20. Binding of the free PCSK9 in the mixed solutions were measured by injecting over the 21B12.1 antibody surface. 100% PCSK9 binding signal on 21B12.1 surface was determined in the absence of mAb in the solution. A decreased PCSK9 binding response with increasing concentrations of mAb indicated that PCSK9 binding to mAb in solution, which blocked PCSK9 from binding to the immobilized peptidobody surface. Plotting the PCSK9 binding signal versus mAb concentrations, K_D was calculated from three sets of curves (0.3, 1 and 3nM fixed PCSK9 concentration) using a one-site homogeneous binding model in KinExA Pro™ software. Although cPCSK9 has lower
protein concentration observed from KinExA assay and SDS-gel, its concentration was not adjusted here since the concentration of cPCSK9 was not used for calculation of $K_D$. The results are displayed in Table 9.5 below and in FIGs. 5B-5D. FIG. 5B depicts the results from the solution equilibrium assay at three different hPCSK9 concentrations for hPCSK9. FIG. 5C depicts a similar set of results for mPCSK9. FIG. 5D depicts the results from the above biacore capture assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>hPCSK9 $K_D$ (pM)</th>
<th>95% CI</th>
<th>cPCSK $K_D$ (pM)</th>
<th>95% CI</th>
<th>mPCSK $K_D$ (pM)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>21B12.1</td>
<td>15</td>
<td>9-23</td>
<td>11</td>
<td>7-16</td>
<td>17000</td>
<td>-</td>
</tr>
</tbody>
</table>

EXAMPLE 10
EFFICACY OF 31H4 AND 21B12 FOR BLOCKING D374Y PCSK9/LDLR BINDING

This example provides the IC50 values for two of the antibodies in blocking PCSK9 D374Y’s ability to bind to LDLR. Clear 384 well plates (Costar) were coated with 2 micrograms/ml of goat anti-LDL receptor antibody (R&D Systems) diluted in buffer A (100 mM sodium cacodylate, pH 7.4). Plates were washed thoroughly with buffer A and then blocked for 2 hours with buffer B (1% milk in buffer A). After washing, plates were incubated for 1.5 hours with 0.4 micrograms/ml of LDL receptor (R&D Systems) diluted in buffer C (buffer B supplemented with 10 mM CaCl2). Concurrent with this incubation, 20 ng/ml of biotinylated D374Y PCSK9 was incubated with various concentrations of the 31H4 IgG2, 31H4 IgG4, 21B12 IgG2 or 21B12 IgG4 antibody, which was diluted in buffer A, or buffer A alone (control). The LDL receptor containing plates were washed and the biotinylated D374Y PCSK9/antibody mixture was transferred to them and incubated for 1 hour at room temperature. Binding of the biotinylated D374Y to the LDL receptor was detected by incubation with streptavidin-HRP (Biosource) at 500 ng/ml in buffer C followed by TMB substrate (KPL). The signal was quenched with 1N HCl and the absorbance read at 450 nm.

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The results of this binding study are shown in FIGs. 6A-6D. Summarily, IC\textsubscript{50} values were determined for each antibody and found to be 199 pM for 31H4 IgG2 (FIG. 6A), 156 pM for 31H4 IgG4 (FIG. 6B), 170 pM for 21B12 IgG2 (FIG. 6C), and 169 pM for 21B12 IgG4 (FIG. 6D).

The antibodies also blocked the binding of wild-type PCSK9 to the LDLR in this assay.

EXAMPLE 11

Cell LDL Uptake Assay

This example demonstrates the ability of various antigen binding proteins to reduce LDL uptake by cells. Human HepG2 cells were seeded in black, clear bottom 96-well plates (Costar) at a concentration of 5x10\textsuperscript{5} cells per well in DMEM medium (Mediatech, Inc) supplemented with 10% FBS and incubated at 37°C (5% CO2) overnight. To form the PCSK9 and antibody complex, 2 μg/ml of D374Y human PCSK9 was incubated with various concentrations of antibody diluted in uptake buffer (DMEM with 1% FBS) or uptake buffer alone (control) for 1 hour at room temperature. After washing the cells with PBS, the D374Y PCSK9/antibody mixture was transferred to the cells, followed by LDL-BODIPY (Invitrogen) diluted in uptake buffer at a final concentration of 6 μg/ml. After incubation for 3 hours at 37°C (5% CO2), cells were washed thoroughly with PBS and the cell fluorescence signal was detected by Safire\textsuperscript{TM} (TECAN) at 480-520nm (excitation) and 520-600nm (emission).

The results of the cellular uptake assay are shown in FIGs. 7A-7D. Summarily, IC\textsubscript{50} values were determined for each antibody and found to be 16.7 nM for 31H4 IgG2 (FIG. 7A), 13.3 nM for 31H4 IgG4 (FIG. 7B), 13.3 nM for 21B12 IgG2 (FIG. 7C), and 18 nM for 21B12 IgG4 (FIG. 7D). These results demonstrate that the applied antigen binding proteins can reduce the effect of PCSK9 (D374Y) to block LDL uptake by cells. The antibodies also blocked the effect of wild-type PCSK9 in this assay.

EXAMPLE 12
Serum cholesterol Lowering Effect of the 31H4 Antibody in 6 Day Study

In order to assess total serum cholesterol (TC) lowering in wild type (WT) mice via antibody therapy against PCSK9 protein, the following procedure was performed.

Male WT mice (C57BL/6 strain, aged 9-10 weeks, 17-27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal chow (Harland-Teklad, Diet 2918) throughout the duration of the experiment. Mice were administered either anti-PCSK9 antibody 31H4 (2 mg/ml in PBS) or control IgG (2 mg/ml in PBS) at a level of 10mg/kg through the mouse’s tail vein at T=0. Naïve mice were also set aside as a naïve control group. Dosing groups and time of sacrifice are shown in Table 12.1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Time point after dosing</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG</td>
<td>8 hr</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>31H4</td>
<td>8 hr</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>IgG</td>
<td>24 hr</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>31H4</td>
<td>24 hr</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>IgG</td>
<td>72 hr</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>31H4</td>
<td>72 hr</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>IgG</td>
<td>144 hr</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>31H4</td>
<td>144 hr</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Naïve</td>
<td>n/a</td>
<td>7</td>
</tr>
</tbody>
</table>

Mice were sacrificed with CO2 asphyxiation at the pre-determined time points shown in Table 9. Blood was collected via vena cava into eppendorf tubes and was allowed to clot at room temperature for 30 minutes. The samples were then spun down in a table top centrifuge at 12,000xg for 10 minutes to separate the serum. Serum total cholesterol and HDL-C were measured using Hitachi 912 clinical analyzer and Roche/Hitachi TC and HDL-C kits.

The results of the experiment are shown in FIGs. 8A-8D. Summarily, mice to which antibody 31H4 was administered showed decreased serum cholesterol levels.
over the course of the experiment (FIG. 8A and FIG. 8B). In addition, it is noted that the mice also showed decreased HDL levels (FIG. 8C and FIG. 8D). For FIG. 8A and FIG. 8C, the percentage change is in relation to the control IgG at the same time point (*P<0.01, # P<0.05). For FIG. 8B and FIG 8D, the percentage change is in relation to total serum cholesterol and HDL levels measured in naïve animals at t=0 hrs (*P<0.01, # P<0.05).

In respect to the lowered HDL levels, it is noted that one of skill in the art will appreciate that the decrease in HDL in mice is not indicative that an HDL decrease will occur in humans and merely further reflects that the serum cholesterol level in the organism has decreased. It is noted that mice transport the majority of serum cholesterol in high density lipoprotein (HDL) particles which is different to humans who carry most serum cholesterol on LDL particles. In mice the measurement of total serum cholesterol most closely resembles the level of serum HDL-C. Mouse HDL contains apolipoprotein E (apoE) which is a ligand for the LDL receptor (LDLR) and allows it to be cleared by the LDLR. Thus, examining HDL is an appropriate indicator for the present example, in mice (with the understanding that a decrease in HDL is not expected for humans). For example, human HDL, in contrast, does not contain apoE and is not a ligand for the LDLR. As PCSK9 antibodies increase LDLR expression in mouse, the liver can clear more HDL and therefore lowers serum HDL-C levels.

EXAMPLE 13

Effect of Antibody 31H4 on LDLR Levels in a 6 Day Study

The present example demonstrates that an antigen binding protein alters the level of LDLR in a subject, as predicted, over time. A Western blot analysis was performed in order to ascertain the effect of antibody 31H4 on LDLR levels. 50-100 mg of liver tissue obtained from the sacrificed mice described in Example 11 was homogenized in 0.3 ml of RIPA buffer (Santa Cruz Biotechnology Inc.) containing complete protease inhibitor (Roche). The homogenate was incubated on ice for 30 minutes and centrifuged to pellet cellular debris. Protein concentration in the supernatant was measured using BioRad protein assay reagents (BioRad laboratories). 100μg of protein was denatured at 70°C for 10 minutes and separated on 4-12% Bis-
Tris SDS gradient gel (Invitrogen). Proteins were transferred to a 0.45 μm PVDF membrane (Invitrogen) and blocked in washing buffer (50mM Tris PH7.5, 150mM NaCl, 2mM CaCl₂ and 0.05% Tween 20) containing 5% non-fat milk for 1 hour at room temperature. The blot was then probed with goat anti-mouse LDLR antibody (R&D system) 1:2000 or anti-β actin (sigma) 1:2000 for 1 hour at room temperature. The blot was washed briefly and incubated with bovine anti-goat IgG-HRP (Santa Cruz Biotechnology Inc.) 1:2000 or goat anti-mouse IgG-HRP (Upstate) 1:2000. After a 1 hour incubation at room temperature, the blot was washed thoroughly and immunoreactive bands were detected using ECL plus kit (Amersham biosciences). The Western blot showed an increase in LDLR protein levels in the presence of antibody 31H4, as depicted in FIG. 9.

EXAMPLE 14
Serum cholesterol Lowering Effect of Antibody 31H4 in a 13 Day Study

In order to assess total serum cholesterol (TC) lowering in wild type (WT) mice via antibody therapy against PCSK9 protein in a 13 day study, the following procedure was performed.

Male WT mice (C57BL/6 strain, aged 9-10 weeks, 17-27 g) obtained from Jackson Laboratory (Bar Harbor, ME) were fed a normal chow (Harland-Teklad, Diet 2918) through out the duration of the experiment. Mice were administered either anti-PCSK9 antibody 31H4 (2 mg/ml in PBS) or control IgG (2 mg/ml in PBS) at a level of 10 mg/kg through the mouse’s tail vein at T=0. Naïve mice were also set aside as naïve control group.

Dosing groups and time of sacrifice are shown in Table 14.1. Animals were sacrificed and livers were extracted and prepared as in Example 11.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Time point after dosing</th>
<th>Number</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG</td>
<td>72 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>31H4</td>
<td>72 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>Group</td>
<td>Treatment</td>
<td>Time point after dosing</td>
<td>Number</td>
<td>Dose</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>-------------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>3</td>
<td>31H4</td>
<td>72 hr</td>
<td>6</td>
<td>1mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>IgG</td>
<td>144 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>31H4</td>
<td>144 hr</td>
<td>6</td>
<td>10mg/kg</td>
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<tr>
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<tr>
<td>7</td>
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<tr>
<td>8</td>
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<td>16</td>
<td>Naïve</td>
<td>n/a</td>
<td>6</td>
<td>n/a</td>
</tr>
</tbody>
</table>

When the 6 day experiment was extended to a 13 day study, the same serum cholesterol lowering effect observed in the 6 day study was also observed in the 13 day study. More specifically, animals dosed at 10 mg/kg demonstrated a 31% decrease in serum cholesterol on day 3, which gradually returned to pre-dosing levels by day 13. FIG. 10A depicts the results of this experiment. FIG. 10C depicts the results of repeating the above procedure with the 10mg/kg dose of 31H4, and with another antibody, 16F12, also at 10mg/kg. Dosing groups and time of sacrifice are shown in Table 14.2.

**TABLE 14.2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Time point after dosing</th>
<th>Number</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG</td>
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<td>2</td>
<td>16F12</td>
<td>24 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>Group</td>
<td>Treatment</td>
<td>Time point after dosing</td>
<td>Number</td>
<td>Dose</td>
</tr>
<tr>
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<td>-----------</td>
<td>-------------------------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>3</td>
<td>31H4</td>
<td>24 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>IgG</td>
<td>72 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>16F12</td>
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<td>72 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>IgG</td>
<td>144 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>8</td>
<td>16F12</td>
<td>144 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>9</td>
<td>31H4</td>
<td>144 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>10</td>
<td>IgG</td>
<td>192 hr</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>11</td>
<td>16F12</td>
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<td>12</td>
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</tr>
<tr>
<td>19</td>
<td>Naive</td>
<td>n/a</td>
<td>6</td>
<td>10mg/kg</td>
</tr>
</tbody>
</table>

As shown in FIG. 10C both 16F12 and 31H4 resulted in significant and substantial decreases in total serum cholesterol after just a single dose and provided benefits for over a week (10 days or more). The results of the repeated 13 day study were consistent with the results of the first 13 day study, with a decrease in serum cholesterol levels of 26% on day 3 being observed. For FIG. 10A and FIG. 10B, the percentage change is in relation to the control IgG at the same time point (*P<0.01). For FIG. 10C, the percentage change is in relation to the control IgG at the same time point (*P<0.05).

Example 15
Effect of Antibody 31H4 on HDL Levels in a 13 Day Study

The HDL levels for the animals in Example 14 were also examined. HDL levels decreased in the mice. More specifically, animals dosed at 10 mg/kg demonstrated a 33% decrease in HDL levels on day 3, which gradually returned to predosing levels by day 13. FIG. 10B depicts the results of the experiment. There was a decrease in HDL levels of 34% on day 3. FIG. 10B depicts the results of the repeated 13 day experiment.

As will be appreciated by one of skill in the art, while the antibodies will lower mouse HDL, this is not expected to occur in humans because of the differences in HDL in humans and other organisms (such as mice). Thus, the decrease in mouse HDL is not indicative of a decrease in human HDL.

EXAMPLE 16
Repeated Administration of Antibodies Produce Continued Benefits of Antigen Binding Peptides

In order to verify that the results obtained in the Examples above can be prolonged for further benefits with additional doses, the Experiments in Examples 14 and 15 were repeated with the dosing schedule depicted in FIG. 11A. The results are displayed in FIG. 11B. As can be seen in the graph in FIG. 11B, while both sets of mice displayed a significant decrease in total serum cholesterol because all of the mice received an initial injection of the 31H4 antigen binding protein, the mice that received additional injections of the 31H4 ABP displayed a continued reduction in total serum cholesterol, while those mice that only received the control injection eventually displayed an increase in their total serum cholesterol. For FIG. 11, the percentage change is in relation to the naïve animals at t=0 hours (*P<0.01, **P<0.001).

The results from this example demonstrate that, unlike other cholesterol treatment methods, in which repeated applications lead to a reduction in efficacy because of biological adjustments in the subject, the present approach does not seem to suffer from this issue over the time period examined. Moreover, this suggests that the return of total serum cholesterol or HDL cholesterol levels to baseline, observed in the
previous examples is not due to some resistance to the treatment being developed by the subject, but rather the depletion of the antibody availability in the subject.

EXAMPLE 17

Uses of PCSK9 Antibodies for the Treatment of Hypercholesterolemia

A human patient exhibiting symptoms of hypercholesterolemia is administered a therapeutically effective amount of PCSK9 antibody, such as 31H4 (or, for example, 21B12). At periodic times during the treatment, the human patient is monitored to determine whether the serum cholesterol level has declined. Following treatment, it is found that the patient receiving the treatment with the PCSK9 antibodies has reduced serum cholesterol levels in comparison to arthritis patients not receiving the treatment.

EXAMPLE 18

Use of PCSK9 Antigen Binding Protein for the Prevention of Hypercholesterolemia

A human patient exhibiting a risk of developing hypercholesterolemia is identified via family history analysis and/or lifestyle, and/or current cholesterol levels. The subject is regularly administered (e.g., one time weekly) a therapeutically effective amount of PCSK9 antibody, 31H4 (or, for example, 21B12). At periodic times during the treatment, the patient is monitored to determine whether serum cholesterol levels have decreased. Following treatment, it is found that subjects undergoing preventative treatment with the PCSK9 antibody have lowered serum cholesterol levels, in comparison to subjects that are not treated.

EXAMPLE 19

PCSK9 ABPs Further Upregulated LDLR in the Presence of Statins

This example demonstrates that ABPs to PCSK9 produced further increases in LDLR availability when used in the presence of statins, demonstrating that further benefits can be achieved by the combined use of the two.

HepG2 cells were seeded in DMEM with 10% fetal bovine serum (FBS) and grown to ~90% confluence. The cells were treated with indicated amounts of
mevinolin (a statin, Sigma) and PCSK9 ABPs (FIGs. 12A-12C) in DMEM with 3% FBS for 48 hours. Total cell lysates were prepared. 50 mg of total proteins were separated by gel electrophoresis and transferred to PVDF membrane. Immunoblots were performed using rabbit anti-human LDL receptor antibody (Fitzgerald) or rabbit anti-human b-actin antibody. The enhanced chemiluminescent results are shown in the top panels of FIGs. 12A-12C. The intensity of the bands were quantified by ImageJ software and normalized by b-actin. The relative levels of LDLR are shown in the lower panels of FIGs. 12A-12C. ABPs 21B12 and 31H4 are PCSK9 neutralizing antibodies, while 25A7.1 is a non-neutralizing antibody.

HepG2-PCSK9 cells were also created. These were stable HepG2 cell line transfected with human PCSK9. The cells were seeded in DMEM with 10% fetal bovine serum (FBS) and grew to ~90% confluence. The cells were treated with indicated amounts of mevinolin (Sigma) and PCSK9 ABPs (FIGs. 12D-12F) in DMEM with 3% FBS for 48 hours. Total cell lysates were prepared. 50 mg of total proteins were separated by gel electrophoresis and transferred to PVDF membrane. Immunoblots were performed using rabbit anti-human LDL receptor antibody (Fitzgerald) or rabbit anti-human b-actin antibody. The enhanced chemiluminescent results are shown in the top panels. The intensity of the bands were quantified by ImageJ software and normalized by b-actin.

As can be seen in the results depicted in FIGs. 12A-12F, increasing amounts of the neutralizing antibody and increasing amounts of the statin generally resulted in increases in the level of LDLR. This increase in effectiveness for increasing levels of the ABP is especially evident in FIGs. 12D-12F, in which the cells were also transfected with PCSK9, allowing the ABPs to demonstrate their effectiveness to a greater extent.

Interestingly, as demonstrated by the results in the comparison of FIGs. 12D-12F to 12A-12C, the influence of the ABP concentrations on LDLR levels increased dramatically when PCSK9 was being produced by the cells. In addition, it is clear that the neutralizing ABPs (21B12 and 31H4) resulted in a greater increase in LDLR levels, even in the presence of statins, than the 25A7.1 ABP (a non-neutralizer), demonstrating that additional benefits can be achieved by the use of both statins and ABPs to PCSK9.
EXAMPLE 20
Consensus Sequences

Consensus sequences were determined using standard phylogenetic analyses of
the CDRs corresponding to the $V_H$ and $V_L$ of anti-PCSK9 ABPs. The consensus
sequences were determined by keeping the CDRs contiguous within the same sequence
corresponding to a $V_H$ or $V_L$. Briefly, amino acid sequences corresponding to the entire
variable domains of either $V_H$ or $V_L$ were converted to FASTA formatting for ease in
processing comparative alignments and inferring phylogenies. Next, framework
regions of these sequences were replaced with an artificial linker sequence
(“bbbbbbbbbb” placeholders, non-specific nucleic acid construct) so that examination
of the CDRs alone could be performed without introducing any amino acid position
weighting bias due to coincident events (e.g., such as unrelated antibodies that
serendipitously share a common germline framework heritage) while still keeping
CDRs contiguous within the same sequence corresponding to a $V_H$ or $V_L$. $V_H$ or $V_L$
sequences of this format were then subjected to sequence similarity alignment
interrogation using a program that employs a standard ClustalW-like algorithm (see,
8.0 was employed along with a gap extension penalty of 2.0. This program likewise
generated phylograms (phylogenetic tree illustrations) based on sequence similarity
alignments using either UPGMA (unweighted pair group method using arithmetic
averages) or Neighbor-Joining methods (see, Saitou and Nei, 1987, *Molecular Biology
and Evolution* 4:406-425) to construct and illustrate similarity and distinction of
sequence groups via branch length comparison and grouping. Both methods produced
similar results but UPGMA-derived trees were ultimately used as the method employs
a simpler and more conservative set of assumptions. UPGMA-derived trees were
generated where similar groups of sequences were defined as having fewer than 15
substitutions per 100 residues (see, legend in tree illustrations for scale) amongst
individual sequences within the group and were used to define consensus sequence
collections. The results of the comparisons are depicted in FIGs. 13A-13J and FIGs.
31A and 31B. In FIG. 13E, the groups were chosen so that sequences in the light chain that clade are also a clade in the heavy chain and have fewer than 15 substitutions.
Example 21

11F1 Binding Specificity

Results from this assay demonstrate that 11F1 binds to PCSK9 and not to PCSK1, PCSK2, PCSK7, or furin, demonstrating the specificity of 11F1 for PCSK9.

Biotinylated PCSK9, diluted in buffer A (25 mM Tris, 150 mM NaCl, 0.1% BSA, 0.05% tween, pH 7.5) was bound to neutravidin coated 96 well plates at a concentration of 0.2 µg/mL, for one hour incubation at room temperature. Separately, 0.4 µg/mL of 11F1 was incubated for one hour at room temperature with various concentrations (ranging from 0 to 20 µg/mL) of either PCSK1, PCSK2, PCSK7, PCSK9 or furin (R&D Systems, Minneapolis, MN) (diluted in buffer A w/o tween). Furin inhibitor, at 4.5 µg/mL, was included with all furin containing reactions. The PCSK9 coated streptavidin plate was washed with buffer A and the antibody/proprotein convertase mixture was added to the plate and incubated at room temperature for one hour. After washing, bound antibody was detected by incubation with goat-α-human Fc-HRP (160 ng/mL, diluted in buffer A) (Jackson Laboratories, Bar Harbor, ME) followed by TMB substrate. The reaction was stopped with 1 N HCl and the absorbance was read at a wavelength of 450 nm on a Spectramax Plus 384 spectrophotometer (Molecular Devices Inc., Sunnyvale, CA).

This assay relied on the ability of proprotein convertase in solution to compete for the binding of 11F1 to plate-captured PCSK9. Pre-incubation of 11F1 and PCSK9 in solution dose dependently and robustly reduced the amount of 11F1 binding to plate-captured PCSK9 detected as reduced OD450 (FIG. 14). All results were expressed as the mean OD450 value ± standard deviation versus concentration of the proprotein convertase. Pre-incubation of 11F1 with PCSK1, PCSK2, PCSK7, or furin, in solution, did not significantly impact the binding of 11F1 to plate-captured PCSK9. Therefore, at the protein concentrations studied, 11F1 binds only to PCSK9 and not to the other proprotein convertase family members tested.

Example 22
Efficacy of 11F1 Inhibition of LDLR:PCSK9 Binding

The example demonstrates that nanomolar concentrations of 11F1 can inhibit binding of both D374Y and wild-type PCSK9 to the LDLR under the conditions of this assay.

Briefly, clear, 384 well plates were coated with 2 μg/mL of goat anti-LDL receptor antibody (R&D Systems, Minneapolis, MN), diluted in PBS, by overnight incubation at 4°C. Plates were washed thoroughly with buffer A (100 mM sodium cacodylate pH 7.5) and then blocked with buffer B (1% non-fat dry milk [Bio-Rad Laboratories, Hercules, CA] in buffer A) for 2 hours at room temperature. After washing, plates were incubated with 0.4 μg/mL of LDL receptor (R&D Systems, Minneapolis, MN) diluted in buffer C (buffer B supplemented with 10 mM CaCl2) for 1.5 hours at room temperature. Concurrent with this incubation, 20 ng/mL of biotinylated D374Y PCSK9 or 100 ng/mL of biotinylated WT PCSK9 was incubated with various concentrations of anti-PCSK9 antibody 11F1 diluted in buffer A (final concentrations ranging from 6.0 ng/mL to 200 ng/mL for the D374Y PCSK9 assay or 3.1 ng/mL to 25 ng/mL for the WT PCSK9 assay). The LDLR-coated plates were washed and the biotinylated PCSK9/antibody mixture was added. The LDLR plate was incubated at room temperature for 1 hour. Binding of the biotinylated PCSK9 to the LDLR was detected by incubation with streptavidin-HRP (500 ng/mL in buffer C) followed by TMB substrate. The reaction was stopped with 1N HCl and the absorbance was read at a wavelength of 450 nm on a SpectraMax Plus 384 Spectrophotometer (Molecular Devices Inc., Sunnyvale, CA). GraphPad Prism (v 4.01) software was used to plot log of antibody concentration versus OD450 to determine IC50 values by nonlinear regression.

11F1 inhibited LDLR:PCSK9 binding. The IC50 values for 11F1 in the D374Y PCSK9 assay ranged from 7.3 nM to 10.1 nM with an average (± SD) of 9.1 nM ± 1.5 nM (n=3). The IC50 values for 11F1 in the wild-type PCSK9 assay ranged from 4.4 nM to 8.1 nM with an average (± SD) of 5.9 nM ±1.9 nM (n=3). It should be noted that these IC50 values are dependent on the amount of recombinant D374Y PCSK9 or WT PCSK9 used in the binding assay. A representative dose response curve for both the D374Y and wild-type assays are presented in FIG. 15 and FIG. 16, respectively.
EXAMPLE 23

Efficacy of 11F1 in Blocking Cell LDL Uptake

11F1 blocks the interaction between PCSK9 and LDLR in vitro and can prevent the PCSK9-mediated reduction of LDL uptake in HepG2 cells.

Briefly, human HepG2 cells were seeded in black, clear bottom 96-well plates (Fisher Scientific CO LLC, Santa Clara, CA) at a density of 5x10^4 cells per well in DMEM (Mediatech Inc., Herndon, VA) supplemented with 10% FBS and 1% of antibiotic-antimycotic solution (Mediatech Inc., Herndon, VA). Cells were incubated at 37°C (5% CO2) overnight. To form the complex between D374Y PCSK9 and antibody or WT PCSK9 and antibody, serial dilutions (1:2) of 11F1, from 666.7 nM to 0.7 nM (for blocking D374Y PCSK9) or from 3.3 μm to 3.3 nM (for blocking WT PCSK9), were prepared in formulation buffer (25 mM HEPES, pH 7.5, 0.15 M NaCl). Either D374Y PCSK9 (2 μg/mL) or WT PCSK9 (25 μg/mL) were diluted in uptake buffer (DMEM containing 1% FBS) and incubated with the various concentrations of 11F1 or uptake buffer alone (negative control) for 1 hour at room temperature with shaking. BODIPY-LDL (Invitrogen, Carlsbad, CA) was diluted in uptake buffer to a concentration of 12 μg/mL. Following overnight incubation, HepG2 cells were rinsed twice with DPBS (Mediatech Inc., Herndon, VA). Twenty-five microliters of the D374Y PCSK9 or WT PCSK9 complex with 11F1 and 25 μL of diluted BODIPY-LDL (Invitrogen, Carlsbad, CA) were added to the cells and incubated at 37°C (5% CO2) for 3 hours. Cells were washed with DPBS 5 times and resuspended in 100 μL DPBS. Fluorescent signals were detected using a Safire plate reader (Tecan Systems Inc., San Jose, CA) at 480~520 nm (excitation) and 520~600 nm (emission) and expressed as relative fluorescence unit (RFU).

GraphPad Prism (Version 4.02, GraphPad Software Inc., San Diego, CA) software was used to plot log of antibody concentration versus RFU and to determine EC50 values by nonlinear regression using the sigmoidal dose-response (variable slope) curve fitting program.

This example shows that 11F1 blocked D374Y PCSK9 or WT PCSK9-mediated decrease of LDL uptake in HepG2 cells in a dose-dependent manner. Adding
recombinant purified D374Y PCSK9 (2 μg/mL) or WT PCSK9 (25 μg/mL) to HepG2 cells reduced the uptake of BODIPY-LDL to ~50 to 60% and ~40% of the level measured in untreated cells, respectively. The antibodies dose-dependently restored LDL uptake to the level observed in untreated cells. The mean (± SD) EC50 value for the ability of 11F1 to block D374Y PCSK9-mediated decrease of LDL uptake was 35.3 ± 9.1 nM (n = 6, FIG. 17). The EC50 value for the ability of 11F1 to block WT PCSK9-mediated decrease in LDL uptake was 124.2 ± 28.5 nM (n = 3, FIG. 18). It should be noted that these EC50 values are a function of the amount of recombinant D374Y PCSK9 or WT PCSK9 used in the cell assay. The EC50 value is lower against D374Y PCSK9 than WT PCSK9 since less D374Y PCSK9 was used in the assay because its binding affinity to the LDLR is 5- to 30-fold greater than that of WT PCSK9 (Cunningham et al, 2007; Fisher et al, 2007; Kwon et al, 2008).

The EC50 values reported here are representative for mean values derived from 3 to 6 separate measurements for 11F1.

EXAMPLE 24
Efficacy of 11F1 and 8A3 in Blocking Human PCSK9 Expressed Via an Adeno-Associated Virus in a mouse model

A single intravenous bolus administration of the anti-PCSK9 antibodies 11F1 or 8A3 leads to a significant decrease in serum non-HDL-C and TC in mice expressing human PCSK9 by AAV. This example demonstrates the effectiveness of both anti-PCSK9 antibodies in blocking the function of human PCSK9 in vivo.

Briefly, 120 C57BL/6 mice expressing human PCSK9 were generated by infection with an engineered adeno associated virus (AAV) coding for human PCSK9, resulting in elevated levels of circulating low density lipoprotein cholesterol (LDL-C). Serum cholesterol analysis was performed using the Cobas Integra 400 plus chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Animals were randomized into treatment groups with similar levels of non-HDL-C (LDL-C and VLDL-C), HDL-C and TC. On treatment day 0 (T=0) a subset of mice was euthanized and serum collected to establish that day’s baseline levels. Remaining mice were then administered 11F1, 8A3 or anti-keyhole limpethemocyanin (KLH) IgG2 control antibody at 30 mg/kg via
tail vein injection. At days 1 through 5 following injection, subsets of mice were euthanized and whole blood was collected from the vena cava and allowed to coagulate for 30 minutes at room temperature. Following centrifugation at 12,000 rpm with a bench top centrifuge for 10 minutes, serum was collected. Serum cholesterol analysis was performed using the Cobas Integra 400 plus chemistry analyzer.

Serum concentrations of PCSK9 were determined using a sandwich ELISA assay. Clear 96 well plates were coated overnight with 2 μg/ml of monoclonal anti-PCSK9 antibody (31H4) diluted in 1X PBS. Plates were washed thoroughly with 1X PBS/.05% tween and then blocked for 2 hours with 3% BSA/1XPBS. After washing, plates were incubated for 2 hours with serum diluted in general assay diluents (Immunochromistry Technologies, Bloomington, MN). Recombinant human PCSK9 (1 ng/ml to 500 ng/ml) was assayed concurrently and used to generate a standard curve on each ELISA plate. A rabbit polyclonal biotinylated anti-PCSK9 antibody (D8773, Amgen Inc, CA) was added at 1 μg/ml (in 1%BSA/PBS), followed by neutravidin-HRP at 200 ng/ml (in 1% BSA/PBS). Bound PCSK9 was detected by incubation with TMB substrate. The reaction was stopped with addition of 1N HCl and the absorbance measured at 450 nm on a Spectra Max Plus 384 Spectrophotometer (Molecular Devices Inc, Sunnyvale, CA). The standard curve (4-parameter logistic fit) generated with recombinant human PCSK9 was used to determine the corresponding concentration of PCSK9 in the serum samples.

Serum concentrations of antibody were determined using a sandwich ELISA assay. Polyclonal goat anti-human Fc IgG and an HRP-labeled goat anti-human IgG Fcγ polyclonal reagent (both from Jackson ImmunoResearch Laboratories Inc, West Grove, PA) were used as the capture and the detection antibody, respectively. A 3,3’5,5’tetramethylbenzidine (TMB) substrate solution reacted with peroxide, and in the presence of horse radish peroxidase (HRP), created a colorimetric signal that was proportional to the amount of the respective anti-PCSK9 antibody bound by the capture reagent. The intensity of the color (optical density, OD) was measured at 450 nm minus 650 nm using a microplate reader (Spectra Max Plus 384). Data was analyzed using Watson version 7.0.0.01 (Thermo Scientific, Waltham, MA) data reduction package with a Logistic (auto-estimate) regression of separately prepared
standard curves. The lower limit of quantification (LLOQ) for the assay was ng/mL. 34.4.

Calculation of Pharmacokinetic Parameters in AAV Mice

Non-compartmental analysis (NCA) was performed on serum concentrations using the pre-determined nominal time points for each subject using WinNonlin Enterprise, version 5.1.1 (Pharsight, St. Louis, MO). Data points for estimating the terminal elimination rate constants and half-lives were chosen by visual inspection of the concentration-time profiles. NCA parameters reported include: apparent half-life (t1/2), area under the serum concentration-time curve from time zero to the last measured concentration (AUC0-t), and apparent serum clearance (CL0-t). AUC0-t was determined using the linear log-linear trapezoidal method, and CL0-t was calculated by Dose/AUC0-t. For 11F1, 8A3, and 31H4 antibodies. Post-study dose solution analysis showed actual doses were within 20% of the 30 mg/kg target. However, for the IgG2 control, analysis showed actual dose was only 40% of the intended target. Therefore, a corrected dose of 12 mg/kg was used for CL0-t calculation for IgG2 control. Parameters were reported to three significant figures, except for half-life which was reported to two significant figures.

Statistical Analysis

All cholesterol results were expressed as the mean ± standard error of the mean. All pharmacokinetic data were expressed as the mean ± standard deviation. The p value of 0.05, determined by 1-way ANOVA was used as a threshold to determine statistical significance between the anti-KLH IgG2 control antibody injected animals and those dosed with anti-PCSK9 antibody at the same time point.

Effect of Anti-PCSK9 Antibodies on Serum non-HDL-C, HDL-C, and TC

To establish a baseline, a subset of mice expressing human PCSK9 was euthanized prior to injection of antibodies and blood was collected. Non-HDL-C, HDL-C and TC levels in these animals were 33 ± 4, 117 ± 4 and 183 ± 9 mg/dL,
respectively (mean ± SEM). Levels of PCSK9 in naïve animals were determined to be 4921 ng/mL ± 2044 ng/mL.

Compared to mice injected with anti-KLH IgG2 control antibody (control animals), injection of 11F1 produced significant lowering of non-HDL-C at days 1, 2, and 4 post-injection (with a maximum of 59%), while TC was significantly lowered at day 4 only (by 22%) (FIG. 19, FIG. 20). No significant lowering of HDL-C was observed at any time point (FIG. 21).

Compared to control animals, injection of 8A3 produced significant lowering of non-HDL-C at days 1, 2, and 4 post-injection (with a maximum of 65%), while TC was significantly lowered at day 2 post-injection (with a maximum of 24%) (FIG. 19, FIG. 20). No significant lowering of HDL-C was observed at any time point (FIG. 21).

**Pharmacokinetics**

At an intravenous dose of 30 mg/kg, 11F1 and 8A3 had very similar pharmacokinetic behavior (FIG. 22). For these two molecules, AUC0-t exposures, estimated CL0-t, and apparent half-lives were equivalent (Table of FIG. 23). The anti-KLH IgG2 control antibody had an unexpectedly lower AUC0-t exposure than 11F1 and 8A3, but this is likely due to the antibody being administered at a lower dose than intended (12 mg/kg as opposed to 30 mg/kg; dose solution analysis showed antibody concentration to be 40% of target. Anti-KLH IgG2 control antibody CL0-t was similar to that of 11F1 and 8A3, when calculated using the corrected dose, and the apparent half-life of the anti-KLH IgG2 control antibody was estimated at >120 hours. These data suggested that affects of the PCSK9 ligand on antibody disposition are less pronounced for 11F1 and 8A3 when compared to other antibodies dosed in the AAV model because 11F1 and 8A3 CL0-t values are more similar to anti-KLH IgG2 control antibody.

**Summary.**

Expression of human PCSK9 by AAV in mice (approximately 5 ug/mL) resulted in a serum non-HDL-C level of approximately 33 mg/dL. Following a 30 mg/kg injection of 11F1, significant serum non-HDL-C lowering was observed at days 1, 2 and 4 post-injection (with a maximum of 59% as compared to control animals).
Significant lowering of TC was seen at day 4 only. Injection of 8A3 resulted in a similar pattern of non-HDL-C lowering with a maximum of 65% as compared to control animals. However, 8A3 administration resulted in significant TC lowering at day 2 only, post-injection, with a maximum of 24%. No significant lowering of HDL-C was observed in animals administered either 11F1 or 8A3. Analysis of serum antibody levels of 11F1 and 8A3 demonstrated a similar profile to anti-KLH IgG2 control antibody.

EXAMPLE 25

Effect of a Single Subcutaneous Dose of 11F1, 21B12 and 8A3 on Serum Lipids in Cynomolgus Monkeys

Single SC administration of 11F1, 8A3 or 21B12 to cynomolgus monkeys leads to the significant lowering of serum LDL-C, and TC. This study demonstrated the ability of antiPCS K9 antibodies to lower serum cholesterol in non-human primates.

Briefly, naive male cynomolgus monkeys were acclimated to their environment for at least 2 weeks prior to experimentation. Animals were randomized into treatment groups based on a pre-screen of their serum TC, HDL-C, LDL-C, and triglyceride levels, and their body weight. After 1 week, animals were fasted overnight, and bled from the peripheral vasculature (cephalic or saphenous vein), for measurement of baseline serum lipid levels at a time point designated T = 0. Animals were then injected SC with either anti-KLH IgG2 control antibody, 11F1, 21B12, or 8A3 (all in 10 mM NaOAc pH 5.2, 9% sucrose) at 0.5 mg/kg (all at 0.4 mL/kg body weight). Fasting blood samples were then collected from animals at designated time points over a 45 day period.

### Experimental Design

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No Males</th>
<th>Route</th>
<th>Treatment</th>
<th>Dose Level (mg/kg)</th>
<th>Concentration (mg/mL)</th>
<th>Volume (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>SC</td>
<td>Anti-KLH</td>
<td>0.5</td>
<td>1.09</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>SC</td>
<td>21B12</td>
<td>0.5</td>
<td>1.19</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>SC</td>
<td>11F1</td>
<td>0.5</td>
<td>1.11</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>SC</td>
<td>8A3</td>
<td>0.5</td>
<td>1.25</td>
<td>0.4</td>
</tr>
</tbody>
</table>
At specified time points, blood was collected from animals under overnight fasting conditions from the peripheral vasculature (cephalic or saphenous vein). Whole blood was allowed to coagulate for 30 minutes at room temperature. Following centrifugation at 3,000 rpm for 20 minutes, serum was collected. Direct serum cholesterol analysis was performed using the Cobas Integra 400 analyzer (Roche Diagnostics Inc, Indianapolis, IN). Apolipoprotein B serum levels were determined at specified time points (day 0, 3, 6, 15, 24 and 33) by Anlytics, MD, with the following methodology. A 17 μL aliquot of the sample (no preparation) was used for analysis with a Hitachi 717 Analyzer using a 6 points standard curve. If the initial value of the sample was higher than the standard curve linearity, then the sample was diluted and repeated with the result multiplied by the appropriate dilution factor. The reagents for the assay (APO-B Reagent Kit # 86071, Antibody Set # 86060, Control Set # 86103) were obtained from DiaSorin (Stillwater, MN).

Antibody concentrations in serum were determined using an enzyme-linked immunosorbent assay (ELISA) with an assay range of 34.4 to 3000 ng/mL (34.4 ng/mL being the lower limit of quantitation [LLOQ]).

Non-compartmental analysis (NCA) was performed on the serum concentrations using the pre-determined nominal time points for each subject using Watson® LIMS, version 7.0.0.01 (Thermo Scientific, Waltham, MA). Data points for estimating the terminal elimination rate constants and half-lives were chosen by visual inspection of the concentration-time profile and best linear fit (typically from 360 h until the antibody concentrations dropped below the lower limit of quantitation). NCA parameters reported include: terminal half-life (t1/2,\(z\)), the maximum serum concentration (Cmax), area under the serum concentration-time curve from time zero to infinity (AUC0-inf), and apparent serum clearance (CL/F). AUC0-inf was calculated using the linear log-linear trapezoidal method. All parameters were all reported to three significant figures, except for half-life which was reported to two significant figures.
Statistical Analysis

A statistical model that considers baseline as a covariate and treatment group as a fixed effect was fit to the log transformed response at each time point for LDL-C, HDL-C, TC, and triglycerides. Tukey's multiple comparison correction was applied to adjust the pair wise comparisons at each time point. The statistical significance was evaluated at alpha=0.05 using adjusted p-values.

Effect of 11F1, 21B12, and 8A3 on Serum LDL Cholesterol

Maximal LDL-C lowering for 11F1 was observed 9 days after injection, with a 57% lowering of LDL-C as compared to anti-KLH IgG2 control antibody-treated monkeys (control animals). LDL-C returned to levels similar to those observed in control animals by day 27. Maximal LDL-C lowering for 21B12 was observed 3 days after injection, with a 64% lowering of LDL-C as compared to control animals. LDL-C returned to levels similar to control animals by day 6. Maximal LDL-C lowering for 8A3 was observed 4 days after injection, with a 54% lowering of LDL-C as compared to control animals. LDL-C returned to levels similar to those observed in control animals by day 27 (FIG. 24).

Effect of 11F1, 21B12, and 8A3 on Serum Total Cholesterol

Maximal TC lowering for 11F1 was observed 9 days after injection, with a 27% lowering of TC as compared to anti-KLH IgG2 control antibody-treated monkeys (control animals). TC returned to levels similar to those observed in control animals by day 27. Maximal TC lowering for 21B12 was observed 3 days after injection, with a 20% lowering of TC as compared to control animals. TC transiently returned to levels similar to those observed in vehicle-treated monkeys by day 4, but were significantly lower between days 14 and 18, inclusively. Maximal TC lowering for 8A3 was observed 9 days after injection, with a 22% lowering of TC as compared to control animals. TC returned to levels similar to those observed in control animals by day 30 (FIG. 25).
Effect of 11F1, 21B12, and 8A3 on Serum HDL Cholesterol and Triglycerides

On average and at each time point, HDL-C or triglyceride levels for animals treated with 11F1 or 8A3 were not significantly different (based on an alpha = 0.05 significance level) from those observed in anti-KLH IgG2 control antibody-treated monkeys. However, 21B12 did induce a statistically significant change in HDL-C at a single time point (day 18 following injection) (FIG. 26 and FIG. 27).

Effect of 11F1, 21B12, and 8A3 on Apolipoprotein B (ApoB)

Serum ApoB levels were measured at days 3, 6, 15, 24 and 33, post-injection. 11F1 and 8A3 were associated with ApoB lowering at days 3 to 24, as compared to anti-KLH IgG2 control antibody-treated monkeys (FIG. 28). 21B12 was associated with statistically significant lower ApoB levels at day 3 only.

Pharmacokinetic Profiles of 11F1, 21B12, and 8A3

A summary plot of the mean concentration-time profiles by treatment is shown in Figure 29. The estimated mean pharmacokinetic parameters for animals receiving 11F1, 21B12, 8A3, and anti-KLH IgG2 control antibody are displayed in Table of Figure 30.

Antibody absorption in all groups was consistent and characteristic of subcutaneous antibody administration. 21B12 pharmacokinetic behavior with regard to CL/F, Cmax, and AUC0-inf was consistent with that observed in previous studies where 21B12 was administered at the same dose. Pharmacokinetics of 11F1 and 8A3 differed significantly from 21B12, where lower CL/F was observed (approximately 15% of 21B12 CL/F) and longer half-lives were estimated (approximately 200 h compared to 40 h for 21B12). Notably, pharmacokinetics of 11F1 and 8A3 were indistinguishable both from one another and the anti-KLH IgG2 control antibody. These data suggest that disposition of 11F1 and 8A3 is impacted to a far lesser extent by association with the PCSK9 target than 21B12, given that 11F1 and 8A3 have the same exposure profile as anti-KLH IgG2 control antibody with no affinity for PCSK9.
Summary of Results

Over the course of the 45 day study, statistically significant lowering of TC and LDL-C was observed in animals administered 11F1, 21B12, or 8A3 as compared to anti-KLH IgG2 control antibody. 11F1 was associated with statistically significant LDL-C lowering (vs anti-KLH IgG2 control antibody) from day 2 to day 24 inclusively. 21B12 demonstrated statistically significant LDL-C lowering (vs anti-KLH IgG2 control antibody) from day 1 to day 4 inclusively. 8A3 demonstrated statistically significant LDL-C lowering (vs anti-KLH IgG2 control antibody) from day 1 to day 24 inclusively. Changes in TC and ApoB mirrored changes observed in LDL-C for all groups. 11F1 achieved a maximal lowering of LDL-C (vs anti-KLH IgG2 control antibody at the same time point) 9 days following injection (-57%). 21B12 achieved a maximal lowering of LDL-C (vs anti-KLH IgG2 control antibody at the same time point) 3 days following injection (-64%). 8A3 achieved a maximal lowering of LDL-C (vs anti-KLH IgG2 control antibody at the same time point) 4 days following injection (-54%). 21B12 lowered HDL-C at a single time point, 18 days after injection. No statistically significant changes were observed in HDL-C levels following 11F1 or 8A3 administration. No statistically significant changes were observed in triglycerides levels following 11F1, 21B12, or 8A3 administration.

EXAMPLE 26

A Two Part Study to Assess the Safety, Tolerability and Efficacy of a Human Anti-PCSK9 Antibody on LDL-C in Subjects with Homozygous Familial Hypercholesterolemia

Study Design: This is a 2 part study. Part A is an open label, single arm, multicenter pilot study. Part B is a double-blind, randomized, placebo-controlled, multicenter, study of antibody, 21B12, (heavy chain, SEQ ID NO:592 and light chain, SEQ ID NO:591) with expanded enrollment but otherwise identical design to Part A. Both inclusion/exclusion criteria and the Schedule of Assessments is the same for Parts A and B.

Inclusion Criteria includes:
• Males and females ≥ 12 to ≤ 65 years of age
• Diagnosis of homozygous familial hypercholesterolemia
• Stable lipid-lowering therapies for at least 4 weeks
• LDL cholesterol >130 mg/dl (3.4 mmol/L)
• Triglyceride < 400 mg/dL (4.5 mmol/L)
• Bodyweight of > 40 kg or greater at screening.

Exclusion Criteria includes:
• LDL or plasma apheresis within 8 weeks prior to randomization
• New York Heart Failure Association (NYHA) class III or IV or last known left ventricular ejection fraction < 30%
• Myocardial infarction, unstable angina, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or stroke within 3 months of randomization
• Planned cardiac surgery or revascularization
• Uncontrolled cardiac arrhythmia
• Uncontrolled hypertension

Schedule of Assessments include, but are not limited to, collection of adverse event (AE) and significant adverse event (SAE) data, vital signs, concomitant medication, laboratory tests, etc.

Subjects who meet inclusion/exclusion criteria are instructed to follow an NCEP Adult Treatment Panel TLC (or comparable) diet and are required to maintain their current lipid lowering therapy throughout the duration of the studies.

The 21B12 formulation is presented as a sterile, clear, colorless frozen liquid. Each sterile vial is filled with a 1-mL deliverable volume of 70 mg/mL 21B12 formulated with 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 5.2. Each vial is for single use only. Placebo is presented in identical containers as a clear, colorless, sterile, protein-free frozen liquid and is formulated as 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 5.2.

In Part A, eight subjects with genetically confirmed homozygous familial hypercholesterolemia on stable lipid-lowering drug therapy for greater than (or equal
to) 4 weeks are enrolled and received open label 21B12 formulation. Table 26.1 shows the genotypes of the patients in the study.

**Table 26.1: Patent Genotypes**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation Allele 1 (Estimated LDL-r Function)</th>
<th>Mutation Allele 2 (Estimated LDL-r Function)</th>
<th>Overall LDL-r Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Asp266Glu (15%-30%)</td>
<td>Asp266Glu (15%-30%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1187-10 G&gt;A‡ (Not determined)</td>
<td>Asp266Glu (15%-30%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Asp224Asn (&lt;2%)</td>
<td>Cys296Tyr (Not determined)</td>
<td>Negative</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Deletion Exon 4-18 (Not determined)</td>
<td>Cys197Gly (Not determined)</td>
<td>Negative</td>
</tr>
<tr>
<td>Patient 5</td>
<td>Asp221Gly (&lt;2%)</td>
<td>Asp227Glu (5%-15%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 6*‡</td>
<td>Asp227Glu (5%-15%)</td>
<td>Asp227Glu (5%-15%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 7*‡</td>
<td>Asp227Glu (5%-15%)</td>
<td>Asp227Glu (5%-15%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 8</td>
<td>Asp175Asn (Not determined)</td>
<td>Asp227Glu (5%-15%)</td>
<td>Receptor defective</td>
</tr>
</tbody>
</table>

*True homozygous patient.

‡Mutation at splice acceptor site 10 nucleotides upstream of the first nucleotide of exon 9, 1187.

‡ Patients share the same genotype.

LDL-r: Low density lipoprotein receptor.
The 21B12 formulation (420 mg) is administered subcutaneously every 4 weeks for 12 weeks, followed by an additional 12 weeks of treatment at 4 week intervals, and then 12 weeks with AMG 145 420 mg administered every 2 weeks. Study visits occur at least every 4 weeks. During these visits adverse event (AE) and significant adverse event (SAE) data, vital signs, concomitant medication, laboratory tests, etc. are collected.

Changes and percentage changes in lipid related parameters are shown in Table 38.2. At week 12 of every 4-weeks treatment, the mean LDL cholesterol by ultracentrifugation decreased from baseline by 16.5% (70.6 mg/dL; 1.8 mmol/L), with a range from +5.2% to -43.6%. Four (50%) patients have a reduction of 15%, with 3 of the 4 (38%) achieving LDL cholesterol reductions ≥30%. Patients with negative LDL receptor activity have no LDL cholesterol reduction.

After 12 weeks of every 2-week treatment, mean LDL cholesterol decrease from baseline was 13.9% (60.8 mg/dL; 1.6 mmol/L). No LDL cholesterol reduction is observed in LDL receptor negative patients, but a greater reduction occurs in patients with receptor defective function (Table 26.3). Three patients (38%) have an LDL cholesterol reduction ≥30%. Receptor defective patients have mean reductions of 22.9% and 23.6% over the 12 week treatment period, respectively, for every 4- and 2-week dosing (Table 26.3).

The changes from baseline at week 12 in apolipoprotein B and related lipoproteins with every 4- and 2-week dosing are shown in Table 26.2. The mean change in Lp(a) is -11.7% and -18.6% with every 4- and 2-week dosing, respectively; this does not appear to be related to LDL receptor activity. Triglycerides decrease by 5.7% and increase by 5.9% with every 4- and 2-week dosing, respectively. HDL-cholesterol and apolipoprotein A1 are essentially unchanged with either every 4- or 2-week dosing (Table 26.2). Treatment with the 21B12 formulation 420 mg every 4 weeks reduces free PCSK9 by 22.7% and 87.6% at week 12 for every 4- and 2-week dosing, respectively (Table 26.2).
Table 26.2: Efficacy Outcomes (Overall).

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Baseline Value</th>
<th>Change from baseline</th>
<th>Percentage change from baseline (%)</th>
<th>Value</th>
<th>Change from baseline</th>
<th>Percentage change from baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21B12 formulation (N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Week 12 Q4W</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (ultracentrifugation), mg/dL</td>
<td>441.7 (40.1)</td>
<td>371.1 (50.4)</td>
<td>-70.6 (32.3)</td>
<td>-16.5 (6.7)</td>
<td>380.9 (56.3)</td>
<td>-13.9 (9.6)</td>
</tr>
<tr>
<td>Mean (SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>218 to 563</td>
<td>190 to 563</td>
<td>-228 to 23</td>
<td>-43.6 to 5.2</td>
<td>196 to 614</td>
<td>-217 to 175</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>33.8 (3.2)</td>
<td>34.5 (3.4)</td>
<td>0.8 (3.0)</td>
<td>4.7 (7.8)</td>
<td>32.9 (3.7)</td>
<td>-0.9 (2.9)</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>269.1 (18.7)</td>
<td>228.8 (21.3)</td>
<td>-40.3 (14.6)</td>
<td>-14.9 (5.0)</td>
<td>235.3 (24.7)</td>
<td>-33.8 (19.0)</td>
</tr>
<tr>
<td>Apolipoprotein A1, mg/dL</td>
<td>99.3 (6.0)</td>
<td>99.3 (4.8)</td>
<td>0.0 (4.8)</td>
<td>1.3 (5.0)</td>
<td>103.8 (6.0)</td>
<td>4.5 (4.2)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>110.8 (22.8)</td>
<td>100.8 (17.8)</td>
<td>-10.0 (7.0)</td>
<td>-5.7 (5.6)</td>
<td>109.1 (14.4)</td>
<td>-1.6 (14.8)</td>
</tr>
<tr>
<td>Lipoprotein (a), nmol/L</td>
<td>246.5 (61.5, 76.0)</td>
<td>170.6 (41.2)</td>
<td>-24.6 (8.2)</td>
<td>-11.7 (3.8)</td>
<td>168.0 (42.4)</td>
<td>-27.3 (7.8)</td>
</tr>
<tr>
<td>Free PCSK9, ng/mL</td>
<td>598.6 (42.8)</td>
<td>447.4 (73.9)</td>
<td>-151.3 (81.7)</td>
<td>-22.7 (13.1)</td>
<td>73.2 (17.0)</td>
<td>-525.4 (43.6)</td>
</tr>
</tbody>
</table>

Values are mean (SE) unless otherwise stated.

* To convert values for cholesterol to millimoles per liter, multiply by 0.0259. To convert values for Apolipoprotein A1 or Apolipoprotein B to grams per liter, multiply by 0.01. To convert values for triglycerides to millimoles per liter, multiply by 0.0113. To convert values for free PCSK9 to nanomoles per liter, divide by 72.
† Median (interquartile range).

Q4W: every 4 weeks; Q2W: every 2 weeks; SE: standard error; LDL: low-density lipoprotein; HDL: high-density lipoprotein; PCSK9: proprotein convertase subtilisin/kexin type 9.

Table 26.3: Efficacy Outcomes Based on Mutation Status

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Week 12 Q4W</th>
<th></th>
<th>Week 12 Q2W</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UC LDL</td>
<td>Apolipoprotein B</td>
<td>Lipoprotein (a)</td>
<td>UC LDL</td>
</tr>
<tr>
<td>Total (N = 8)</td>
<td>-16.5 (6.7)</td>
<td>-14.9 (5.0)</td>
<td>-11.7 (3.8)</td>
<td>-13.9 (9.6)</td>
</tr>
<tr>
<td>Defective LDL receptor (N=6)</td>
<td>-22.9 (7.2)</td>
<td>-18.3 (6.1)</td>
<td>-10.0 (4.7)</td>
<td>-23.6 (7.6)</td>
</tr>
<tr>
<td>Negative LDL receptor (N=2)</td>
<td>2.6 (2.6)</td>
<td>-4.5 (2.5)</td>
<td>-16.8 (5.7)</td>
<td>15.3 (24.6)</td>
</tr>
</tbody>
</table>

Average of Week 4, 8, and 12 Q4W

<table>
<thead>
<tr>
<th></th>
<th>UC LDL</th>
<th>Apolipoprotein B</th>
<th>Lipoprotein (a)</th>
<th>UC LDL</th>
<th>Apolipoprotein B</th>
<th>Lipoprotein (a)</th>
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<td>2.1 (5.6)</td>
<td>-22.7 (7.9)</td>
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Q4W: every 4 weeks; Q2W: every 2 weeks; SE: standard error; UC LDL: Ultracentrifugation low-density lipoprotein

Approximately 51 new subjects are enrolled into Part B. Subjects enrolled are randomized to a 2:1 allocation into 2 treatment groups: 420 mg 21B12 Q4W SC or placebo Q4W SC. Randomization is stratified by baseline LDL-C levels. Study visits occur every 4 weeks, with two optional visits occurring at week 2 and week 10. Visits entail collection of AE and SAE data, vital signs, concomitant medication, laboratory
tests, etc. A fasting lipid panel is collected at week 6 to assess the nadir LDL-C level in response to 21B12 treatment. 21B12 formulation is administered at day 1, week 4, and week 8. The end-of-study (EOS) visit and the last estimation of lipids occurs at week 12 for all subjects.

I Incorporation by Reference

All references cited herein, including patents, patent applications, papers, textbooks, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain preferred embodiments of the invention and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.
WHAT IS CLAIMED IS:

1. A method of lowering serum LDL cholesterol in a patient diagnosed with homozygous familial hypercholesterolemia comprising administering at least one anti-PCSK9 antibody to the patient in need thereof at a dose of about 120 mg to about 3000 mg, thereby lowering said serum LDL cholesterol level by at least about 10%.

2. A method of treating a patient diagnosed with homozygous familial hypercholesterolemia comprising administering at least one anti-PCSK9 antibody to the patient in need thereof at a dose of about 120 mg to about 3000 mg, thereby treating the homozygous familial hypercholesterolemia in said patient.

3. The method of claim 1, wherein the serum LDL cholesterol level of said patient is lowered by an amount selected from the group consisting of a) at least about 15%, b) at least about 20%, c) at least about 30%, d) at least about 40%, and e) at least about 50%.

4. The method of claim 3, wherein the anti-PCSK9 antibody comprises,
   a) a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:23, a CDRL2 of the CDRL2 sequence in SEQ ID NO:23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:23, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 49, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO: 49;
   b) a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:465, a CDRL2 of the CDRL2 sequence in SEQ ID NO:465, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:465, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 463, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 463, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:463;
   c) a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:12, a CDRL2 of the CDRL2 sequence in SEQ ID NO:12, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:12.
NO:12, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:12, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 67, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 67, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:67;

d) a light chain complimentarity region (CDR) of the CDRL1 sequence in SEQ ID NO:461, a CDRL2 of the CDRL2 sequence in SEQ ID NO:461, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:461, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 459, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 459, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:459;

e) a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:485, a CDRL2 of the CDRL2 sequence in SEQ ID NO:485, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:485, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 483, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 483, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:483; or

f) a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:582, a CDRL2 of the CDRL2 sequence in SEQ ID NO:582, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:582, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO: 583, a CDRH2 of the CDRH2 sequence in SEQ ID NO: 583, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:583.

5. The method of claim 3, wherein the anti-PCS9 antibody comprises,

(a) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 23 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:49;
(b) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 12 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 67;

(c) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 461 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 459;

(d) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 465 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 463;

(e) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 485 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 483; or

(f) a light chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 582 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO: 583.

6. The method of claim 3, wherein the anti-PCSK9 antibody comprises:

(a) a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 23, and a heavy chain variable region that comprises and amino acid sequence, SEQ ID NO: 49;

(b) a light chain variable region that comprises an amino acid sequence, SEQ ID NO: 12, and a heavy chain variable region that comprises an amino acid sequence, SEQ ID NO: 67;

(c) a light chain variable region that comprises amino acid sequence SEQ ID NO: 461 and a heavy chain variable region that comprises amino acid sequence SEQ ID NO: 459;
(d) a light chain variable region that comprises the amino acid sequence of SEQ ID NO:465 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:463;

(e) a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 485 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:483;

(f) a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 582 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:583; or

(g) a light chain variable region that comprises an amino acid sequence, SEQ ID NO:591, and a heavy chain variable region that comprises an amino acid sequence, SEQ ID NO:590;

7. The method of claim 4, 5 or 6, wherein the anti-PCSK9 antibody further comprises:

(a) the light chain constant sequence of SEQ ID NO:156;
(b) the light chain constant sequence of SEQ ID NO:157;
(c) the heavy chain constant sequence of SEQ ID NO:154;
(d) the heavy chain constant sequence of SEQ ID NO:155;
(e) the light chain constant sequence of SEQ ID NO:156 and the heavy chain constant sequence of SEQ ID NO:154;
(f) the light chain constant sequence of SEQ ID NO:157 and the heavy chain constant sequence of SEQ ID NO:154;
(g) the light chain constant sequence of SEQ ID NO:156 and the heavy chain constant sequence of SEQ ID NO:155; or
(h) the light chain constant sequence of SEQ ID NO:157 and the heavy chain constant sequence of SEQ ID NO:155.

8. The method of claim 6, wherein the anti-PCSK9 antibody further comprises a glycine residue at the C-terminal end of the light chain variable domain.

9. The method of claim 6, wherein the at least one anti-PCSK9 antibody is selected from the group consisting of 21B12, 11F1, 31H4, 8A3, and 8A1.
10. The method of claim 4, wherein the anti-PCSK9 antibody is administered to
the patient at a dose selected from the group consisting of: a) 120 mg to about
700 mg, b) about 140 mg to about 600 mg, c) about 140 mg to about 450 mg,
d) about 420 mg, e) about 450 mg, f) about 600 mg, g) about 700 mg, h)
about 1400 mg, i) about 1200 mg, j) about 420 mg to about 3000 mg, k)
about 1000 mg to about 3000 mg, or l) about 3000 mg.

11. The method of claim 10, wherein the anti-PCSK9 antibody is administered
to the patient on a schedule selected from the group consisting of: (1) once a
week, (2) once every two weeks, (3) once a month, (4) once every three
months (5) once every six months and (6) once every twelve months.

12. The method of claim 10, wherein the administering step comprises
administering the at least one anti-PCSK9 antibody parenterally.

13. The method of claim 12, wherein the administering step comprises
administering the at least one anti-PCSK9 antibody intravenously.

14. The method of claim 12, wherein the administering step comprises
administering the at least one anti-PCSK9 antibody subcutaneously.

15. The method of claim 14, wherein the at least one anti-PCSK9 antibody
comprises a light chain complementarity determining region (CDR) of the
CDRL1 sequence in SEQ ID NO:23, a CDRL2 of the CDRL2 sequence in
SEQ ID NO:23, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:23,
and a heavy chain complementarity determining region (CDR) of the CDRH1
sequence in SEQ ID NO:49, a CDRH2 of the CDRH2 sequence in SEQ ID
NO:49, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:49.

16. The method of claim 14, wherein the at least one anti-PCSK9 antibody
comprises a light chain variable region that comprises an amino acid sequence
that is at least 90% identical to that of SEQ ID NO: 23 and a heavy chain
variable region that comprises and amino acid sequence that is at least 90%
identical to that of SEQ ID NO:49.

17. The method of claim 14, wherein the at least one anti-PCSK9 antibody
comprises a light chain variable region that comprises the amino acid
sequence of SEQ ID NO: 23 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:49.

18. The method of claim 14, wherein the at least one anti-PCSK9 antibody comprises a light chain variable region that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO:590

19. The method of claim 17 or 18, wherein the at least one anti-PCSK9 antibody further comprises:

(a) the light chain constant sequence of SEQ ID NO:156;
(b) the light chain constant sequence of SEQ ID NO:157;
(c) the heavy chain constant sequence of SEQ ID NO:154;
(d) the heavy chain constant sequence of SEQ ID NO:155;
(e) the light chain constant sequence of SEQ ID NO:156 and the heavy chain constant sequence of SEQ ID NO:154;
(f) the light chain constant sequence of SEQ ID NO:157 and the heavy chain constant sequence of SEQ ID NO:154;
(g) the light chain constant sequence of SEQ ID NO:156 and the heavy chain constant sequence of SEQ ID NO:155; or
(h) the light chain constant sequence of SEQ ID NO:157 and the heavy chain constant sequence of SEQ ID NO:155.

20. The method of claim 19, wherein the anti-PCSK9 antibody further comprises a glycine residue at the C-terminal end of the light chain variable domain.

21. The method of claim 15, wherein the anti-PCSK9 antibody is administered to the patient at a dose of about 70 mg to about 450 mg subcutaneously once every two weeks, and wherein the serum LDL cholesterol level of the patient is lowered at least about 10 - 50% for about 7-14 days.

22. The method of claim 15, wherein the anti-PCSK9 antibody is administered to the patient at a dose of about 70 to about 450 mg subcutaneously once every month, and wherein the serum LDL cholesterol level of the patient is lowered at least about 10 -50% for about 21 to 31 days.
23. The method of claim 21, wherein the anti-PCSK9 antibody is administered to the patient at a dose of about 420 mg.

24. The method of claim 22, wherein the anti-PCSK9 antibody is administered to the patient at a dose of about 420 mg.

25. A method of lowering serum LDL cholesterol in a patient diagnosed with homozygous familial hypercholesterolemia comprising administering to a patient in need thereof an anti-PCSK9 antibody comprising a light chain that comprises the amino acid sequence of SEQ ID NO:591 and a heavy chain that comprises the amino acid sequence of SEQ ID NO:590 at a dose of about 420 mg, thereby lowering said serum LDL cholesterol level by at least about 10%.

26. The method of claim 25, wherein the anti-PCSK9 antibody is administered to the patient once every two weeks.

27. The method of claim 25, wherein the anti-PCSK9 antibody is administered to the patient once every month.
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**FIG. 1B**
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FIG. 1B3
FIG. 1B4
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<td>G2Y</td>
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31H4

Nucleotide sequence of heavy chain variable region:
5'GAGGTGCAGCTGGTGGAGTCTGGGAGGCTGTCATGAGCCCTGGGGGCTGCAAGCTGGCTG
GACTCTCGTGTGACGCGCCCTCTGGAATCCACTTCGATAAGCTATAGCTAGCAACTGGG
GCCAAGGCTCCAGGAGAGGGGCTGGAGTGTGCATCTCAATTAAGTAGTAGTAGTAGT
TACATTTCTCTACGCCAGACTCGATGAAAGGCAGATTACCCACACTTCCAGACACAGCC
AAGAACTCACTGTATCTGCAAATGAACAGGCTGAGACCCAGAGACGCCTGTTA
TTCTGTGCGAGAGATTACGATTTTGGAGTGCTTATATGATGCTTATGCCG
GGCCAAGGGGACAATGCTACGGTCTCTTCA3' (SEQ ID NO: 152)

Amino acid sequence of heavy chain variable region:
EVQLVESGGGLVQPGSSLRLSCAASGFTSSYSMNNVRQAPGKGLEWVSSISSISISY
ADSVKQRFTSRIIDRKNSLYSLQMNSLRAEDTVYYFCARDYDFWSAYYDAPDVWQGT
MVTVSS (SEQ ID NO: 67)

Nucleotide sequence of light chain variable region:
5'CAGTCTGTGCTGAGGAGGCACAGCGCCTCCATGCTGCGGCCCCAGGAGAGGGTCA
CCATCCTGCTGAGCTGGGAGGAGCAGCTCCAACTCGGGGAGGAGTTATGATGATCCTG
ACCAGCAAGCTCCAGGAGACAGCCTCCAAACTCCCACTCTCTGGIACAGCAATCGGC
CCTCAGGGGTTCCCTGACCCGATTTCCTGTGGCCCTCAGCTGCGACTGACCTGACCCCTG
CCATCAGTGGGCTCCAGGCTGAGGAGTGCAGGCTGATATTACCTGCGACTCCATGAC
GCAAGGCTGAGTGTTCTGATTTCCGGCAGGGCAACAGCTGAGCTCTTCA3' (SEQ
ID NO: 153)

Amino acid sequence of light chain variable region:
QSVLTPPSVSGAPQQRTVSITSCGSSSNIGAGYDIVVWYQQGTPAKILLISGNSRPSGV
PDRFSKGSKSASLASTGLAQEDAEYYCQSYDSSLSSGSGVF GGKTTLTVL (SEQ ID
NO: 12)

FIG. 3E
Nucleotide sequence of heavy chain variable region:
5'CAGATTCAGCTGGTGACAGTCGAGGAGCAGGGAAGGAAGGCTGGGCCTCAAGTGA
AGGTCTCTGCAAGGGCTTGGTTACCCCTTGACACAGTATCTAGTCAGCTGGTGAC
GACAGGCCCTGGACAAGGGCTTGGGATGCTGAGGATGCTGACGCGTTCACAAATGT
AACAAACTATGCACAGAAGGTCAGGGCAGCTACCATGACCAGACACATTC
CAGAGCAGTCACTATGGAGCTAGGGACGCTGAACTGACGACGACGCGCTGAT
ATTACACTGTCAGGGCTACAGTGATAGTGAGCTGGGCGCAAGGACCCAGGCTCACC
GTCCTCTGCT3' (SEQ ID NO: 92)

Amino acid sequence of heavy chain variable region:
QQLVQGAEVKKPGAVKVSCKAAGAPLTSYGISWVRQAPGQGLEWMGWISAYNGNTY
AYQKVQGSVTMTDSTSTTYMEISSLRSDDLCTAVYYCARGYGMVDVWGQGTTTV
SS (SEQ ID NO: 48)

Nucleotide sequence of light chain variable region:
5'CAGTCTGCGCTGCATCAGCCGGCTCCGTGCTGCTGCTGGTCTCTCTGCAGTCGATCAC
CATCTCCTGCACCTGAAACCCGACAGTGGAAGTTGGTGTTATAACTCTGTCCTGCTA
CCAACTATGACACGCGGGAAAAACCAACATTAGTATGAGTTGAGTCAGATACGGC
CTCTACAGGGTTCTAATCGCTCCTCGGTCACAAAGTCTGGCAACACGGCTCCCTGTA
CCATTCTGTCGGCTCAAGAGGAAGAGGCTGATAATTCTGCAAGCTCATACAA
GCCACAGCATGGCTCTGGCCGAGGAGCCCAAGCAGCGTCACCTCT3' (SEQ ID NO: 93)

Amino acid sequence of light chain variable region:
QSALTQPASVSGSPQSWSTSTSTDDVGVGNVSVGQYQPGPKKLYEVSNRFSGGV
SNRFSGSKGSNTASLTISGLQAEDEADYFCSSTSTSMVFGGTKLTVL (SEQ ID NO: 19)

FIG. 3F
Nucleotide sequence of heavy chain variable region:
5' CAGGTTCAGCTGGTGACACGCCCTGGCCTCTGGTCAGTTTTATATGCTTAT
AACTATGTGCAAGAGCTGGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
ATTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
GTCCTCCTCT3' (SEQ ID NO: 94)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVQSSGLLTVSP
NTVEMQKGLLQGRGTMSSTSTAYMELRSRLSDTAVVYCAR
YMDVWVSFSFYNGVSS (SEQ ID NO: 49)

Nucleotide sequence of light chain variable region:
5' CAGTTGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
GTCCTCCTCTCT3' (SEQ ID NO: 95)

Amino acid sequence of light chain variable region:
QSLTQPASVSPGGGGLEDISTTCSTGSSDVGGYNSVSVYQQHPGKAPKLMYEVSNRPSGV
SNRSQGSGKNTASLTGLQADEADYYCNSYTSMTSMVPFGGTLLTVL (SEQ ID NO: 23)

Alternative Nucleotide sequence of light chain variable region (26E10v1):
5' CAGTTGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
CAGTTAGCTGGGCTGAGTTGATGAGAGGACACCCCTTCTAG
GTCCTCCTCTCT3' (SEQ ID NO: 293)
Nucleotide sequence of heavy chain variable region:
5' CAGTTTCACTGGTGCAATCTGGAGGAATGAAAGCCGGTCAGAGTA
AGGCTCTTCCAGCTCTCGGTCACGCCCTTACACCTGAAAGCTATGGATATCAAGTG
GACAGGCCCCTGGACAAAGGGCTTGGATGGAATGGAATGGAACCTGCTGT
AACAAACTATGCAAGAAGGCTTTCAAGTCTGACCTGACCAGACAGACATCC
CACAGACACTATACATCTACAGTTGAGGAGATGAGATGAGCTCTGACCGAAGG
ATTACTGTGGAGAGGGCTAGGTATGGACGCTCTGGGGGCAAGGACCACGCT
GTCTCCTCCT3' (SEQ ID NO: 96)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVTKVSCKASGYTVTLTSYGPSWSVQRAPGQGLEWMGWISFYNGN
TYNAQRKVRQRTMTTIDSTISSTYIMELRSLRSDTAVAAYCARGYGMMDVVGQGTVTV
SS (SEQ ID NO: 51)

Nucleotide sequence of light chain variable region:
5' CAGTTCTGTCCTGACCTACGCCCTCGGCTGGTCTCTGGACAGTATCC
ACACCCCTGGTACCTGAAGCAACAGGACGATGATGGTTATATACCTGTCTGCTCC
CCAAACAGACACCAAGGCAACCCCAACACATGATTATGAGGATGCTAATCGGC
CCTCAGGGTGTTCGTTGGTCCTGTGCAAGTCTGGCAACAGCCGACCGTCCTGAC
CACTCTGGGCTCCAGGCTGGAGGCTGGCTGTTATATCTGACGTCATATACAA
GACCAGCATGGCTTCCGGCGGAGGACCAAGGCTGACCCTCCTA3' (SEQ ID NO: 97)

Amino acid sequence of light chain variable region:
QSALTQPSVSGLPGSQGSITSGTCGTSDDVGGYNSVSVWYQQHPGKPMLYIVSNRPSGV
SIRFSGSGNTADSLTSLGLQAEDÄADYFCSSYTSTSMVFSGGTKLTVL (SEQ ID NO: 17)

FIG. 3H
Nucleotide sequence of heavy chain variable region:
5' CAGATTCACTGGTCAGTCTGGAGTGAAGGCTCGCTCGCTTACACCAGCTATGGTACAGCTGGGTGC
GACAAGGCGCTGGAGCAAGGCTTGGTGATGGATGGATGGAFCAGCTTGTACATGGT
AACACAAACTATAGCAAGAGTCAGGCAGACAGTGCAACCATGACGACAGACACATC
CAGAGCAAGTCTACATGGAGTGAAGCAGCCTGAGATCTGACACGACAGGCGCTGT
ATTCTCTGTCAGAGAATGGATACGGATGGACGTCTGGGGCCAAAGGACGACGGTACC
GTCCTCTCA3' (SEQ ID NO: 98)

Amino acid sequence of heavy chain variable region:
QIQLVQSGAEVKKPGASVKVSCAKASGYTLTSYGISWVRQAPGQGLEWMGWISFYNGNT
NYAQKTVGRETMITIDSTSTSTVYMELRSLQSDTAVYFACRGYGMDVWGQGTTTVS
S (SEQ ID NO: 53)

Nucleotide sequence of light chain variable region:
5' CATCTCTGCGCTGACTCACTGGCTCTCCGATGCTCGCTCGCTGAGTACGTCAGTCAAC
CATCTCTGCACTGGACAGCAGTGACAGTTGCTGATATAAATCTGCTCTGATGGTA
CCACAGAGCAGCGAAGCCACCCCAACCACACACTAGTATGTTAGAGTCAGTAAACGC
CTCAGGGTTCTACTCGCTTCTGCGCTCGCTCGCTCTGACCACGCGCCTGCA
CCATCTCTGCGCTCAGCGCTAGGGACGAGGCTGATATTCTGTGACGTCATATAAC
AGCAGGAGCATGGTCCTCGGCGAGGAGCAGGCAAGGCTGCGCTA3' (SEQ ID NO: 99)

Amino acid sequence of light chain variable region:
QSIDLQAPSVGSQPQSTSIKLYICTGSSDVGGYNVSVQNYQHPGLAFQPPYQLYKVMYVEVSNRPAG
SNFSGKSGNTASNLTSQGLAQEDDEAAYFCCSYTSTSVMFGGKTAL (SEQ ID NO: 18)
Nucleotide sequence of heavy chain variable region:
5' CAGGTTTACGCTGGTGCAGCTGGTGGCAAGCTAGTTGGAAGCAAGCCTGAGGCTCAAGTGA AGGTCTCTGCAAGGCTCTTCTGGTTAACACCTTAACCAGCTATGGTATCGCTGGTGTCG GACAGGGCCCTGGCAAGAAGGCTTTGCTGGATGGAATGGATGACCGACAGTACCTTTATTATGGT AACAACAAGACTATGCACAGAGCTCAGGGCGAGGACGAGGCCACATGACCACAGACCACATC CACGAGCACAGCCCTACATGGAGCTGAGGGACCTGAGATCGACGAACAGGGGCTGCTGATTATGGGAGAGGTCTCGTGGGAACAGCTGGATGACACAGGCTACCAGCTCCCTCA3' (SEQ ID NO: 100).

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVQYVKVSCKASGYVTLYSGWVRQAPGQGLEWWMGWSFYNG NTNYAQKILGFDTMTPSTAYMELRSLLSDDTAVYYCARCYGRGMDIFFWGGQGTVT VSS (SEQ ID NO: 50).

Nucleotide sequence of light chain variable region:
5' CAGTCTGCCCTGACTCAGCTGCCCTGCTGGGCTGCTTCCTGGACAGTGATCAGC CATCTCTGCACTGGAAACCAGCAGTGACGTTGGTATATACCTCTCTCCCTGTA CACACAGCAACCAGCGCAAGCCCAACCATGATATTATAGAGCTGACACTAACAGGC CCTAGGGGTCTTCTATCGCTCTGGCTCCTCAAAGCTGGCAAACAGGCCCTCTGCA CCATCTCTGGCCCTAGGCTAGCAGGCTGATTAACCTCAGACACTCTATACAAA GCACACAGCATGGTCTCGGCGGAGGCGACAGCTGGACCGCTCCCTCA3' (SEQ ID NO: 101).

Amino acid sequence of light chain variable region:
QSALTQPASVSFGPSQSITISCTGTSDDVGGINSVSVYQQHPGKAPKLMITIVETNRPSSGV SNLPSGSKSGNTSLSTISGIAAEDADYYCNSYTSSTMVFGGGTLTTLV (SEQ ID NO: 26).
Nucleotide sequence of heavy chain variable region:
5'CAGGTGCAGCTGCAGGAGTCGGCAGCGCCAGGACTGTGAGCGGCCACGACCTCACTACAGCCCTGT
CGGTCACTCCGACTGTCCTCTGTGCTGCAGCAGTGGTGGTCTACTGCTGGACTGTGAGCCTG
GGAGAACAGCCCTGGGTCCGCTCACTCAGTGAGAGTACGTTCTATTGCAGAGCTGAGCA
GATTACGTGTCGGAGAGGAGATACAGCTATGGTCCTTTACTTGGTCGAGCTGAGCA
GGGAAACCTGGTCACCAGTCTCTCA3' (SEQ ID NO: 102)

Amino acid sequence of heavy chain variable region:
QVQLQSEGPGLVKPSQTLSTACTSVGGGSSGGYWSWRQHPGKLEWIGYIVNSGTY
YNPSTKSRVTISYDSNKQFSLKLSSVTAAADTVYYCAREDAMPYFYDVPDYWGQGTKV
VSS (SEQ ID NO: 87)

Nucleotide sequence of light chain variable region:
5'CAGCTGTCAGCGCAGCGCCCTCAGCTGCTGGCCACGACGAGGGTCA
CCATCTCCTGACTGGGAGAGCTGCAACATCGCGGCAACCATGATGTGACACTGGT
ACCAGCAAGGTCCAAGACAGACCCACAAACTCCTGCTATGTAACACCTATCGGC
CCTGAGGGTCAGGAGCGTCTCTTTATCTTGCTCCAGTGTTACATATCATCCAG
CCATCTGTCAGGCCGCTAGCTGAGATGAGCTGATTACGTCAGTCCACTATGACA
ACAGCCCTGAGCTGGTGTTACGGGAGGGACCAAGCTGACCTCA3' (SEQ ID NO: 103)

Amino acid sequence of light chain variable region:
QSVLQTQPGSAGPQYRTISCTGSSNSNAGHYDVHYQWPQGTAKPOKLIIYGMTYRPSG
VPDRFSGSKSGTASLAIITGLQAEDDEADYYCQSYDNSLSGTVFVGCGTKLTVL (SEQ ID NO: 13)

FIG. 3K
Nucleotide sequence of heavy chain variable region:
5’ CAGGTGCACCTGGTGGAGCTGTCACGGCTTGGAGGTCCCTGA
GACTCTCTGTCGACGCCTGCTGGATTTCACTTCCAACAGCTTGGCATTGCCAGCTGGTCC
GCCAGGCTCCAGCAAGGGCTGGAGGTGGGACCTATCTGCTGATGAAAGT
GATGATACATTAGCACGACTCCGGTGAAGGCGCATTACACCATTCCTCAGAGACAATTC
AAGAACACAGCTGTATCTGCAATAAGACAGCCTGAAGCITCAGTCACGGACACGGCTGTGA
TTACTGTGGCAGAGCCATAGCAGACGCCCTCTACTACTACTAGGTATGGACGTCTGGGG
CCAAGGGACACCAGGTCCACCGTCTCTCCTCA3’ (SEQ ID NO: 104)

Amino acid sequence of heavy chain variable region:
QVHLVESGQGVYQPGRSLRLSSACASSGFTFNSFGMHWVRQAPGGLEWVALWSDGSD
EYYVADVVKQRFTISRDNKNTLYQLMNSLRAEDTVYYCARAIAALYYGMDVWGQ
GTTVTVSS (SEQ ID NO: 79)

Nucleotide sequence of light chain variable region:
5’ CAGCTCTGTGTGGACGACGGCCTCTGATCTGGCGGCCCGCAAGACAGGTCAC
CCATCTCTCTGCTGGAAAGCACGCTCCACAGCTGGAAATTTTGATTTGCCCTGGFACC
AGCAGCTGGCTGGATAGTATATTAGCTTAATAAGCGACCCCT
CAGGGATTTCTGACCCAGATCTTCTGTGGTCACAAGTCTQGACGTCAGCCACCCGGGCA
TACCCGAGTCCCACTGGGAGGAGGGCGATTATAGCTGGGAACTAGGATAGC
AGCTGAGTGGTTATGTCTGGAACTGGGACACCAGGTCAACGGTCTCTCCTCA3’ (SEQ ID NO: 105)

Amino acid sequence of light chain variable region:
QSVLQPPSVSAAAPQKVTVICSGSSSSNIGNNFLVNYQQLPGTAPKLLIYDYYNKRPSGIPD
RFSGSKSGTSAIQLGTGLQTGDADYYCGTWSSLSAYVFGTGTRTVTL (SEQ ID NO: 35)
Nucleotide sequence of heavy chain variable region:

```
5' CAGGTGCAGCTGCTGAGTGCTGCTGTCAGCCCTGGGAGCTGCGGTTCCCTGAG
GACTTCTCTTGACGCGTGTTGAGTCTTTGGGATGACCTCGTTCACGCTTTGAGGTTCC
GCCAGGCTCAGGGACAGGGCAGTGCTGGGGCTAGCATTATGGAATGATGAACT
AATAAACTATGACAGTCCCGTGAAAGGGCAGTATCCACCATCTCCAGAAACTATTCC
AAGAACACGCTGTAATTCTGCAAATGAACAGCAGGAGACCGAGGAGACGGCTGCTGTA
TTACGTGGGAGGCCATGAGCCGGCCCTCTACTACTACTACTAGGTATGAGCCTCTGGG
CCAAGGAGCCACGCTCCGTCCTACCTCA3'  (SEQ ID NO: 106)
```

Amino acid sequence of heavy chain variable region:

```
QVQLVESGGGLVQPGRSLRLSCAASGFITFSSFSAGHWVRQAPGKGLEWVLWLFWNDGSN
KYYADSVKGRFTISRDNSKLYLQMQNLRAEDTAKEYCARRAIAALYNYVGMDVGWQL
GTTVTVSS  (SEQ ID NO: 80)
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Nucleotide sequence of light chain variable region:

```
5' CAGTCTCTGCTGCTGAGCGCAGGCCCGCTCAGTGCTGCTGCCCCAGCAGAAAGGCTCA
CCTCTCTCTGCTGGAGACGACCTCCAAACTGGGAATATTITGTAATTCCTGGCTACC
AGCAGCTCCAGAAGCGCCCGCCAAAATCTCACTAATAAGCGACCCCT
CAGGGATATCTCCTCCAGATTCTCTGCTCGTCAAGCTGCTGGCACTGCACTCCGGCCA
TCACCCGGTCTCCACAGCTGGGAGCCAGGCGATTACTCCTGGGAAACATGGGATAGC
AGTCTGAGTGGTTATGTTCTGGGAACCTGGAACCGGTGCACGGGTCTCCACCTA3'  (SEQ ID NO: 107)
```

Amino acid sequence of light chain variable region:

```
QSLVTLQPSVSAAPQKVTSCSGSSSNIGNNFVWSYQQLGQPDKKLLYDYNKRPGD
RFSGSKSGTSAATLGITGLQGTDEAVYVCGTWDSSLSGYVFGLTGVTVLS  (SEQ ID NO: 36)
```

FIG. 3M
Nucleotide sequence of heavy chain variable region:
5'CAGGTGCACCTGGTGAGCTCTGGGGAGGCCTGGTCCACGCTGGAGGCTCCCTGA
GACTTCCTGTGACGCCGTCTGATTCACTTTCAACAGCCTTGGCATGACCTGGTCC
GCCAGGTCCAGGGAAGGGCTGGATGGGGCTGCCATATTAGTGCTGATGGAGAT
GATAAATAGATGACACCCGTGAGAGGCGGGATACCACTCCAGAGGACAAATCC
AAGAACCAGCGTTACTGCAAATGAAACAGCCTGAGAGCGGACGACAGCCTGGA
TTACTGTCGCGAGACGATAGACGACCTCATTACTATTACGGTATGACGTCTGGG
CAGGGGACCACGGTACCCCTCTCTCA3' (SEQ ID NO: 108)

Amino acid sequence of heavy chain variable region:
QVHLVESGGGVMVQPSRLSLCASAAGFTFNSFGMHWVRQAPKGLEWVALIWSDGD
KYYADSVKGRFTISRDSNKLTYLQMNSLRAEDTAVYYCARAIAYYYGMDVWQ
GTVTLEVSS (SEQ ID NO: 76)

Nucleotide sequence of light chain variable region:
5'CAGITCGTGTGACGACGCCGCCCTCAGTGCTCGGCCAGGACAGAAGGTCA
CATCCTGCTCTGGAAGCATTCCACATGGGAATAATTGTATTCGTGATTCC
AGGACGTCACGGAAAGCCCGCCAAACTCCTCATTTATGGACTATAATAAGCGAACC
TCAGGATTTCTGGATTCTCTGGCTCTGAAGCTGGCAGGTCAAGCTGCACTGGG
CTACCCGATCTACGGAGGACGGGCGATTATTACGTGGAGGAATGATAGC
AGCCTGACTTATATGCTCCGAACTGGGAACGGGTTAACCCTCTCTCA3' (SEQ ID NO: 109)

Amino acid sequence of light chain variable region:
QSVLTQQPSVSAAPQQRTTSUCGSSSNIGNNFSWYQQFPGTAPKLLYDYNCRPSGIPD
RFSG5KSQTSATLIGTQLQGDEAYYYCCTGWDSSLSSYVFVGTGTRVTVL (SEQ ID NO: 37)

FIG. 3N
Nucleotide sequence of heavy chain variable region:
5' CAGGTGCACTGGTGGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTGAGCTGGTG4

Amino acid sequence of light chain variable region:
QSVLTQPSVSAAAPGQKTVTSC50SSNSNIGNNFVSWYQQLPGTAPKLLIYDYKRPSPGIPD
RFBGSKS6GTSATLGITGLQTTGDEADYICYGTWDSSLSGYVFGTGYVTYL (SEQ ID NO: 38)
Nucleotide sequence of heavy chain variable region:
5' CAGGTGCAGCTGGTGAGGCCTGGTCCAGCTGAGGGAGCTCCTGA
GACTTCTCTGCTAGCTGGTGATTCACTCCCTGGGATCGCATTTCCTGAG
GGACAGGCTCACGGCTGGGATGCTGCTGGTGGCGAATTACTATGACGAGAAGT
AAAGAAATACTATGAAGACTCCCGTGAAGGCGGATTACACTTTCCAGAGACAAATCC
AGGAAACCGCCGAAATCTGAAACACGCCTGAAGCGGGAGAAGCGGCTTGCTA
TTACTCTGCGAGGCCCATAGCAGCCCTCTACTACTACTACCGGTATGGACGTCTGGG
GCAAGGGACACCGTCACCGTCTCTCA3' (SEQ ID NO: 112)

Amino acid sequence of heavy chain variable region:
QVQLVESGGGLVQPSRLRLSCAASGFTSFSGMWVRQAPGKGLEWVQLIWNNGSN
KYADSVKGRFTISRDNSKLTVMLQNSLRAEDTAAYYCARALAEYYYGMDVVGQ
GTTVTSS (SEQ ID NO: 78)

Nucleotide sequence of light chain variable region:
5' CAGTCCTGTGTACAGCAGCAGCGCCAAGAGAAGCTCCTCAAGACAATGGGAAATAAATTTTCTGCTGATT
CGCACAGCCTCAAGAACAGGCCCCAAAACCTCTCATTTAGACTATAAATAGCGACTCT
CAGCAGATCCCTGACCGATTACTCTGCTGCTCAAGTGCAGTGCACGACGCAACCCTTGCCG
TCAACCGGACTCCAGACTGGGAGACAGGCCGATTACACTCTGGGACACATGGGATAGC
AGCTGGAGGTGTTATGTCTTGGAGACTGGGACAGAGYTCAACCGTCTCA3' (SEQ ID NO: 113)

Amino acid sequence of light chain variable region:
QSLLTQPVTSAAPGKVTVICSGSSSNIGNNYFVSWYQLPPTAPKLILYDYNKRPSEGIPD
RFSGKSGTATSALTGIGTGLQTDREADYYCGTWDSSLSGYVFGTGTRTVTL (SEQ ID NO: 39)

FIG. 3P
31G11

Nucleotide sequence of heavy chain variable region:
S*CAGGTGCACTTGTTGGAGTCTGTTGGGGAGGCCGTGGTCCAGCTGGGAGGTCCCTGAGA
GACTCTCTTGATGAGTCTCTTCTATTACACCTTACAGAGCTATGGCAGCTGTGGCTCC
GCCAGGTGCTCCAGGAAAGGGCTTGGAGTGCTGGCTGCACTATATATGGCTAGATGAGT
AAATGCTTCTAATGTAGAGTCTCCCGTGAAGGCGGCTATCCACTCCAGAGACAAATC
AAGAAGACCGCTGTATCTGCAAATAGGAAAGCCTCTGAGAGCCGAGACAGGTGTGTA
TTCAGTGGCAGAGGTATAGCAGTGGTCTTACTACTACAGGTTATGGACGCTGTGGGG
CCAGGGACCACGGTCACGGTCTCCTCATTCA3' (SEQ ID NO: 114)

Amino acid sequence of heavy chain variable region:
QYVLVESGGGVYQGRSLRLSCEAASGFTFRSYYGHRWVRQAPGKLEWVALIHWDGSN
TYYVDSVKGRTIISRDSKNTYLLQMNSLRAEDTAAYYCARGIAYAVYYGMDVVGQ
GTTTVDSS (SEQ ID NO: 83)

Nucleotide sequence of light chain variable region:
S*CAGTCTGTGGTACGCAACCCCTGAGCTGGCCGCCAGGAGAGGTGAC
CCATCTCTGTGCTGGAACAGCTCCAAACCTTCCTTATGAGAATAATATGGCTACTCC
AGCAGCTCCCAACGCAAGCCTAAACTTCTCTTATGACAGTAAATAGGCGAACC
CAGGGATTCCCGACGGATCCTCTGCTGCTCAGTCGTCAGCTGGCCACAACGCTGACA
TCAGCCGAAGCTTsCTGGAGAGGGACGAGGCGATTATCTTGCGAGAACATGGGATAGC
AGCCTGAGTCTTTATGTCTTCAGGACTGGGACCAGGTACCCCTA3' (SEQ ID
NO: 115)

Amino acid sequence of light chain variable region:
Q5VLTVQPSVSAAAGQKVTSICSGSSNSQNFVWSWYQQLPGTAPKLLYDAYSNKREVP
DSGSKGTSATLLDIVLQYGDADYAYVYCGTWDSDSAYAYVFGTGTKTVL (SEQ ID NO:
40)

FIG. 3Q
**23B5**

**Nucleotide sequence of heavy chain variable region:**

5’GAGGTCCAGTGTGGAGTGCAGCCTGGGGAGAGTCTGGTACAGACTGGGGCCGTCAGCTGGAGCCAGCAGCTCCAGTGGAGATGGTCTCAGACTATTAGTGATGATGATGATGATGAT
AACAAGGGCAGACTTCGTGGAGGGCGCGTTCACTCATCAGAAGAGCAGACATAC
CAAAACAAGCCTGTATCTGCAAATGAAACAGCCTGAGAGGCGCAGGACACGCGCTAT
ATTACTGTGCGAAAAGTGTGTCAGTAAATGTTGTGATGCTATGCTGCTGAGACTACGGGCCC
AGGGAACCCCTGGTCCACGGTCTCCTCA3’ (SEQ ID NO: 116)

**Amino acid sequence of heavy chain variable region:**

EVQLLESGGGLVQPGSGSLRLSCAASGFTSSYAMNWVRQAPGKGLEWVSTISGSDNT
YYADSVKGRFTISRDNSKNTLYQMNLSRAEDTAAYYCAKFKVLHYAMLYWGOOD

**Nucleotide sequence of light chain variable region:**

5’GACATCCCTGATGGCCAGACCATCCTGCACCTCTGTCCTGGATCTCTGGAGACAGAGT
CACCAGATCTGCGGAGGAGAACTAGCAGACTGATTTCAGATTAAATTTGGTATCAGCA
GAAACAGGGCAGACCCCAAGCTTCACTCTGCTGCTAATCTGGCGCCTCAGTTGGAAGTGG
GGTGCCATCAAGTTCAGAGTGGGATCAGTGGAGAGATTTCACTCTCCACTCAACCAC
CAAGCTGCAACACCTGAGATTGGCTAAGCTACTACTGCAAGACGATTACAGTTCCCAT
CTACACTTCCGCAAGGGACAGCACTTGAGATTAAA3’ (SEQ ID NO: 117)

**Amino acid sequence of light chain variable region:**

DILMTQSPSLASAVGDRYVLTCRASQSSYLYNLWYQQKPGKAPKVLYAASSLQSGVS
RFSGSGSTDFTLTINSQPEDFATYVCQQSYSSPITFGQGTRLEIK (SEQ ID NO: 9)
25G4

Nucleotide sequence of heavy chain variable region:
5'GAGGTGCAGCTTTGGAGGTCTGGGGGAGGCTTTGATACAGCGGAGGCTCCTGA
GACTCTCTTTGGAGCCTGAGCCTTGATTACACCTCTTAGACGATAGCATCGGTCT
GCGAGGCTCGAGGAGGGGTAGGGGTGGGTCTCAACTTATGAGTGGATGGGTGT
AACACATACACGGAGCTCGGTGAAAGCCGTTGACCACATCGAGACAAATTCC
CAAGAAACACGGCTATCTGCAAACTGAAACAGCTGAGACGCGAGACAGCAGCT
ATTACTGTCGAGAAAAGGTTTGTACTAATGTGTATGTGCTATGCTTTGACTACTGGG
AGGGAAACCTTGGTACCCGTCCTCTCCTCA3' (SEQ ID NO: 119)

Amino acid sequence of heavy chain variable region:
EVQLVESGGGLVQPGSGRLSLCAASGFTTSSYAMNWVRQPGRGLEWSIDNGSNT
YYADSVKGRFTISRDNSKNTLYQMLRRAEDTAVYYCAKPGPLMVYAMLDYWQQ
TLVTVSS (SEQ ID NO: 72)

Nucleotide sequence of light chain variable region:
5'GACATCCAGATGCAGCCAGCTCCTCCTCCCTCTATCTGCAATGGAGACAGAAGT
CACCATCTCTGGCCGGAAGTCAGCAGCATATAGCCTATATTAAATGATTGCAGC
GAAGCCAGCGGGAAAGCCCCTTTACCCCTCTGATCTATGCTGCAAGCCATTGG
GTTCCATTCAAGGTCTCGTGCTGGGACAGATTTCATCTCACCACATCAG
CAGTCGCAACCTGAAAGATTTCGCAACATTACTACTGTCACAAGGTACAGCG
CTCCTCGGGAAAGCCACAGACACTGGGAGATAA3' (SEQ ID NO: 119)

Amino acid sequence of light chain variable region:
DIQMTQSPSLSLASVGDRVTITCRASQSISIVLYNWWYQQKPQKPAYLLIYAAASLQSGVPSR
FSGSQSGTIDFTLTISSLQPEDFATYYCQQQSYASAPITFQGQTRLEIK (SEQ ID NO: 10)

FIG. 3S
Nucleotide sequence of heavy chain variable region:
5' CAGTTCAGCTGGTCCATGCTGAGCTGGGAGAAGCTGGGTTTGAAGAGCTGGGCTTGGAGCTGGGGCTG
GACAGGCCCTGTGCACAGGGTGCAGGGGATGCTTGGATGCTTGGGACAGGCTTGGGGCTG
TACAGAAACTATGCAAGAGTGCCAGGCAAGAGCTGACACAGCAGACAGCT
ATATTAGGCGAGGCTACGTATGGACGTCTCGGGCCAGGGCACCAGCTGACACC
GTCTCCTCA3' (SEQ ID NO: 120)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVKKVSQKASQIGSLTYSGGWVRQAPGQQLWWMGVISAYNGN
TNYAQKVKV QVGRVTMTTDTSTSTVYMEVSLRSDTDGTVYYCARQGYGMVWGWQQT
SS (SEQ ID NO: 54)

Nucleotide sequence of light chain variable region:
5' CATCTGCCTTCACTCGCCCTCTCCCTCCTTGGTCTGGCTCTCCTGGGACAGTCACTCA
CAGCGCCTGCACTGACACAGAGTGACGTTGGTGTGTTAACAATCTGTCTCCCTGTG
CAAGACCGGCGCTCTTCAATGGGGCTTTCTGCTTGGGTGCCCAGGCAATACGGCCCTCTCTGA
CAATCTCCTTGGTGTTCGCTAGGACAGCAGCTGATTATTTCTTGCAGCTCATATAC
AGCAACAGCATGTCCTCGGCGGACAGGACCAGCTGACCCCTCA3' (SEQ ID NO: 121)

Amino acid sequence of light chain variable region:
QSLTQPASVSGPSQSSICSTTGTSSDVGYSNSVSWYQQHPGKPPKLMVSELYEVSNRSAGV
SFRSFGSKSGNTASLTISGLQAEDEADYFCSSYTSTSMVFPGGKTGLTVL (SEQ ID NO: 20)

FIG. 3T
Nucleotide sequence of heavy chain variable region:
5'CAGTCTGCTGACTAGCAATCAGGTTGCTGGCTGTCTCCTGACAGTCCATCAC
AGCTGCTGACACTGAAACAGACTGAGCTGGCTGTCTCGTGTCGTCGTCG
GACACCTCTGCGAAGGGCTTGGAGGGATGGATGAAGGGCGGGTTACACAT
ATTACTGCGAGAGGCTACGATGGACGTCTGGGCAAGGACCACGCTGACC
GCTCTCCTCA3' (SEQ ID NO: 122)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVTKASQTVLTIAPGQGLEWIMQTWNLSTSSY

Nucleotide sequence of light chain variable region:
5'CAGTCTGGCCCTGACTCA GGCTGGCTGTCTCCTGACAGTCCATCAC
CATCTCCTGCACTGAAACAGACTGAGCTGGCTGTCTCGTGTCGTCGTCG
GACACCTCTGCGAAGGGCTTGGAGGGATGGATGAAGGGCGGGTTACACAT
ATTACTGCGAGAGGCTACGATGGACGTCTGGGCAAGGACCACGCTGACC
GCTCTCCTCA3' (SEQ ID NO: 123)

Amino acid sequence of light chain variable region:
QSALTQPSVSGPSQITTSCTGTGSDVGYYNSVWYQQHPGKPPLMIYEVSNRPSGV
SIFPSGSKSNTASLTISGLQAEDEADYFCSSYTSTSMVFGGKTGLTLV (SEQ ID NO: 16)
Nucleotide sequence of heavy chain variable region:
5’CAGGTTCAGCTGGTGCACTGCTAGCTGAGGTTGAAAGAAGCTGGGGGCTCAGTGAA
AGGTCTCTCAGCAAGGCCTTCTGTTACCCTTGGACCAAGCTATCGTATCGTGCTG
GACAGGCCCTGGACAAAGGGCTTGAAGTAGGATGGAGTGGATGCGATCAACGGCTTACAAATGGT
AACACAAACTATGCAACAGAAAGCTCAGAGGCAGATCACCAGAGCACAGATCA
CAGCGAACAGCTCAGATGAGTGAGGGAGCCTGAGATCAGACAGCCCGGTGT
ATTACTGTGGAGAGGCTACAGTATGGAGCGTCTGGGGGCAAAGGGAGCCACGCTCACC
GTCTCTCA3’ (SEQ ID NO: 124)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVKVSCKASGYPLTSYGISWVRQAPGQGLEWMGWISAYNGT
TNYAQKVQQGRVTMTIDTSSTSTYMLERLSQDDTAVYYCARGYGMDVWGQGTTTV
SS (SEQ ID NO: 55)

Nucleotide sequence of light chain variable region:
5’CATCTCTGCGCCTGACTAGCCTGCTCCGTTCTGGCTCTGGTGGTCTCTGCTGGAGCAGTCGATCAC
CACTCCTCGAATGAGAACAGCAGAAGCTAGGTGAGGTGTTATACTCTGCTCTGCTGTA
CAAACGACCACAGCTCACTCGCCTCGGAGAAGGAGCTGCTGTTATTGATGTTGAGAGCTG
CTGCTGCTCCGCTCGCCTGCTGCTGGTCTTCTGGCTCGCCTCGTACAAATGG
GCACACGACTGGCTCTTCGGGGAGGAGACAGCTGAGGCTCTCA3’ (SEQ ID NO:
125)

Alternative Nucleotide sequence of light chain variable region:
5’CATCTCTGCGCCTGACTAGCCTGCTCCGTTCTGGCTCTGGTGGTCTCTGCTGGAGCAGTCGATCAC
CACTCCTCGAATGAGAACAGCAGAAGCTAGGTGAGGTGTTATACTCTGCTCTGCTGTA
CAAACGACCACAGCTCACTCGCCTCGGAGAAGGAGCTGCTGTTATTGATGTTGAGAGCTG
CTGCTGCTCCGCTCGCCTGCTGCTGGTCTTCTGGCTCGCCTCGTACAAATGG
GCACACGACTGGCTCTTCGGGGAGGAGACAGCTGAGGCTCTCA3’ (SEQ ID NO:
294)

Amino acid sequence of light chain variable region:
QSALTQPSVSGSPQSTISCTGTSDDVGGYNSVSWYQQHFKPCKLMAYEVSNRPSGV
SNRFSGSKSGNTAFLTISGLQAEDEAYFCSSYTSSTMVFGGGTTLTVL (SEQ ID NO:
21)

FIG. 3V
Nucleotide sequence of heavy chain variable region:
5' CAGGTTTACATGGTCAGCTTGGAGCTAGGATTGAAGAAGCTGGGCTCAGTGA
AGGCTCTCTGCAAGGCTTTGTGCTCTCCCTTGAAGCAAGTCTTGAGCTCGGCTG
GACAGGCCCTGGACAAAGGGTCTTGAAGTGGAATGGATGATCTCCCCCACTCTGGA
ATTACTTGGCGAAGGCTTACGGTGTTAGAGCTCTGCTGCGGGCCAAGGACACACGTAC
GCTCTCCCTA3' (SEQ ID NO: 126)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVQLVKASGGTYLTSYGLISWVRQAPGQGLEWWMGWTSAYNGN
TNYAAKTVQRVTTMPSTSTSTVYMELRSLRSDDTAVVYCAROYGMDVWGQGTTVTV
SS (SEQ ID NO: 56)

Nucleotide sequence of light chain variable region:
5' CAGTCTCGCCCTGACTAGCTTCAGCTCCGGCTCTGGTTACTTGCTCTGCTGGCAAGTCATCAC
CATCTCTGCACTGGAACCAAGACTTGACCTGGGTCTTTAATACCCTGGCTCTCTCGTA
CCACACTGACCCAGGCAACCACCCCAACCTACATGATTTATGCGTCAATGAATCAGC
CCTCAGGGATTTCTAAATCGCTTCTGCTCAAGCTGGCCAACACAGCGCTCCCTGA
CCTACTTGGGCTCCAGGCTAGGGACAGGGCTGATATTCTGCAAGCTCATATACAA
GACACAGCTATGGCTCTCGGGCCAGGGACACAGCTGGACGCTCTCTA3' (SEQ ID NO:
127)

Amino acid sequence of light chain variable region:
QSLTVQPSVSQSGSIQVSVSVQTSNVDSVGGGYSVSYQHPGKPPKLMYEVSNRPSG
SNRFSGSKEAALSLQGQAEDADYFCSSYTVSTSMVFHHGGKTLVL (SEQ ID NO: 22)
17C2

Nucleotide sequence of heavy chain variable region:
5' CAGGTTCAGCTGGTGACAGCTGGAGTTAAGAGCCCCTGAGTGAAGGTCTCTGCAACGGCCTCTG
AGTCTCTCTGAAGCCTTCTGTGTTCACTACCTTACAAGCTATGGTATCAGCCTGCGCTG
GACAGGCCCCTGGACACAGAGCTTGGAGTTGGATTGGAGTTGGTACAGGCTTTACAAATGG
AACACAAACTATGCAAGAGCTACTTCAGGCAAGAGTCAACTGACACGACACAGGACATAC
CAGAGCAACAGCCCACATGGAAGAGCCTGAGATCTGAGCAGACAGACCCGCTGT
ATACGTGACAGAGCTACGTATGAGCAGCTGGGCCCAGGCCAGAATCCGTCACC
GTCTCTCA3' (SEQ ID NO: 128)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVVKVSAKQSEFYGGSLGWMGWYSAYNG
NTNYAQKFSQGRVMTITGVDDTSTAYMLRSLRSDDTAVYYCARGYVMDVWGQGTV
RTSS (SEQ ID NO: 57)

Nucleotide sequence of light chain variable region:
5' CAGTCTGACCTGTACACTGCTGCTGGTTCTGCTCTGGAACTCAGTGATCAC
CATCTCTGCACCTTGAACAGCTACTCCTGTGCTCTGATGGTGCTTTAATACTCTGATTCTCTG
GCCAACGACACCGACATGTTTATCAGTTGGACATGATATCGG
CCTCAGGTTTCTAACatCCTGCTGCTGCTGCAATACGCTGAAACAGGCTCTCCTGA
CCATCTGCTGCTGCTGCTGAGGATGACCTGCTGATTATACCTGCTGCTGATTACCA
GCACCAAATCTGTTATCAAGCGGAGGACAGCTGACCTCCTA3' (SEQ ID NO:
129)

Amino acid sequence of light chain variable region:
QSALTQPASVSGPQGITITCTGTSSDVGAYNVSWSVYQHPGKAAPKRMIYEVSNRPGV
SNRSFGSKSNTASLTISSLQAEDEAYCYSYTVSTNMVFGGGTKLTQL (SEQ ID NO:
24)

FIG. 3X
13H1

Nucleotide sequence of heavy chain variable region:
5’TACGTACAGTTCAGTGCAGTGCTACGGGACTGGTGAAAGCCTCAGCAGCACCTCT
CACTCACCTGAGCCTCCGAGGACTGCTCCTAGCAAGATGCTGCTGGTGAAGCT
GGATCCAGCAGTCTCCCTATCGAGACGCTGCTGGGGGAGGACGATACCTCCAG
TCCAGTGATTAAATATATCAGTATCTGAGAAAGCACTGCAAATACCATCAACCA
GCCACATCGAGAGACGACATCTCTGCATCAGCTGACAGCTCAGCCGGGAGACAG
GCTGACTCTGCTGGCAAGAGGCGGCGCAGACTGCTGGCTTTTGACATCGGCGGCAG
GGAAACCTGCTGTCACCCGGTCTCTCAGA3’ (SEQ ID NO: 139)

Amino acid sequence of heavy chain variable region:
QVQLQPSGGLVKPSQTLSTCAGSVDGSSNAAAWNWRQPSRGSLEGWLGRTYRYSK
WYKNYSVSVKRSRTNPDTSDKSNQFLQLNSVTGDTAVYYCARSGYPDAAFDYWQGTL
VTVSS (SEQ ID NO: 91)

Nucleotide sequence of light chain variable region:
5’CTCTCTGGCCTCACGGCCAGCTGCTCTCCCTTGGCTGCTCTGGGACTGCGATCAC
CATTCTGGCCACACGCGAGCTGGTGGGAAGCTAACCTGGCCCTGCTGGGAGCA
CCACAGAGCTCGCCAGGCAAGCCCGCAGAAGCTGATAACTGACTGACTGACTG
CCTGAGGAGTCTTCTAACGCAGCCTTGGCCCTACAGCGGCTGGGACAGCGCTCGA
GAATCTGCTGGCATTGAGGTGGAGCTAATTACTGCTGCTCTATGCAA
GTAGTAGAGACTTTTCGGCGGGAGGACCAAGCGTAGGGCTCCCTCA3’ (SEQ ID
NO: 131)

Amino acid sequence of light chain variable region:
LSALTQVASVSGPSQIISSCTGSSDVGVNYNLVSWSYQSOGAPKLMNYEVSNSPGV
SNRFSGKSGNTASLTRSLSGIAEDADYCCSYAGGSLTFLVGGGTGKVTILV (SEQ ID NO:
28)

FIG. 3Y
Nucleotide sequence of heavy chain variable region:
S'GAGGTGCAGTTGGTGGAGCTGCGGGGAGCTGGTGGTGGCCACGGCTTGCGAGAGGAGTGAAGT
GAGAATATCTATGGAACCTCCTGGAAGGCGGATTCAAAGCTCCAGACGAAAGCG
CAGAACTCTGCTATCTGCAAAATGAAACAGCTGAGAGCCGAGAGACACGAGCTGAT
ATTACTGTCGAGAGAGTCAACACTGGGGAATTTGTCCTTGATATCTGGGGAACAGGGA
CAATGGTCACCCTCCTCTCA3' (SEQ ID NO: 132)

Amino acid sequence of heavy chain variable region:
EVQLVESGGGLVQPGSSRLSLSSVCVVSGFTFYWMSWFVRQAPGKPLEWVANIQLQDSYE
KYVYDSDKGRFTISRDNAKNSLYLQLMNSLRADTVYYCARESNWSVFGDFWQGTM
VTYSS (SEQ ID NO: 64)

Nucleotide sequence of light chain variable region:
S'CAGTCTCTGCTGACTCGACCCACCTCGAGCTGAGGACCGGCGCAGAGGGTCA
CCATCTCTGTCCAGCTCAAGACAGCTGCAAATGGAAGTAAAGACATGTAACCTGACCC
AACAGGGCTCCAGAGCGCCGCCACACCTCCCATCCTATGGAATAATCAAAGCGGCCCC
TTAGGGTCCCTCGACCCGGATTCTCTGGCTCAAGTCGACCTCGTCGCTCCGCTGC
AATCAGTGGCGCTGAGCTCAAGATGAGGCTGATATTATTGTGCACCTCGGATGAC
AGGCTGAAATGGTGTTGGCGGAGGAGACAAAGCTGACCCCTCA3' (SEQ ID NO:
133)

Amino acid sequence of light chain variable region:
QSYLTQPSSAGTPQRYVTSCGSSSSNGKTVNWYQQVPGTAPKKLYRNNQRPLGVP
DRFGSNGTASLSLAIQSLQSEDEAYYCAAWDDSNWVFGGGTKLTVL (SEQ ID NO:
30)

FIG. 3Z
9H6

Nucleotide sequence of heavy chain variable region:
5'QAGCTGCAGCTGGAGCTCAGGGGGGCTTTATAGCTGAGCTGGGAGTACCTCAGCCT
GACCTCGCTGACGCACTTTTAAAGGCTCTGAGGAGGTAAGAGGGTGCCCG
CCAGGAGCAGGGAGGAGGGTGATGGCAGCACATAAGACTGAGGAAAG
AGAAATAGACATGACTTTGAAAGGGCTGCTGACGACAGCAAGCCTGAGGAGGACACGGGCTGTA
TTACTGAGGCAAACCTGAAATGCTGGGGATTTGCTTTTGATGCTGGGAGCCACGGGAC
AATGGTCACCGCTTCTTCA3' (SEQ ID NO: 134)

Amino acid sequence of heavy chain variable region:
EVQLVESGGGLVQPGGLSLRLSCASAGFTSFQRYWMSWVRQAPGKGLEWVANIHKDGSE
KYYVDSVKGRFTISRDKNSLYLQMNWILLQRAEDTAAYVYECRESWGGFAFDVWGHGT
MVTSS (SEQ ID NO: 62)

Nucleotide sequence of light chain variable region:
5'CGATCTGGCTGAGCAGCCACCTCAAGCTGCTGCGCCAGGACAGGCTCA
CCATCTGCTGCTGAGGACAGCTCCACACCTGGAAGTAAACTGTAACCTGTAAC
AGCGATCCACGAGCAAGCCAGGAAACTCCTCCTGCTATAGTAATAATCGGCGGCTC
CAGGGGGTCCCTGACCGATTCTCTGCTGCAAGCTACCCCTGCCTGCCTGGCCA
TCAGGGCTGCTCAGCTGAGGAGGCTGATTATATTGCTGACGACTGGGATGACA
GCCCTAAATTGGGTGGGGAGGACACAAAGCTGACCCTCCTCA3' (SEQ ID NO:
135)

Amino acid sequence of light chain variable region:
QSVLTQPSAGPGQPQRVIAGSSSNNQSNVTNYWYQQLPGTAPKLILYSNNRRHPSGVVPD
RFSGSKSGTSAASLISGLQSEDEAYYCAAWDDSLNWVFVGGGTLTVL (SEQ ID NO:
31)

FIG. 3AA
13B5

Nucleotide sequence of heavy chain variable region:
5’GAGGTGCACTGTTGGAGTCTGGGGGGGAGGTGCTCTCGGCCTCGTGA
GACTCTCTCTGGAGAGGCTTCTGCCCTCTGAGATTCTACCTTGAAGCACTGATCGCC
GCCAGGCTCAGGAGGGGTCTGGAGAGGCTCAGTCAACTATTAGTGGTGATTGGTG
AGGACATATTAGCAGACCTCCTGTAAGGGCCGCTTCACCATCTCCAGAGCACATT
CAAGAACACGCTGTATCTGCAAAATGAAGAAGGCTTAGAGAAAGCGCCAGAAGGCTCA
ATTACGTGGAGGAAGAAGTGGGCAAGTCCCTTTGACTACTGGGGGCAAGGACACCTCTGG
TCACCGTCTCCTCA3’ (SEQ ID NO: 136)

Amino acid sequence of heavy chain variable region:
EVQLESGGLYVPQPGSLRSLCAASGFTFSSYAMSWVRQAPKGLEGWVSTISGGYRTY
YADSVKGRFTISRDNSKNTLYLQMNLSRAEDTAIVYYCAKEVGSFDFYWGQGTLVTVSS
(SEQ ID NO: 69)

Nucleotide sequence of light chain variable region:
5’CAGTCTGGTTGACGAGGCCGCTTCCACTCGCTTCGAGCCCAAGGAAAGGTCA
CCATCTCTGCTTCCAGGAAACTCCACACATTGGGAATATTGATTCTCTCGTACC
AGCAGCTTTCCAGGAAACGCCCTTCAACTCTCTTATATGACAAATATAAGCGACCT
CAGGGATTCGTTCTCAGCTACCTCGTACATGCCATACGCACGTCAGCCACCTGGGA
TCACCGGACACTCCAGACTGGGGAAGAGGCCCTATTACTGCGGAACATGGGATAGC
AGCCGAGTCGCTGGTATTCCGCGGAGGAGCCAAAGCTGGACCCGCTCTCA3’ (SEQ ID
NO: 137)

Amino acid sequence of light chain variable region:
QSVLTQPSVSAAAPQKVTVTCSGSGNSNIONNYVSYYQQLPGTAPKLICIDNNKRP
g SIP DRFSGSNPSATLQITGLQTGDEADYYCGTWDSSLASAVVFGGKTLTVL (SEQ ID
NO: 42)

FIG. 3BB
Nucleotide sequence of heavy chain variable region:
5' CAGGTGACAGCTGGTGAGTGGAGTGGTGTCACGTCCTGGAGGATCCTGTCGCTTGCCCTAG
GACTCTCTGCTGAGCAGCTCTGATCCCTCAGTATGGCATGCACTGGGCTCC
GCCAAGCCTCAGGAGAGGGCTGCTGGAGTGGGGCATATTATGGTATGATGAGAT
AATAAATACTACGAGACTCGTGAAGGGCCGATTACCTACCCCTCCAGACAGAAACC
AGAAGAACTGTATCTCCATAGAACAGCTGAGAAGCCGAGAACACGGCTCGTAG
TTACGTGGAGGAGGGAACCCGCACGCTGCTCGGGCCGGGTATGGACGTCTGGO
GCCAAGGACACAGCCACGCTGCCCTCCTCA3' (SEQ ID NO: 138)

Amino acid sequence of heavy chain variable region:
QVQLVESGGGLVQPSRLSLCAASGFTSSYGMHWVRQAPGKLEWAVAILWYGDSN
KYYADSVKGFRITISRDNNSKNTLYLQMNLSRAEDTAVYYCARRRGLAAPGMDVWG
QGSTTVTSS (SEQ ID NO: 81)

Nucleotide sequence of light chain variable region:
5'TCTATGAGCTGACTAGGAGACTACCCCTAGTTCTGCTTGCCCTTCCAGAGCAAG
AACTCTCTGGCTCTGAGGAGATAAATTGGGGGGAATAATATGCTGCTGGTATGACAGAA
ACCAGGCACTGCCCCTCTGTGGCTCTGCTCTACATGAAATACCAAGTGGCCCTTAAAGGAT
CCCTGAGGGAGTTTCCTGCTGCTTACCATACGTGACAGACAGACTGAG
TGTATTTCGGCCGAGGGAACAAAGTGCTGCCAGTCCTCA3' (SEQ ID NO: 139)

Amino acid sequence of light chain variable region:
SYELTQPSVSTVSQPQGQTRICGDSKDGYACWYYQKPGQPQPVLYIQNYETQIVPLGIPE
RFSGKSGNTVTLLTSGTQAMDEADYQCQAWDSSTTVFVGGTKLTVL (SEQ ID NO: 44)

Alternative Nucleotide sequence of light chain variable region:
5'TCTATGAGCTGACTACCCCTAGTTCTGCTTGCCCTTCCAGAGCAAG
AACTCTCTGGCTCTGAGGAGATAAATTGGGGGGAATAATATGCTGCTGGTATGACAGAA
ACCAGGCACTGCCCCTCTGTGGCTCTGCTCTACATGAAATACCAAGTGGCCCTTAAAGGAT
CCCTGAGGGAGTTTCCTGCTGCTTACCATACGTGACAGACAGACTGAG
TGTATTTCGGCCGAGGGAACAAAGTGCTGCCAGTCCTCA3' (SEQ ID NO: 295)

FIG. 3CC
3C4

Nucleotide sequence of heavy chain variable region:
5’CAGGTGCACTGCAGGAGTGCGCAGGCCAGGACTGGGCTGGAACCTTCACAGACAACCTGT
CCCTCACTGCACCTGCTCTGCGCTCAGCCAACGAGATGTGATTACCATCTGGAGCT
GGATCAGCCGCCAGCCACGGGAGGGCCTGAGGATGTCGTGTGGTACAATCTATTAG
GGAGAGACACTACTCAACCCGCTCCCTCAAGACTGAAATTACCATATGAGAACA
CTCTAAGAAAGTGGTTCCTCGAAGCTCGTGACTGCGCTCGGACACGGGCGCT
GTATTACCTGCGAGAGGGGTTGACTACTACTACTACCTATGGACGCTGGG
GCCAAGGACCACGGTACAGGTCTCCTCCTCA3’ (SEQ ID NO: 140)

Amino acid sequence of heavy chain variable region:
QVQLQESGPGLVKPSQTLSTLTCTVSQSISSSDYYWWSWIROHPGKLEWIGYIYYSSTY
YNPSLKSRTISVVDTSKNLFLSLLSSVIAADTAASYCARGGVTTYYYAMDVWQGTTV
TVSS (SEQ ID NO: 85)

Nucleotide sequence of light chain variable region:
5’GACATAACAGATGACCAGTCTCCTCCATGCTCCTGCTCTGGAACATGGAGACTAGT
CAAGCTACACGGCCGGCGAGTCACATTAGCAACATTTAAGGGGATCCTGCA
AAAACAGGGATCTTGCTGAACATGCTGCAAGTCCAGATGGG
GGTCCCATCAAGGTTCTAGGCTGCTGACATGAGATTTTCCTCCTCCTACTACAG
CAGCTCTGCAACTTGAAGATTGCGCACTTACTACTGTGCAACAGAGTTACAGTTACCC
GCTCATTTTCGCGGAGGAGGACAGTTGGGACATCAAA3’ (SEQ ID NO: 141)

Amino acid sequence of light chain variable region:
DIQMTQSPSSLSASVGDRVTITCRASQIRSNLYSLWYLYKPIAPKLILYAAASSLQSGVPSR
FSQGSGSTIDFTLTISSQSEDATYCYQYQSYSTPLEFVGCGGTVK (SEQ ID NO: 7)

FIG. 3DD
Nucleotide sequence of heavy chain variable region:
S'CAAGGTGCACTGTGGAGTCTGGGAGGAGATGGCTCCAGGGCTGGGATGCTCAGGGCAGGGATGCTCC
GACCTCTCGTGACGAGCTCTGGATCTCCACTTCCTCAGTAGCTATGGCAGTACGACTGGCC
GCCAGGCCTCCAGAAAGGGCTGGAAGGGGCGTGGCCAGTTATATGTAATTGAGAGAT
GATAAATACTATGCAACTCCGTGAAAGGCGCGTCCATCAACATCTACAGGAACAGATACTCC
AAGAAACACGCTGTAATGCAAATGAGACGCTGAGGCAGCGACACCCCGCTGTA
TTACACTGGCAAGAGACAGACTGGCTCCCTGTGAAACTCAACTACTACGGTGATGGACGGCTTG
GGGCGCAAGGGGACACGGTCACCCGGCTCTCTCAA G' (SEQ ID NO: 142)

Amino acid sequence of heavy chain variable region:
QVQLVESGGVQPGSGRLSLCAASGGFTSSYGGMHWVRQAPGKGLEWAVIYWYGSD
KYYADSVKGRFTISRDSKNTLPLQLNLAESETGPEKLPSVLYEMMDWVQG
QGTTVTVS (SEQ ID NO: 74)

Nucleotide sequence of light chain variable region:
S'GATATTGTGATGACTCAGTCTCCACTCCTCCTGCTCGTCACCCCTGGAGAAGGGCC
CTCCACTCCTGCTCGAGATGCGAGCCTCCTGCAAGTAGGAGATACAAACTTTTGG
AATTGCGCTCGCAAGGCGAGGCGTGTCCTCTACAACTCTGTCATATTTTGGC
CAGGCGGCTCAGCTGCTACAGTCCATGATGGCCAGTGATAGCAACAGATTTT
ACACTGGAAATCAGCAAGTGGCTGGAGTGTCTGGGTATTACGTCATGCA
AGTTCTCATAACTACCTCATTTCTGGCCCTGGAGAGAAAAGGATGATATCAAA (SEQ ID NO: 143)

Amino acid sequence of light chain variable region:
DIVMTQSPISLSLVFTGEPSSCSRSQSSLHSGYNFNLWLYQKPGQSPQLILYLYSLH
GVPRDFGSNGTDFIIEISVEAEADVGVYYCMQVLQTPTFPGGTKVDIK (SEQ ID NO: 5)

FIG. 3EE
Nucleotide sequence of heavy chain variable region:
S'GAGGTGCACGTGGTGAGTGGGAGGGAGCCTTGTCAGCTCGGCGGCTGCTG
GACTCTCCCTGACGCTTGACTACCTTCAAAGTCTACCTTGAGCTGGCGC
CCAGGCTCAGGGAGGCGCTGAGTGGGCTGCGACAAGTAAAGGAAATAG
GAAGATACTATTGAGAGCTGAGCTGCAGGCGGATTCCACATCCTCGAGAGACAG
CAAAGAATTCAGCTGCTGGAAGCGGAGTGGCTGCTGAGCTGCTGCTGCTG
AATCCCTGACTAGAAGAGTCAAAACTGGGGGATTGCGCTTGTATATCTGGGGCCACAGGA
CAATGGTCCACGGTCTCTTCA3' (SEQ ID NO: 144)

Amino acid sequence of heavy chain variable region:
EVQLVESGGGLVQPGFLSRLSCAASGLFTSDKWMSWVRQAPKGLVESWYANIKDGS
KYYVDSVKGRFTISRNLKNSLYLQMNSLRAEDTAVSYCTRESNFWGAQFHIDWKGQTM
VTVSS (SEQ ID NO: 65)

Nucleotide sequence of light chain variable region:
S'CATCTCTGGCTACTACCCCTCCAGCTGAGGCCACGACAGGCTCA
CCATCTCCTGTTCTGAAAGCTCTCAAGACCATCGGAAAGTAAACTGTGATACC
AGCAGTCCAGGGAGCGCCCACCAACTCTCTCATCTAGTAAATCTGGGCCGCT
CAGGAGTTCCCTGACGAGATCTCTTGCCACATTACCTGAGACAGCTGAGGCT
CAGCAGTGGCTCTCAGCTGAGATGAGCTGAATTACCTGACAGCAGAGCTGACAC
GGCTGAAATTGGGTCTCCGCGCCAGGGACCCAAGCTGACCAGCTGCTCTTCA3' (SEQ ID NO: 145)

Amino acid sequence of light chain variable region:
QSVLQPSASAGPTQGVRVTSICSNSNIGKTVNYIYQOMPSTAKLLLYSNNRPSGVPD
RFSGSKSGTSAASLAIISGLQSEDEADYYCAAWDSDLNWVFGAGTKLTL (SEQ ID NO: 33)

FIG. 3FF
3B6

Nucleotide sequence of heavy chain variable region:
5'CAGGTTCACCTGGTGCAGTCAGGTGAAAGCTTGGGCTTGAGCTCG
AGHTCTCTCAGCAAGGGCTTCTGGTTACACTTTAACCACTGATATGC
GACAGCCCTGGGACAGGTGGATGGAATGGGATGACCTGAGCACTCG
AACACAAACTATGCAACAGGAAGTCCAGGAGAGTCACATAGCAGACACATCC
CAGACGACAGCAGCTGAGGAGCCTGAGATCGAGACGCCTGAGAATGTG
ATTACTGTCGAGAGGATTATCTCGGGGACTACTGGGGCCAGGGAACTCTGTCACC
GTCTCCCTCA3' (SEQ ID NO: 146)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVKVSCKASGYTFTSSYGISWVRQAPGQGLEWMSGWISTYNQNSYNAQKTVQGRVTMTDSTSTAYMLERLSLDDLYAVYVSLCGRGTVRDYWGQGTLVTVSS (SEQ ID NO: 60)

Nucleotide sequence of light chain variable region:
5'CAGCCCTGTGACGACGGCTCTTTGCACTAGCCCTGGAGCGCTGTCAC
ACTCACTGCAAGGAGAGGCTACACTAGTATGAGTGGACTGGATACAGCA
GAGACCAGGAAAGGCACTCCCTGTCTGGAATGGGATGATGGATGGCATTGG
GATCAGAGGGGAAAGCATCCCTGATCGGCTTCAGTCTTTGCGCTAGGCGCTGAACTC
GGTAATCGGACCACAAGAAATCCAGAAAAAGGATGAGTGGACTACACTGTGG
GCAGACCAGCTGGAGGCAACTCTGCTGTTGGTATTCCGGGAGGGCAACAGCT
GACGCTCTCA3' (SEQ ID NO: 147)

Amino acid sequence of light chain variable region:
QPVLTIPLFASLGLGASVTLCTLSGGYSSYEVDMYQYQPGRFVMRVDGGIVGSK.
GEGIPDRFSLGSLNLYLTIKNQFEDESDHYHCAGADHSGTNNFVVFGGGTTLV (SEQ ID NO: 46)

FIG. 3GG
Nucleotide sequence of heavy chain variable region:
5'-CAGGTGCAGCTACAGCAGTGGGGCGACGACTGTTGAAACCTTCCGGAGACCCTGGTCCCTACCTGCCTGTCTATGGTGCCTCCTCAGTGGCTACTACTGGAATCCGCCAGCCCAAGGGAAGGGGCTGGAGTGGAATGGGGGAAATCAATGATGGAAGAACCGACTACAACCCTGTCCTCAAGAAGTCAGTCAGTCAAGCTAATGACGAGAACGTCTCACAAG

Amino acid sequence of heavy chain variable region:
QVQLQWAGGLKLPSCTLSTCLAVGGPSFSAYWYNIRPRQPKLGEWIGENKHSGRTDDYNPSLKSRTISGVDTKKQSFKLNSVTAAADTAVYCARQGLVPDYYWGQGTTLVTVSS

Nucleotide sequence of light chain variable region:
5'-CAGTCTGTGCTGACCTCAACCCACCTCAGCCTGAGGGACCCCGGGCAGAGGGTCA

Amino acid sequence of light chain variable region:
QSVLQTQPSASGTPGQRVTISCSSIGNSSNTVNVQQLPGTAPKLIIYSNNRPSGVPDSFGSGSSTASLAISSLQSED1EYCAVIWDDSLSNGWVFGGTKLTVL

FIG. 3HH
Nucleotide sequence of heavy chain variable region:
5' CAGGTTTCACTGGTGATGCTGAGTCGAGGTGAGTAAGCGCTGGGCTCTAGTGAA
AGTCTCTGCAAGGCCTCTCTACATGGGATGGTGAAGTCAGCAGGCTGCTACAATG
AACACAAATGCAGAGAGCGTCCAGCGAGACTCAAGATGAACGGGATGCTAGC
CAGAGCAGACACGCCACAGATGGGTAGGGAGGAGCTGAGATCTGAGCALACACGGCGCTG
TTCGACTGCGAGAGGCGTACGTTATGGAGCTGCTGGGCAAGGACCACACCGGTCA
GCTCTCTCT3'  (SEQ ID NO: 150)

Amino acid sequence of heavy chain variable region:
QVQLVQSGAEVKPGASVKVSCKASGYTFSYGVISWVRQAPGQGLEWWMGWISAYNGN
TNYAELKLQGRVTMTVTSTAYMVEVRSLRSDATYFVTCARGYVMDSMVWQQQTVTS
S  (SEQ ID NO: 58)

Nucleotide sequence of light chain variable region:
5' CAGTCTGGCTGACTGAGCCTGCCCTGCTGGCTGGCTGCTTGGACAGTGATCAC
CATCTCTGCACTGAAACCAGCGTAGCAGTGCTGGCTCTATATTCTGCTCCTCGTC
CAACACCAAGGCAAGCGCCCAAAGCTACATGATTTATGAAGGTGCTAGTAAATCGGC
CTCAGGGTTTTCTACCTGCTCTGCGCTCAAGTCTGGCAACACGGCGCTGAC
CATCTCTGCGCTTCCAGCTGAGGAGAGGCTGATTTACTGCGACAGCTATATCAAG
CAACAGCGGTTGTATTGCGCGAGGGACAAACCTGACCCGCTCTCA3'  (SEQ ID NO:
151)

Amino acid sequence of light chain variable region:
QSALTQPASVSGSPQGSIHCCTGTSSDVGYNSVSVYQHPGKAPKVMIFYEVSNRPSGV
STRFSGSKSGNTASLTSGLQAEDADYYCCSYTSSSVVVFGGTGLV  (SEQ ID NO:
15)

FIG. 3I1
Nucleotide sequence of heavy chain variable region:
5'CAGGTTCCAGCTGGTGCAGTTGGCTAGTAGGAGAAGACGCCTGGGCTCCAGTGA
AGGCTCCCTGCCAGGGCTCTTCTGTTACACCTTAAACCAGCTATGGTATACGCTGGGTCG
GAGAGGCCTGGGAACAGGGCTTTTAGGCTAGTGAGGATGGTGCACTGGTATTATGAT
AACACAAACTATGCAAGAAGCCTCCAGGGAGAGGACCAGTACCACAGACAGACACCATT
CAGGAGCAGCCAGCCATCACTGAAAGCTGAGGAGGACCTGAGATCTGAGACAGCAGCAGGGG
ATTACTGTGGAGAGGGCTACGAGTATGGACGTCTGGGGCAAGGACAGACCCAGCTACC
GTCTCTCTCT3' (SEQ ID NO: 94)

Amino acid sequence of heavy chain variable region:
QVQLVQQSGAEVKPGASVQLTVSCKASGYTLTSQGWSWRQAPGQGLEWWMGWVSFGNG
NTNYAQKLEGQRGTMSTPSTAYMLERLSRLSDDTAVYVCARGYGRMDVWGVT
VSS (SEQ ID NO: 49)

Nucleotide sequence of light chain variable region:
5'CATCTGCCTGACCTCAACCCCTCTCCGGTCTGGGTCTCCCTGGACAGTGACATCAC
CATCTCCTGCAACTGGAACACAGACGTCGTGGTTAATACCTCTTCTGGTTA
CCAAAGCACCCAGGAAAAGCCCAACTACATGATTTATAGGTACGTGATACCCGC
CCTCAAGGGTCTTAACTGCTCTCTGCTGCCACAGTCGCAACCAACGCTCCCCTGGA
CCATCTCCTGGCTCGGCTCGGCTCCAGGGCTAGGGACGGGCTTATTACTGCAAATCATACAA
GCACCAGCATGATTTCCGCGAGGGACACAGCTGACCCGTCTCTA3' (SEQ ID NO: 296)

Amino acid sequence of light chain variable region:
QSLTQPASVSGPQGSSITSCSTGGGDVSYSNYQSHQPAGPKLMIYEVSNRPGV
SNRFSGSKSGNTASLTISGLQAEDADYYCNYTSITSMVFGGGKTLTVL (SEQ ID NO: 23)

FIG. 3JJ(i)
Amino acid sequence of heavy chain variable region (CDRs underlined):

EVQLVQSGAE VKKPGASVKV SCKASGYTLT **SYGIS**WVRQA PGQGLEWMGW - 50
**VSFYNQNTNY AOKLOG**RTGTM TTDPSPTSTAY MELRSLRSDD TAVYYCARGY -100
**GMDV**WGQGTT VTVSS (SEQ ID NO:590)

Amino acid sequence of Heavy Chain (CDRs underlined):

EVQLVQSGAE VKKPGASVKV SCKASGYTLT **SYGIS**WVRQA PGQGLEWMGW - 50
**VSFYNQNTNY AOKLOG**RTGTM TTDPSPTSTAY MELRSLRSDD TAVYYCARGY -100
**GMDV**WGQGTT VTVSSASTKQ PSVFPLAPCS RSTSESTAAL GCLVKDYFPE - 150
PVTVSWSNAGA LTSGVHTPFA VLQSSGLYSI SSVTVPSN FGQTQYTCNV - 200
DHKPSNTKVD KTVERKCCVE CPPCPAPPVA GPSVFLFPPK PKDTLMISRT - 250
PEVTCVVVDV SHEDPEVQFNY WVVDGVEVHN AKTPREEQF NSTFRVSVL - 300
TVVHQDWLNG KEYKCKVSNK GLPAPIEKTI SKTKGQPREP QVYTLPPSRE - 350
EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP MLDSDGFFL - 400
YSLKTLVDKS N WQQGNVFSCS VMHEALHNY TQKSLSLSPG K - 441
(SEQ ID NO:592)

Amino acid sequence of light chain variable region (CDRs underlined):

ESALTQPASV SGSPGQSI **TCTGSSDV**G **GYNSV**WYQQ HPKGAPKLMI - 50
**YEVSNRPSGV** SNRFSGSKSG NTASLTISGL QAEDADYYC **NSYTSTSMV**F - 100
GGGTKLTV L (SEQ ID NO:591)

Amino acid sequence of Light Chain (CDRs underlined; Insertion between light chain variable region and constant domain italicized and underlined):

ESALTQPASV SGSPGQSI **TCTGSSDV**G **GYNSV**WYQQ HPKGAPKLMI - 50
**YEVSNRPSGV** SNRFSGSKSG NTASLTISGL QAEDADYYC **NSYTSTSMV**F - 100
GGGTKLTVL QPKAAPSVTLPFPPSEELQANKATLVLCS DFYPGAVTVA - 150
WKADSSPVKA GVETTTPSKQ SNKYAAASSY LSLTPEQWKS HRYSNCQVTH - 200
EGSTVEKTVA PTECS - 215 (SEQ ID NO:593)

FIG. 3 JJ(ii)
Constant Domains

Human IgG2:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSSGALTSGVTHTFPAPVLSQGLVSLSSVT
VPSNFTGQTVDHKSNTKVDRVKCCCESPPCPAPPVAGPSVFFLFPPKDTLTIEMYRTPEVC
VVVDVDHDEPEVQFNWYVDGEVHNAKTKPREEQFNSFTRVSVLTVHQQQLNQKEVKKVC
SNKGLPAPIEKTI86KTQCPREPQVYTLPPSREEMTKQGSLTCLVKGFYPSDIAMEVESNQGQPENNYYKTTPPMLD
SDGSFFLYSLKTLVQSRWQQQGVFSVCSYMHEALNHYTQKSLGLSGK (SEQ ID NO: 154)

Human IgG4:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSSGALTSGVTHTFPAPVLSQGLVSLSSVT
VPSNFTGQTVDHKSNTKVDRVKCCCESPPCPAPPVAGPSVFFLFPPKDTLTIEMYRTPEVC
VVVDVDHDEPEVQFNWYVDGEVHNAKTKPREEQFNSFTRVSVLTVHQQQLNQKEVKKVC
SNKGLPAPIEKTI86KTQCPREPQVYTLPPSREEMTKQGSLTCLVKGFYPSDIAMEVESNQGQPENNYYKTTPPVL
DSDGSFFLYSLRVTQSRWQQQGVFSVCSYMHEALNHYTQKSLGLSLGK (SEQ ID NO: 155)

Human lambda:
QPKAAPSVTLPSPSSEELQANKATLVLCLISDFYPGAATVAVKADSSPVKAGYETTTPSKOSNNKYAASSY
LSTLPEQWKSRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 156)

Human kappa:
TVAAPSFVFPSDEQLKSQGTAASVCLNSNFPREAKVQWYDNALOQGNSQESYTEQDSDKSTYSLSST
TSLSKADYEHKCVAYACEVTHQLSSPVTKSFNREGC (SEQ ID NO: 157)

FIG. 3KK
SH5.1

Nucleotide sequence of heavy chain variable region:

5’CAGGTGCAGTGGTGTACATGCCTGGGCTGGTGAAGAAGCCTGGGCTCA
AGTGAGTTCTCTGCAAGCTTGCTGAATAACATCTCTCCGCTACTATAC
ACTGGGTGCCAGCCCTTGAGAAGGGCTTGGAGTGAGTGAGTACCA
CCCTCACTGTTGCGCGCAAGACTATGCAACAGAGTTTGCGCAGGAGGCTACC
ATGCACCAAGGAACACATCAGACAGGCAACCAGCTACATGGAGTGACGAGGCTGA
GAATCTGACGACAGCGCGGTATATCTCTGCGAGAGGCAACTGGAACACTCA
CTACTACGGTATGGCAGCTCTGGGGCAGAGGACACAGGTCACCGTCTCTCTCA
3’ (SEQ ID NO:418)

Amino acid sequence of heavy chain variable region:

QVQVAQQAGSVEKQPGASVQSKASGSYGYFTGGYYIHWVRQAPQGQGLEWMGW
PHSSGANYAQKFQQRVMTMRTDSISTAYMELSRLSDDDTAVYVYCGARGN
YYMGDVWQGQTTVTSS (SEQ ID NO:419)

Nucleotide sequence of light chain variable region:

5’GACATCCAGATGACCCAGTCCATCCCTCCCTGCTGGTCTGATGGAGAC
AGAGTGACCCATCATCCTGGGGGAGTCAGGACATTTGCCAATATTATGCTT
GGATACGACAAACGAGGGAAAAATTCCTAAAGCTCTGGTCATGCTGGACT
CAGCCCTGCACTGACGGTTCTCCAGCTGTGGGACAGTTGCTAGGCATCGGACA
GATTCACATCTCAGCAGCCAGCTACACAGGGTGGCTGGGACAGTGCTGGGACA
GATGTCAAAAGAGTAAGAGATGGCAGGGCTACATTTTCGGGACACGAGGTTG
ATATCAAA3’ (SEQ ID NO:420)

Amino acid sequence of light chain variable region:

DIQMTQPSLSSASVGDRVTITCRASQDISNYLAWYQQKPGKVPDLKIIYAASTLQ
SGVFSRFSGSGTDFMODISSLQFEDVATYFCQRYQIAAPFTFGPGTVDIK (SEQ ID NO:421)

FIG. 3LL
24F7.1

Nucleotide sequence of heavy chain variable region:

5'CAGGTCGACGCTGAGTGGGAGATCTCTGGGAGCGGCTGTCATCTGGGAGGTGTCCTAGACTCAGTTATGGCGATGC
ACTGGGTCCTGCCAGGCTCAGGAAGGGCTGAGTGGTGGCAGTTATCTG
GTATGTGGGAAGTACTAAATACTATGCGATTCGCCTGGAAGGGCCGATCCAC
ATCTCCAGAGACAATTCACCAGAAGACACTGTATCTGCAATGAGACGGCTGA
GAGCCGAGGAGACACGCGGGACTCGTATTACTGTGGGAAGGGCTGCTGGGTATCACA
CTACTACTAGGTTATGACAGTCTGGGGCAGGACCAGGTCACCGTCTCTCT
TCA3' (SEQ ID NO:422)

Amino acid sequence of heavy chain variable region:

QVQLVESGGGTYVQPSRLSLCAASGFTTFSSYGMRMHRQAPGKGLEGWAVIYW
YDGSTKYADSKVGRSTISRDNKNTLYLQMNSLRAEDTAVYYCARSVAGYHY
YYGMDVWVGQGTTVTVSS (SEQ ID NO: 423)

Nucleotide sequence of light chain variable region:

5'TCTTCTGAGATGCTGACTCAAGGGACCTGCTGGGCTGTTGAGGACAGACA
GTCAGGATACATGCGCAAGGAGACAGGCTGAGGCTAATTATGCAACCTGGAT
ACCAGCAAGACGCAAGACAGGCCCCCTGTGACTTGCACTATGTAAGAATACTA
CCGGCCTCAGGATCCGAGACCGATTCTCTGGCTTCAAAGCTCAGAGACACA
AGCTCTCTGACCACTACGAGTGGCTAGGCGGAAAGATGAGGCTGACTATTACT
GTAACTCCGGAGAGATTTGACCATCTGGGTCTGCGGAGCGGACCAAGGCTGAGG
GACACTCTCTCTCTCTCTCTCATCA3' (SEQ ID NO:424)

Amino acid sequence of light chain variable region:

SSELTQLDPAVSVALGQTVRITCQGDSLRGYATWNYQKPRQPAPVLYVIYGNRYP
SGIFDRFSGTSNNTASLTTGQAEDCDEAYYCNSRDSIGNLTVFLFGGGKTLTVL
(SEQ ID NO:425)

FIG. 3MM
22B11.1

Nucleotide sequence of heavy chain variable region:

\[ 5'\text{CAGGTGCACTGGTGAGTCTGGGGGAAGGCTGGTCCAGCCTGGGAGGTC} \\
\text{CCTGAGACTCCCTGTGACGGCTGATTAACCTAACGTAGCCTG} \\
\text{ACTGGGTCGCCAGGCTCAAGGCCAGGTAGGCTGGTCAGTATAG} \\
\text{GTAGATGGAAGTAATAGATACCTAGACTCCGTGAAGGGCGACCCACC} \\
\text{ATCTCCAGAGCAATTCCAAGAACAGCTCCTATCTGCAAATGACAAGCCTGA} \\
\text{GACCGGAGACACGTCGCTATTACTGTCGGAGTACGTGGCCTTACCA} \\
\text{CTACTACTACGGTATGGACGTCTGGGCGCAAGGACCCAGCCTACGTCC} \\
\text{TCA3'} \quad (\text{SEQ ID NO:246}) \]

Amino acid sequence of heavy chain variable region:

QVQLVESGGGLVQPGSRSLSLCASTASGETFYGLHWVRQAPGKGLEWVAVIWL \\
DGSKYYADSVVGRHTISRDNSKNTLYQSSQV兰LRAEEDTAVYYCARSVAGYHYY \\
YGMDVWGVQGTTVSS \quad (\text{SEQ ID NO:247})

Nucleotide sequence of light chain variable region:

\[ 5'TCTTCTGAGCTGACTGAGCCCGCTCTGGGGACTGAGCCTGCTTGCCCTTGAGACAGACA} \\
\text{GTCAGGATCCATGCAAGGACACAGAGCCCTGGTACAGAGCCTGGGCTT} \\
\text{ACCAGCAGAAGCCAGACAGCCGCTCTGTTCTTGCTTCCTGGCTAGACACCA} \\
\text{CCTCCCTGACATCGAGCTGGCGGGGATGAGCTAGAGCGGCTGACTATTACT} \\
\text{GTAATCTCAGGAGACATCTGTTCTGACATCTGGCTGGGAGGACGCAAGGACTG} \\
\text{CTGACGTCTCAA3'} \quad (\text{SEQ ID NO:248}) \]

Amino acid sequence of light chain variable region:

SSELTQDPAVSVALGQTVRITCQGDSLSRYGWSYQQKPRQAPVVLVIFGKNRP \\
SGPDRFSQGTSQTATLSLTITGAAQAEDEAYCNSRDIIDHDLLFGGGTKLTVL \quad (\text{SEQ ID NO:249})

**FIG. 3NN**
Nucleotide sequence of heavy chain variable region:

5’CAGGTGCGACTGTGGGATCGCTGGGGAGGGCTGTCGCTGACCTCTCAAGACTAGCTTACCAC<br>TTTGAGAGATTAATATATCTAGCAGACTCGTGAGGCGACTGAACCTTACCA<br>TCAGAGACATTTCTAAGAAACTCGGCTGTATTGATGAGCTGAGTACAGTGTGTTACC<br>CA3’ (SEQ ID NO:430)

Amino acid sequence of heavy chain variable region:

QVQLVESGGGLVQPSRLSLSCAASGFTFRNYGMHWVRQAPGKGLEWVAVWL<br>DGSLRYYADSKGRSTISRDNSKNTLYLLMNSLRAEDTAVYYCARSGVAGYHY<br>YYGMDFWGWQGTTVS (SEQ ID NO:431)

Nucleotide sequence of light chain variable region:

5’TCTTCTGAGCTGACTCAGGACCTGCTGCTGTGCTGACCTTGGAGCAGACA<br>GTAGAATACAGCAGAGAGACACCTCAAGAAGCTATTAGCAAGCTGT<br>ACCAGCAGAAGCAGAACAGCAGCGCTGTACTTGTACATCTATGTTAAAACAA<br>CCGGCTCTCAGGATCCAGAGAATCTCTGCGCTCACCAGGAAACACAGCA<br>GTCTTCTGACCATACACTGGCAGGGCAAGATGAGGCTGACTATTACTG<br>TAATCCGGGAGCATCTTTGCTGACCATCTCTGGTGTCGGCAGGGGACCAA<br>ACTGACCGTACTA3’ (SEQ ID NO:432)

Amino acid sequence of light chain variable region:

SSELTQDPAVSVALGQVTIRICQGDSLRSYYASYWQYQQKPRQPVPVLVYGGKNRRP<br>SGIPDIQSGTSGNTASLITGQAEDAYCYYCKSRDIIGDHLVFSGGGTTLTVL<br>(SEQ ID NO:433)

FIG. 300
24B9.1

Nucleotide sequence of heavy chain variable region:

5’CAGTGTCAGCCTGAGTCAGTGCTGGGAGGCCTGGCTGCAGCTCCGCTGGGAGGTC
CCTGAGACTCTCTCTGTGCAGCGTTGATTACACTTGCACTGATGCTATGCG
ACTGGCTCCAGCCAGCTCCAGCAGCCAGGGGCTGGAGTGGTGGCAGTTATATG
GTATGATGGAGTAATAATATATAGCTGAGACTCCGCTGAGAGGCGGATTCACC
ATCTCCAGAGACAAATTCAGGAAACAGCGCTGTATGCTCAGAATGACAGCCCTGA
GAGCCGAGGACACGCGCTGTATTACGTGCTGAGAGATCGGGGACTGGACTG
GGGCCAGGAAACCTTGTCAGCCCTGCTCTCTCA3’ (SEQ ID NO:434)

Amino acid sequence of heavy chain variable region:

QVQLVESGGGVVFQGRSLLAASGLVFSSYGHMWVRQAPGKLLEWVAVIW
YDGSKVYADSVKGVRFTISRDNSKNTLQLMNSLRAEDTAVYYCVRDRGLDW
GQGTLVTVSS (SEQ ID NO:435)

Nucleotide sequence of light chain variable region:

5’TCTTTCTGAGCTGACCTGACCTGCTGCTGTCTGGCCTTGGGACAGACA
GTCAGGATCACTGCCAAGGAGACAGCCTGAGGCTATTATATGCAGACTGCT
ACCAAGCAAGCCAGCACGCGCCCTGTACCTGTCACTATATGTAAGACAA
CCCGCCCTCAGGATCCAGGCTGATTCTCCTGGCTCCATCCAGGACACACACAG
GCTTCTTGAGACTACTGGGCTCAGCCGGAAGATGAGGCTGACTATATC
GTAAGTGTCGGGACAGCTGGTACCACTCGTGCTGGGAGGACACCCAGCGTACGCGGAGG
GCTACGCCTCCTGA3’ (SEQ ID NO:436)

Amino acid sequence of light chain variable region:

SSELTVQPDAPSVALGQTVRITCQGDSLRYAYSWYQQKPRQAPVLIVYGKNRP
SGIPDRFSGTSGNTASLTITGAQAEDDEDYCKSRDSSGDHLVFLGGGKTCLTVL
(SEQ ID NO:437)

FIG. 3PP

20A5.1

Nucleotide sequence of heavy chain variable region:

5’CAGGTGCAGCTGGGATCTGGGAGGACGCGTGGTCACGGGCTCCAGCCTGGGAGGTTCCTGAGCTCTCCCTGACGCTGGAGATTCACCTGACTAGTCTAGGCGATTGCTACACTGTTAGTTAAAAAGACTATGCGAAGCTCCTGGAAGGCCAGTCCACCACAGCAAGCACTCCAGAGCCAGGCTGATAGAAATGAAAGCCAGGCTCTACTACGTGACTTCACTCCA

Amino acid sequence of heavy chain variable region:

QVQLVESGGGLVQPSRLSLCASAASGFTFSSYGWMHVVRQAPGKGLEWVAIYYDSYKDYADSVKGSRSTIRDSRNKNNTLYLMNQLRAEDTAVYCSVAVYHYYGMDVWQGTTVTVSS (SEQ ID NO:443)

Nucleotide sequence of light chain variable region:

5’TCTGCTGACGCTGACTACAGGACCCCTGCTGCTGGCTTGGGACAGACA GTCAAGGATACATGCCAGAAGGAGGAAGCCTCCAGAACCTATTAGTGCAGCTGGTGACCGCAAGCCACGGCCCTATTCTCTGTCATCTATGGATAAAAACAA CCAGGCCTTGAGCCAATCAGGCTGACTTCACTTAGGATGGGTACCAAATCACA GTCTGCTGACGCTGACTACAGGACCCCTGCTGCTGGCTTGGGACAGACA

Amino acid sequence of light chain variable region:

SSELTQDPASVVALGQTVRTCGQDSRITYYASWYWYQKPRQAPILVIVYGKNNRPSGIPDRFSGTSIGSALTITGAQAEDAYYCKSRLIDIGNHLLFGGGTKLTVL (SEQ ID NO:445)

FIG. 3RR
Nucleotide sequence of heavy chain variable region:

5' CAGGTGCAGCTGGTGCCGCTCGGGGAGGCTGTCAGCCTGGAGGGGACGTC
CTGAGAATCTCCTCGTCAGCGTCTGGATTTCACCCGATGATGATGATGATGCA
CTGGGTCCGACAGTCACGACGGAAGGCTGGGCTGGAATGCGGGCGAGCTCATGG
TATGATGGAAGTAAACAAATGACTATGCAAGCCCATCGTGAAAGGGCCGATTCACCA
TCTCCAGAGACAAATCCAGAGACGCTGTATCTGCAAATGACACGTTGAG
AGCCGAGGACACGGGCGTGTATTACGTCTGCAGAAGGGGTTGTTCCGGGAGT
CATCGCTACTACTACTACGATATGGACGTCTGGGGCAGCGCCACGCGTCA
CCGTCCTCCTCA3' (SEQ ID NO:446)

Amino acid sequence of heavy chain variable region:

QVQLVASYGKVYQPSRLSLCAASGFLSSYGMHWRQAPGQGL';EHWVAVW
YDGSNKYYASVKGRTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGGSGSH
RYYYGMDVWGGQGTTVTSS (SEQ ID NO:447)

Nucleotide sequence of light chain variable region:

5'TCTTCTCGAGCTCAGCTAGACCCCTGGCTGGTGCTGTCGCTGGCTGCTG
GGGAGCCACGTAGAATCTACAGACGCAAGCCCATCGTGAAATGGCAACGTC
ACCAGCAAGACCCGAGCAGCCCGCCTATCTCTCTCTCTCTCTCTCTACGATG
ACGGGGCCTCCAGGGATCCAGACGGGATTCTCTCTCTCTCTCTCTCTCTA
CCGTCCTTGGACATCCTGACGCGGGTCCTAGGGAAGATGAGGCTGACTTACT
GTAACATCCCGGAGCATCATTGGTAAACATCTCTCTCTCTCTCTCTCTCTCTCT
ACTAAACCTCCTCGGAGGACTAAACCTCCTCGGAGGACTAAACCTCCTCGGAGGACTAA
GCTGACCGCTCTCA3' (SEQ ID NO:448)

Amino acid sequence of light chain variable region:

SSELTQDPAVSVALGQTVCITCQGDRLRTYAYSWYQPKPRQAPILVIYQKNRPS
GIPDRFGSTSGITASLITGQAEDDEAYYCKSRIDSNHLFQGKTLTVL (SEQ
ID NO:449)

FIG. 3SS
Nucleotide sequence of heavy chain variable region:

5'CAGGTGCAAGTGACTGCTGGAGGCGCCGCTGTCCAGCGCTGGAGGTC
CTGTCGCTGGCAGGGTCGACGTTTCTAACCTAGTATGAGCATC
ACTGGGGTCCGCAAGCTCCAGAGAAGGGCTGGAGTTGCTGAGTGTTAATG
GTATGGAGGATTAATATACCTAGACTCGTGATTCGGAAATTTAAGATCCTG
ACGGGAGGACACGCGCTTATATGTGCGAGGTACCTGCCGCT AACG
TTATAGCTGAGCTGTGGGGCAGGGACACACGTGACCGTGCTGCC
TCA3' (SEQ ID NO:450)

Amino acid sequence of heavy chain variable region:

QVQQVESGGGVSQPGRLSLCAASGFTFSNYGMDHVRQPAGKGEWVAVWIM
YDGGMNYMLARPSYKRRSLISRSNWSTLYLQMNSRRAEDTAATTCYCCSVVAGYHY
YGMDOVWQGTTTVAS (SEQ ID NO:451)

Nucleotide sequence of light chain variable region:

5'CAGTCTGCCCCTGACTGACCCCTGCTGCTTGGACAGCAGCTGA
TGACCCTCTTGCTGACGTGGCTGGATGTAGTATAGCTGCTGCA
CTCTGTGCACACAGCGCCAGCAACCCCAACTCGACATTTATGAGG
TCAGATTACGCTGCTCAGCGGTATTTATCCATCTGCTGCTGACAGTGGC
AACACGCGCTCTCGACCCATCTAGCGTTGCGCTGCAAGCGAGAGCGCTGGT
ATTTCTGACGTCATACCAGACCCAGCATGCGCTTTGGCGGAGGGACCAA
GCGGCGGCTCTGA3' (SEQ ID NO:452)

Amino acid sequence of light chain variable region:

QSAALTQPSVSRSQPQTSSTISCTGTSYDGGYNSVSWYQQHIPQPKLMYEVSN
RPSGISNRPSGSKSNTASLTISGLQQADEADYFCSSTSTSMVFGGKTKLAVL
(SEQ ID NO:453)

FIG. 3TT
**20E5.1 — version2 (v2)**

**Nucleotide sequence of heavy chain variable region:**

```
5' CAGGTGCAAGTGCTGGAGCTCTGCGGAGGCTGGCTCCAGCTGGAGGCTC
CTGAGACCTCTCTGTCAGCTGGCTTCCCCCTGAGGTCTGGGCTGCTGTTG
GATGATTGGAGCTTTATAATATATATGCAAGACCTCCTGAGAGGCGGAT
ACTCCAGCTCAGCGCAGGCTGATACCTGCAAATGACACGGCGTC
```

Amino acid sequence of heavy chain variable region:

```
QVQVVEGQVEQSGRSLRSCLCASAEGFTFSNYGMHWVQRAPGKGLEWVAVIW
YDGGNKYYADSVKGRSIISRDNKSTLYLQMNLSREDAEYVYYYCARVASVG
YYGMDVWGGQGTITVVAS (SEQ ID NO:455)
```

**Nucleotide sequence of light chain variable region:**

```
5'TCTTCTGACAGCTGACTGAGACCCCTGCCTGTGCTGTGGCCTTGAGCAGCA
GTTGGATCACTGCAAGAGAAGACAGCTACAGGCTTTATATATGCAACGTG
AACCAGCATAGGAGGCCCTGGGCTTCACTCATCTGTATGTAAGAACAA
CCGGCTCAGGGATTCCAGAGACCAGTCTGCGCTCCAGGCAGAAACACA
GCTTCCCTGAGACCACTGGGCCTAGGCGGAAGATGAGGGCTGACTATTACT
GTAACATTCCGGGACACATATTGTGACCACATCTGTGTTGGCCAGGGAGCAAA
GCTGACGCTCCTA3' (SEQ ID NO:456)
```

Amino acid sequence of light chain variable region:

```
SSELTDPAVSLQTVGRCDSLRTGYYASWYQQKPQLPQLLVEYQGKNNRP
SGIPDFRSGSTGNTSLTTLIGQAEDAYYCNRSRDNYGNDLHVFGGGKTLV
L (SEQ ID NO:457)
```

**FIG. 3UU**
8A3.1

Nucleotide sequence of heavy chain variable region:

5'GAGGTGCAGCTGGTGAGTCTGGGAGGCTTGTCATCCAGCTGGGGGCTCC
CTGAGACTCTCTCAGCTGGCAGCTCAGGATACCTTTAGTGTAGTATGAG
CTGGTGCCACCGCTCCAGGAAAGGCGCTGAGTCTGGGCTGCGACAGCATAAA
AACAAGATGGAGAAGAATCATATGCTGGACTCTGTGAAAGGCGATCCAC
ATCTCCAGAGAGAACTGCAAGGAACACTCAGTGATCTGCAAATGAAAAGGCTGA
GAGCCGAGGACACGGCGTTGTATTACTGCTGCGAAGAGATCTTTGTTATTAGT
GATATAGTAGACTACTACTACTACTACGGTGATAGCCTGGGCGAAGGACC
ACGGTCACCGTCTCTCTCA3' (SEQ ID NO:458)

Amino acid sequence of heavy chain variable region:

EVQLVESGGGLVQPGGLSLRLSCAASGFTSSYWMVWSWVRQAPGKGLEWVASIKQ
DGSEKYYVDSVKGRFTISRDARNARNSLYLQMNSLRAEDTAAYVYGCGDLMVYD
IDYVYYGMDVFQPQGGTHTTVSS (SEQ ID NO:459)

Nucleotide sequence of light chain variable region:

5'GATATTTGTAGACTCAGTCTCCACTCTCTCTCTCCGTCACCCCTGGAAGGC
CGGCCTCTACTCTGGAGGCTTAAGCTACATGTAGATTAGTAACTTAC
AATTAGTTAGCTTACGGTCTCAGAAAGCCAGGCGAGTCTCCAGAGCTTCTGAC
CTCTGGTTCTAATCGGCGTCTGCGCTTCTGTGACAGGTTTCACTGGCAGT
GGATCAGCGACAGATTTTACACTGAAATCAGCAGAGTGAGGGCTGAGGATG
TTGGGCTTATTTACTGCTGTCAGGCACACTACAAACTCCGGCGTCACTTCCGCGGA
GGGCACAGGTAGAGATCAAA3' (SEQ ID NO:460)

Amino acid sequence of light chain variable region:

DYMVTQSLPLVTGEPASPSSCRRSSQSSLHSGNYNYLWDWLQPKPGQSPQQLLYLG
SNRASGVPDRGFSGSSGTDTFLKISRVSEAEDVGVYYCMQALQTPLTPGGGTKVEIK (SEQ ID NO:461)

FIG. 3VV
Nucleotide sequence of heavy chain variable region:

5’ GAGGTGACGTGGTGGGAGGCTTTTGCTCCAGCTGGGAGAGCTGCCC
CTGAGAATCTGCTTGGCACGCATGCTACCTCTTTTATAATTTTGAAGCT
CTGGTCGCCAGGTCCAGGACTTCTGGGCCTGAGGATGGTGGCTTCTCAAAA
AAGGATGGAAGTTGGAAAAATACCTATGTGCACTCTGTGAAGGCGGATTCGGC
ACCTCCAGAGAAGCAAGGAGAAGAATCCTACGTCTTTCTGCAATAGAGCCTGA
GAGGCGAGGATAGCGCTGTGACTACCTGTGAGACCTCTTAATGTGT
GATGATATAGACTACTACTACTACTACTACTAGGTAGTGGACGCTCTGGGCAAGGACC
ACGGTCAGCTCCTCCAC3’ (SEQ ID NO:462)

Amino acid sequence of heavy chain variable region:

EVQLVESGGGLVQPGGLRLSCAASGFTFVSSNYWMSWFVRQAPGKLEWVQIKQ
DGSEKYYVDSNKGRFASRDNAKNLSNKLQMNSLRAEDTAVYYCARDLQLMVYD
IDYVYYGMDVWGQGTTVSS (SEQ ID NO:463)

Nucleotide sequence of light chain variable region:

5’GATATTGTGATGACATGAGTCTCCTCCCTGCTGTCACCCCTGGAGAGCC
CGGCCCTCATCTTGGTAGCTAGTCAGAAGCTCCTCTGCAATAGTAGGGTAC
AACTATTGGTAGTTTCACTCCAGAAGGCAAGGCAAGTCACAGCTCCCTGA
TCTATTGGTGTCTAAGGGCTTCGGCTGAGGTCTAGGTGGCGAGT
GGATGACGCAACACTCTTAACTACAGAATCGAGGCTGAGGATG
TTQGAGTATTTATACTGCACTCAACACTCCTACAAAACCTCCGCCTCTTTGGCGGGA
GGACCAAGGCGGAGATCAA3’ (SEQ ID NO:464)

Amino acid sequence of light chain variable region:

DIVMTQSPSLPVPPTGEPASICRSSHQQSSLHSNGYNVLWDYLOKPGQGPQLLLYLG
SNRASGVDPDGFSGSGTGLHTKLVRVEAREVGGVYVCMQTQLTQLTFTGGGTKVEIK (SEQ ID NO:465)

FIG. 3WW
12H11.1

Nucleotide sequence of heavy chain variable region:

5' CAGGTGCACGCTGTTGAGTCTGCTGGGGAGGCGTGGCCACGGCTGGGAGGTC
CGTGACACTCTCTCTGACGCTGATCGAAATTCCTTACTGATAGCTATGCTGAC
ACTGGTGCCCGGAGGCTCCAGGAGAGGCTGAGTGCTGGGGAGCAGTTATA
CTATGATGGAATAAGAAACACTATGCAACACTCCGAGAAGGCGGATTCAC
ATCTGAGAGAATGCAAGAACACGCTGTATCTGCAATGAAAGACGCTGA
GAGCCGAGGACACCGGCTGTTATTACTGTGCGAGAGATCCGGGACTG
GGGCAGGGAAACCTGGCTACCCGCTCTCCCTCA3' (SEQ ID NO:466)

Amino acid sequence of heavy chain variable region:

QVQLVESGGGLVQPSRLSLRLRASGGFTSSYGHHWVRQAPGKGLEWVAVYY
DGKHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAAYVYYCARDRGGLWQQ
GTLVTVSS (SEQ ID NO:467)

Nucleotide sequence of light chain variable region:

5' GACATCGTGATGACGACCAGCTCCAGACTCCCTGCTGCTGCTGCTGCGAG
AGGGCCACCATCAGACTGCAACAGGCTGACGAGGCTCTACAACTGCA
GATGACATCTCTGAGCGCCCGCTAACCAGGATCGGGCTGCTCCCGAGATCGT
GCAGCGGTGCTGACAGATTTCACTCTCACCACATCAGGAGCGCTGAC
AGATGCTGGAGTTCTTACTGTGCAAATATTATATAGTACTCGTGGACGGG
GGAAGGGAAACCCGCGAACGAAATCCAAA3' (SEQ ID NO:468)

Amino acid sequence of light chain variable region:

DIVMTQSPDSLAVSLGERATINCKSSQVLYSSKSNLYWLWYQQPKGQPPLLIY
WASTREGVPDFSGBGTDFTLTISLQAEDVAVVYCCYQYYSTPWTGFQGK
VEIK (SEQ ID NO:469)

FIG. 3XX
11H4.1

Nucleotide sequence of heavy chain variable region:

5'GAGGTGCAAGCTGGTGAGTCTGGGGAAAGCTTGGGCACGCTGGGCTGGCCCTGGCCCGGCTCC
CTGGACACTCTCTCTGTGCGCTCTCTATGACCTACCTTATGAATCTTGATGAG
CTGGTCCGGCCAGCTCCAGGAAGGGGCTGAGTGGTGAGGGGCAACACATAA
GCAAGATGGAAATGATGAATAAACATATGTGAGCTGCTGGAGGCGCATTCACC
ATCTCCAGAGACACAGCAGCAAGAAATTCACTGCTACTGCAAAATGAAACAGCTGTA
GAGCCGAGGACACGGCTGTATTACTCTGCGGAGAGACTAAAACATGGGGATT
TGCTTTTGTATATCTGGGGCCAGGGACAAATGGTCAACCGTCCTCTCA3' (SEQ ID NO:470)

Amino acid sequence of heavy chain variable region:

EVQLVESGGLVQPGGLSLRLSCAASGLFTSWKEWYVNNKQ
DNDKYYVDVSKGRFTISKDKNKLNLQMKSNLRAEDTAVYYCARESNWGFCAF
DIWGGQGTMVTVSS (SEQ ID NO:471)

Nucleotide sequence of light chain variable region:

5'CAAGCTCTGTGCTGACTGACGGCAACCCTCAGCTGGGACCCCGGCCAGAGG
GTACCCACTCTCTCTGAGGACAGCTACCCAACTCGGAAGTAAAACTGTCA
ACTGGTCACCAGCAGTCCGGACAGGGCCAGGCCCCAAACCTCCTCATCTATGAA
TAATCGGGCGCCCTCAGGGCTCTCTGACGATTCTCTGCTCTCAAGTCTGGCA
CCTGAGCTCCTGCGCATACTGGGGTCCAGTGCTAGGATGTGATGATGATAT
TACTGTGAGGAGTGCATGACGGCTGAAATTGGGCTGTCGGGCGCAGGACCA
AGCTGACCGTCCTA3' (SEQ ID NO:472)

Amino acid sequence of light chain variable region:

QSVLFTQPSASGTGQVRVTSCSGSNNIGSKTVNVWYQQFPTAPKLIYSSNRRP
SGVPDRFSKSGTSAALSAISQEDADYYCACAWDDSSLNWWFAGTKLTVL
(SEQ ID NO:473)

FIG. 3YY
Nucleotide sequence of heavy chain variable region:
5'GAGGTGCAGCTGCTGGAGTCTGGGGGAGGTGTCTGCAGCTGGGGCTCCCTAGGCTGAAGCTGGCTGGTCTGGGGGTGCCTGGGTTCCCTAGGTCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGC
**FIG. 3AAA**
8A1.2

Nucleotide sequence of heavy chain variable region:

5’GAGGTGCAGCTGTTGAGTCTGGGGAGCGTTTGCTGAAAGCTGGGAGTCC
CTGAGACTCTCTCTGAGCAGAGCTCTGGTACCTAACTGATTAGAGAC
CTGCGCTCCAGCCAGCTTGTGGAGGTGGGTGGTGAAGACAGAGCA
GCAAGATGGAAGTGAAGATATGACTATGTGGAAGCTCTGGTAAGGCGGAATCC
ACTCTCCAGACACCCGCAAGAAACTCTCTGTAATCTACAAATGACACGGCTGC
GAGCCAGAGACACCGCTGTTAATTACTGTGCAGACCTCTTGTACTATGTG
GTATGCTCTACACTACTACTACTACTACGCTATGGACGTCTGGGGCCACCGGAC
ACGCTACCCGTCCTCTCA A’ (SEQ ID NO:482)

Amino acid sequence of heavy chain variable region:

EVQLVESGGGLVQPGSSRLSCAASGFTFSSLPIFWVRQRPAKGLEWVASIKQ
DGSERYVVDSDVKGRFTISRDATKNSLYLQMNSLRAEDTVYYCARPLVMVYA
LHYYYVGDMDVWGHGTGTVVSS (SEQ ID NO:483)

Nucleotide sequence of light chain variable region:

5’GATTTTGATGATGACTGACCTCACTCTCCACCCTGGGAGAC
CAGGCTCCATCTCGTGAGACTGCTAGACAGCTCGTCTAGAGATGAC
AACTAAAGTGATTTGACACTGCAAGCAAGGCGAGGCGATCCTTTCAAGCTTGAGGAGT
TCTTGGGTTAACTTATCGATCAAGCTACTACAAACTCGGCTACCTTGGGGAGA
GGCACCAAGGTGGAAGTACAA A’ (SEQ ID NO:484)

Amino acid sequence of light chain variable region:

DIYMTQSPSLSLPVTPEPASICRASSQSLHSGNYNYLDWYLQKPGQSPQQILLYLG
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K (SEQ ID NO:485)

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  - 11H2.1: VH1.307/VH1.307
  - 11I1.1: VH1.307/VH1.307
  - 11J1.1: VH1.307/VH1.307
  - 12H1.1: VH1.3-33/VH1.3-33

- CH/CH:
  - 11G1.5: CH2/CH2
  - 11H1.1: CH3/CH3
  - 11H2.1: CH3/CH3
  - 11I1.1: CH3/CH3
  - 11J1.1: CH3/CH3
  - 12H1.1: CH3/CH3

**Hypervariable Domains:**

- VH: 46-55, 59-67
- VL: 22-32
- CH: 76-99

**Framework Regions:**

- FR1: 1-30
- FR2: 99-126

**CDR Domains:**

- CDR1: 31-45, 68-79
- CDR2: 46-58, 80-98

**Fig. 3GGG**
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**FIG. 3JJJ**
Fig. 3LLL

21B12

**Light Chain**

ESALTQPSAV SGSPQCSITI SCTGTSDDVG GYNSVSWYQQ HPGBPKPQMI YEVSRRPSGV SRRFSGSGSKG
NTASLTTSL QAEDAEYYCY NYSTSTSMVF GGGTKLTVLG QPKAAPSVTFL PFPSSSELQA NQATLVCLIS
DYPYPAVTVA WKADSPPKVA GVUVTTPSQK SNKYYAAST LSLTPEQWKS HRSYSQCVTH EGSTVEKTVA
PTECS (SEQ ID NO:297)

**Heavy Chain**

EVQLVQSGAE VKKPGASVKV SCKASGTYLTL SYGISWWRQA FPQQGLEWMGW VSFYNGNTNY AQKLQRGRGM
TTDFSTSTAY MEERSLRSDDD TAVYCAARY GM涯WSQQGTIT VTVSSAASRPF PSVFFLAPSS QSTSGGTSAAL
GCLVVKDYFPFE VTVSSWNLGA LTSGVHTFPA VLQSSGLYSL LSVTVFESSS LGTQTVICN VHKPSNYKVD
KKVFKSCAAR DEVDHHHHHH (SEQ ID NO:298)

31H4

**Light Chain**

ESALTPQPSV SGAPQRVTI SCTGSSSNIG AGYDVHWQQY LGTAPKLLI SGNSSRFSGV PDRFSGSKSG
TSASLALTGL QAEDAYCYY QCYDSSLSAG VFQQGTLTIV LQREPAAAPV TIFPPSSEEEL QANKATLVCL
ISDFYPCAVT VWAADSPSV KAGVETTTF KS QSNKHYAS YSLTPEQWK SHRSYSCQV TEGSTVEKT
VAPTECS (SEQ ID NO:299)

**Heavy Chain**

EVQLVESGGG LVKPGSSRL SCAASGFTFS SYSMMWVRQA FPGKLEWSS SSSSSTIT ACSVKGRFTI
SRDPAKNSLY LQMNSLRAPD TAVYCARQY DFWASYDSAF DWSQGSTMV TTTASTKGSF VPLAESGKS
TSQHTAALGC LVKDYFPEPV TSQWNSALTC SGRHHTPAVL QSSGLYSLSS VTVFSSSLG TGTVICN VH
KPSNTKVDK VEERKSCAAR DEVDHHHHHH (SEQ ID NO:300)

31A4

**Light Chain**

ALQSVLTPQP SASGPTGPQRV TISCSSSSSN IGSNTVNYQ QLPTAPKLL IYSSNNQREPSG
VPDFRSGSKS GTASLAISSG LQSEDEAMY CAVWDDSLING WVFGGTTKLT VLGQPKAAPS
VTFPPSSEE LQANKATLVC LIDSFYPGAV TVAWKADSP VKAGVETTTP SKQSNKHYAA
SSYLSLTPEQ WKSRRHSCQV VTIEGSTVEKT TVAPTECS (SEQ ID NO:301)

**Heavy Chain**

QVQLQQOGAG LLKPSSETLAL TCAVYGGGFS AAYWNNWIRQP FPGKLEWIGE INHSGRTDYN PSLKSRTVTIS
VDFSKKQPSL KLNSVTAAAT AVYVCCAOQG VDFYWQGQT LTSSAASTK GSFVLPALS SKESTSGTAA
LGCLYDIFCPF ETVSSWNSAG ALTSVGHTFP AVLQSSGLYS HSSVTVPSS SLGTVICY NVHKPSNTKVK
DKVFKSCAAR DEVDHHHHHH (SEQ ID NO:302)
10 or 100pM 31H4 + (0.05pM ~ 5nM) cPCSK9

K_D = 2 pM
95% CI: 1~3 pM

FIG. 4D
100pM 31H4 + (2.6pM - 25nM) mPCSK9

$K_D = 500$ pM

95% CI: 400 - 620 pM

FIG. 4F
12% Tris-Glycine gel, QuickBlue stain, 1μg/Lane

1. Marker 12
2. hPCSK9
3. mPCSK9
4. cPCSK9
5. 16F12
6. 21B12
7. 31H4
8. Marker 12
9. hPCSK9
10. mPCSK9
11. cPCSK9
12. 16F12
13. 21B12
14. 31H4
15. SeeBlue Plus2

FIG. 5A
Binding of 1.56-100 nM 21B12 to mPCS9 captured on penta-His Ab.

$K_{on} = 8.7 \times 10^4$
$K_{off} = 1.5 \times 10^{-3}$
$K_0 = 17 \text{ nM}$

FIG. 5D
Sigmoidal dose-response
Best-fit values

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31H4 IgG2

![Graph showing percentage inhibition against [Ab] ng/ml](image)

FIG. 6A
Sigmoidal dose-response

Best-fit values

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3H4 IgG4

% Inhibition

[Ab] ng/ml

FIG. 6B
FIG. 11A

D0
Sacrifice n=6

D3
Sacrifice n=6

D10
Sacrifice n=12

D13
Sacrifice n=12

Dose 31H4
10mg/kg
(Into all mice)

Dose 31H4
or IgG
10mg/kg

Dose 31H4
or IgG
10mg/kg
FIG. 12A
FIG. 12B
FIG. 12C
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**FIG. 13A**
FIG. 13B
### FIG. 13C

| Heavy Chain: |  |
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| 20D10 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 3039 heavy cdr | GYPLTSYGIS (SEQ ID NO:169)  |
| 2757 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 1989 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |
| 21512 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 2301 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 2685 heavy cdr | GYPLTSYGIS (SEQ ID NO:169)  |
| 3101 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 2715 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 17C2 heavy cdr | GYPLTSYGIS (SEQ ID NO:169)  |
| 25A7 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 3B6 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 3C9 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |
| 9H6 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 1A12 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 2335 heavy cdr | GYPLTSYGIS (SEQ ID NO:169)  |
| 25G4 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 1395 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 22B2 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |
| 28812 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 15F1 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 27A6 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |
| 31G11 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 30A4 heavy cdr | GYPLTSYGIS (SEQ ID NO:169)  |
| 31B12 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 31H4 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 27B2 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |
| 3C4 heavy cdr | GYPLTSYGIS (SEQ ID NO:168)  |
| 31A4 heavy cdr | GYPLTSYGIS (SEQ ID NO:166)  |
| 13H1 heavy cdr | GYPLTSYGIS (SEQ ID NO:167)  |

**Consensus**

|  |
| --- | --- | --- | --- |
| GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS |
| GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS |
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| GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS |
| GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS | GYPLTSYGIS |

**R.GLAARPGGMIV** (SEQ ID NO:254)
FIG. 13D
FIG. 13E
Consensus for Group 1:

20D10_light-heavy_cdr
3089_light-heavy_cdr
2747_light-heavy_cdr
1929_light-heavy_cdr
21812_light-heavy_cdr
2347_light-heavy_cdr
2694_light-heavy_cdr
3101_light-heavy_cdr
2785_light-heavy_cdr
1762_light-heavy_cdr
2547_light-heavy_cdr

Consensus for Group 2:

22E2_light-heavy_cdr
2391_light-heavy_cdr
2463_light-heavy_cdr
16F12_light-heavy_cdr
27A6_light-heavy_cdr
31G1_light-heavy_cdr

FIG. 13F
FIG. 13G
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**FIG. 13J**
FIG 14
FIG 15
FIG 16
FIG 18

![Graph showing RFU x 10^-4 vs. concentration with error bars and bars for Con and WT groups.](image-url)
FIG 19
FIG 20
FIG 21

A bar graph showing HDL-C (mg/dL) levels over time following injection (days) for different groups labeled as IgG2, 8A3, and 11F1.
FIG 22
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<tr>
<th>Antibody</th>
<th>AUC₀₄ (μg·h/mL)</th>
<th>CL₀₄ (mL/h/kg)</th>
<th>Apparent t₁/₂ (h)</th>
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<td>IgG2</td>
<td>23,500</td>
<td>0.511</td>
<td>&gt;120</td>
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<td>11F1</td>
<td>34,700</td>
<td>0.865</td>
<td>83</td>
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<td>8A3</td>
<td>36,700</td>
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FIG 23
FIG 24
FIG 25
FIG 27
FIG 29
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<th>Antibody</th>
<th>$t_{1/2,z}$ (h)</th>
<th>CL/F (mL/h/kg)</th>
<th>Cmax (µg/mL)</th>
<th>AUC$_{0\rightarrow\infty}$ (µg*h/mL)</th>
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<td>Anti-KLH</td>
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<td>0.234 ± 0.036</td>
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<td>6.22 ± 2.21</td>
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<td>11F1</td>
<td>210 ± 110</td>
<td>0.228 ± 0.081</td>
<td>6.72 ± 0.62</td>
<td>2,440 ± 910</td>
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<td>8A3</td>
<td>190 ± 40</td>
<td>0.255 ± 0.039</td>
<td>6.63 ± 0.66</td>
<td>1,990 ± 290</td>
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FIG 30
Light Chain Variable Sequence Comparison and Consensus Sequence

(HCDR1, HCDR2 and HCDR3 are underlined):

8A1: Light Chain Variable Sequence (SEQ ID NO:485); LCDR1 (SEQ ID NO:503); LCDR2 (SEQ ID NO:504); LCDR3 (SEQ ID NO:558)
8A3: Light Chain Variable Sequence (SEQ ID NO:461); LCDR1 (SEQ ID NO:503); LCDR2 (SEQ ID NO:4); LCDR3 (SEQ ID NO:558)
11F1: Light Chain Variable Sequence (SEQ ID NO:465); LCDR1 (SEQ ID NO:503); LCDR2 (SEQ ID NO:504); LCDR3 (SEQ ID NO:505)
Consensus: Light Chain Variable Sequence (SEQ ID NO:582); LCDR1 (SEQ ID NO:503); LCDR2 (SEQ ID NO:504); LCDR3 (SEQ ID NO:584)

8A1: 1-DIVMTQPLSLPVTGEPASISRCRSSQSLHSGNYNYLWQLQPGGQSPQLLIVLGSNRA
8A3: 1-DIVMTQPLSLPVTGEPASISRCRSSQSLHSGNYNYLWQLQPGGQSPQLLIVLGSNRA
11F1: 1-DIVMTQPLSLPVTGEPASISRCRSSQSLHSGNYNYLWQLQPGGQSPQLLIVLGSNRA
Consensus: 1-DIVMTQPLSLPVTGEPASISRCRSSQSLHSGNYNYLWQLQPGGQSPQLLIVLGSNRA

8A1: 5GVPDRFSGSSGDTFLKISRVEAEDVGVVCMQAQLTPTLFGGTKEIK-112 (SEQ ID NO:485)
8A3: 5GVPDRFSGSSGDTFLKISRVEAEDVGVVCMQAQLTPTLFGGTKEIK-112 (SEQ ID NO:461)
11F1: 5GVPDRFSGSSGTHTLKISRVEAEDVGVVCMQTLTPTLFGGTKEIK-112 (SEQ ID NO:465)
Consensus: 5GVPDRFSGSGTX1X2TLKISRVEAEDVGVVCMQX1X2TLPTTFTGGGTKEIK-112 (SEQ ID NO:582)

X1 is H or D; X2 is F or L; and X3 is T or A

FIG 31A
Heavy Chain Variable Chain Sequence Comparison And Consensus Sequence
(HCDR1, HCDR2 and HCDR3 are underlined):

8A1: Heavy Chain Variable Sequence (SEQ ID NO:459); HCDR1 (SEQ ID NO:513); HCDR2 (SEQ ID NO:528); HCDR3 (SEQ ID NO:534)
8A3: Heavy Chain Variable Sequence (SEQ ID NO:461); HCDR1 (SEQ ID NO:513); HCDR2 (SEQ ID NO:501); HCDR3 (SEQ ID NO:502)
11F1: Heavy Chain Variable Sequence (SEQ ID NO:463); HCDR1 (SEQ ID NO:513); HCDR2 (SEQ ID NO:501); HCDR3 (SEQ ID NO:502)
Consensus: Heavy Chain Variable Sequence (SEQ ID NO:583); HCDR1 (SEQ ID NO:585); HCDR2 (SEQ ID NO:586); HCDR3 (SEQ ID NO:587)

8A3: 1-EVQLVESGGGLVQPGGLRLSQAASGFTSSYWMWSVVRQAPGKGLEWVASIKQDGSEA YYY
11F1: 1-EVQLVESGGGLVQPGGLRLSQAASGFTS NYWMWSVVRQAPGKGLEWVASIKQDGSEA
8A1: 1-EVQLVESGGGLVQPGGLRLSQAASGFTSF NMTWVRQAPGKGLEWVASIKQDGSE RYY
Consensus: 1-EVQLVESGGGLVQPGGLRLSQAASGFTFSX,YWMX,YVRQAPGKGLEWVASIKQDGSEX,YY

8A3: 61-VDSVKGRTISRDNARNSLYLQMNSLRAEDTAVYYCAR DLVLMYD IDYYYYGMDVW
11F1: 61-VDSVKGRFAISRDN AKSLFLQMSLRAEDTAVYYCAR DLVLMYDIDYYYYGMDVW
8A1: 61-VDSVKGRTISRTDANKSLFLQMSLRAEDTAVYYCARPLVMV YALHYYYYYGMDVW
Consensus: 61-VDSVKGFRX,ISRDX,A,X,NSLX,LQMNSLRAEDTAVYYCARX,LVLMVYX,X,XX,YYYYYGMDVW

8A3: GQGTTVTVSS-127 (SEQ ID NO:459)
11F1: GQGTTVTVSS-127 (SEQ ID NO:463)
8A1: GHGTTVTVSS-127 (SEQ ID NO:483)
Consensus: GX,G TTVTVSS-127 (SEQ ID NO:583)

X, is S or N; X, is S or T; X, is K or R; X, is A or T; X, is N or T; X, is K or R; X, is Y or F; X, is D or P;
X, is D or A; X, is I or L; X, is D or H; and X, is Q or H

FIG 31B
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61P3/66 A61K39/395 C07K16/40

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)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>WO 2012/154999 A1 (AMGEN INC [US]; CHAN JOYCE CHI YEE [US]; GIBBS JOHN P [US]; DIAS CLAPT) 15 November 2012 (2012-11-15) page 2, line 20 - page 37, line 2; claims 1-70; figures 2A-D, 3A-D, 3G66, 3HHH, 3JJJ, 3JJJ; example 37</td>
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<td>WO 2012/109530 A1 (IRM LLC [US]; NOVARTIS AG [CH]; GOLDSMITH JOSHUA [US]; COHEN STEVEN BR) 16 August 2012 (2012-08-16) page 2, paragraph 0084 - page 6, paragraph 0033; claims 1-44 paragraph [0137]</td>
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[X] Further documents are listed in the continuation of Box C.  
[X] See patent family annex.

* 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
  
  **P** 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
  
  **A** document member of the same patent family

**Date of the actual completion of the international search**

16 October 2013

**Date of mailing of the international search report**

05/11/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5618 Patentien 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, 340-2907
Fax: (+31-70) 340-3016

Authorized officer

Hermann, Patrice
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