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(54) **Title:** ANTI-PCSK9 AND METHODS FOR TREATING LIPID AND CHOLESTEROL DISORDERS

(57) **Abstract:** The present invention provides compositions and methods for treating disorders of cholesterol and lipid metabolism by administration of an anti-PCSK9 antibody or a peptide inhibitor of PCSK9.

WO 2009/055783 A2

Anti-PCSK9 and Methods for Treating Lipid and Cholesterol Disorders

This application claims the benefit of U.S. patent application no. 60/982,922, filed October 26, 2007, which is herein incorporated by reference in its entirety.

Field of the Invention

The field of the present invention relates to methods and compositions for treating disorders of cholesterol homeostasis by administering an anti-PCSK9 antibody or antigen-binding fragment thereof.

Background of the Invention

Atherosclerotic coronary heart disease (CHD) represents the major cause for death and cardiovascular morbidity in the western world. Risk factors for atherosclerotic coronary heart disease include hypertension, diabetes mellitus, family history, male gender, cigarette smoke, high serum cholesterol, high low density lipoprotein (LDL) cholesterol levels and low high density lipoprotein (HDL) cholesterol levels. In general, a total cholesterol level in excess of about 225-250 mg/dl is associated with significant elevation of risk of CHD.

A variety of clinical studies have demonstrated that elevated levels of total cholesterol or LDL cholesterol promote human atherosclerosis. Epidemiologic investigations have established that cardiovascular morbidity and mortality vary directly with the level of total cholesterol and LDL cholesterol.

One method for lowering LDL cholesterol levels is by administration of HMG-CoA reductase inhibiting drugs. These drugs antagonize HMG-CoA reductase and cholesterol synthesis in the liver and increase the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL. A drawback of such an approach is that these drugs commonly suffer from a disadvantageous side-effect profile, including, for example, liver toxicity. An alternate approach is to modulate the LDL receptor pathway directly.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a serine protease family member that binds to and regulates LDL receptor expression on the surface of cells. Inhibition of the LDL receptor-PCSK9 interaction is an attractive approach to the treatment

of cholesterol disorders. Inhibition of interactions between large proteins (*i.e.*, protein-protein interactions or PPI) by the use of antibodies or small molecule inhibitors is, however, generally regarded as being particularly difficult and challenging. Large proteins such as PCSK9, with a molecular weight of about 74 KDa, and LDLR, with a molecular weight of about 160 KDa (glycosylated on cell surface; 115 KDa in immature form), are likely to exhibit extensive intermolecular contacts over a large area. The existence of extensive contacts makes it unlikely that a given antibody or small molecule inhibitor will successfully block their binding.

Summary of the Invention

The present invention surprisingly has overcome the technical difficulties associated with the blocking of intermolecular interactions between large proteins and has demonstrated that blockage of the PCSK9-LDLR interaction with an antibody or peptide is possible. As discussed in detail herein, this, in turn, provides a novel method by which to treat cholesterol disorders.

The present invention provides, in part, a method for reducing total cholesterol level, low density lipoprotein cholesterol level, apolipoprotein B level, total cholesterol/high density lipoprotein ratio or low density lipoprotein/high density lipoprotein ratio, in a subject (*e.g.*, a human), comprising administering, to said subject, a therapeutically effective amount of an antibody or antigen-binding fragment thereof (*e.g.*, monoclonal antibody, polyclonal antibody or recombinant antibody) or EGF-A polypeptide that binds specifically to PCSK9 which antibody or fragment or polypeptide inhibits binding between PCSK9 and LDL receptor; optionally in association with a further chemotherapeutic agent (*e.g.*, ezetimibe and/or simvastatin). In an embodiment of the invention, the antibody or fragment or EGF-A polypeptide binds specifically to a PCSK9 catalytic domain or to a domain of PCSK9 which interacts with an LDL receptor EGF-A domain.

The present invention further provides, in part, a method for treating or preventing hypercholesterolemia, hyperlipidemia, hypertriglyceridaemia, sitosterolemia, atherosclerosis, arteriosclerosis, coronary heart disease, vascular inflammation or xanthoma, in an subject, comprising administering, to said subject, a therapeutically effective amount of an antibody or antigen-binding fragment thereof (*e.g.*, monoclonal antibody, polyclonal antibody or recombinant antibody) or EGF-A polypeptide that binds specifically to PCSK9, which antibody or fragment or polypeptide inhibits binding between PCSK9 and LDL receptor; optionally in association with a further therapeutic agent (*e.g.*, ezetimibe and/or simvastatin). In an embodiment of the invention, the antibody or

fragment or EGF-A polypeptide binds specifically to a PCSK9 catalytic domain or to a domain of PCSK9 which interacts with an LDL receptor EGF-A domain.

The present invention also provides, in part, a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof (*e.g.*, monoclonal antibody, polyclonal antibody or recombinant antibody) or EGF-A polypeptide which binds specifically to PCSK9, which antibody or fragment or polypeptide inhibits binding between PCSK9 and LDL receptor, and a pharmaceutically acceptable carrier; optionally in association with a further chemotherapeutic agent (*e.g.*, ezetimibe and/or simvastatin).

The present invention also provides an isolated polypeptide comprising an amino acid sequence comprising about 90% or more amino acid sequence similarity to a fragment of the human LDL receptor which fragment consists of amino acids beginning at about amino acid position 314 and ending at about amino acid position 355 of said receptor wherein said polypeptide; wherein said polypeptide optionally comprises one or more properties selected from the group consisting of: (i) binds to PCSK9; (ii) competes with LDL receptor or an anti-PCSK9 antibody or antigen-binding fragment thereof for binding to PCSK9; (iii) reduces total cholesterol level when administered to an animal; (iv) reduces low density lipoprotein cholesterol level when administered to an animal; (v) reduces apolipoprotein B level when administered to an animal; (vi) reduces total cholesterol/high density lipoprotein ratio when administered to an animal; and (vii) reduces low density lipoprotein/high density lipoprotein ratio when administered to an animal; or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier. In an embodiment of the invention, the polypeptide consists of the amino acid sequence of SEQ ID NO: 3; or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier.

The present invention provides an isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:
(i) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 11B5 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 10;
(ii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 75B9 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 18;
(iii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 77D10 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 26;
(iv) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 29C10 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 34;

(v) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 22D11 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 42;
(vi) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 1F11/1G11 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 50;
(vii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 11B5 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 14;
(viii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 75B9 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 22;
(ix) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 77D10 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 30;
(x) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 29C10 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 38;
(xi) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 22D11 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 46; and
(xii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 1F11/1G11 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 54. The present invention also provides an isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:
(i) HCDR1 comprising the amino acid sequence G F N I K D T Y M H (SEQ ID NO: 11), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q D (SEQ ID NO: 12) and HCDR3 comprising the amino acid sequence S Y F G S I F A Y (SEQ ID NO: 13); (ii) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 19), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q G (SEQ ID NO: 20) and HCDR3 comprising the amino acid sequence S Y Y G S I F A Y (SEQ ID NO: 21); (iii) HCDR1 comprising the amino acid sequence G F N I K D Y Y I H (SEQ ID NO: 27), HCDR2 comprising the amino acid sequence W I D P E N G D T E Y A P K F Q G (SEQ ID NO: 28) and HCDR3 comprising the amino acid sequence Y Y R Y D D G T W F P Y (SEQ ID NO: 29);
(iv) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 35), HCDR2 comprising the amino acid sequence W I D P A N G Y T K Y A P N F Q G (SEQ ID NO: 36) and HCDR3 comprising the amino acid sequence G Y Y R Y Y S L D Y (SEQ ID NO: 37); (v) HCDR1 comprising the amino acid sequence G F T F S N H D M A (SEQ ID NO: 43), HCDR2 comprising the amino acid sequence S I T P S G

G T T Y Y R D S V E G (SEQ ID NO: 44) and HCDR3 comprising the amino acid sequence Q N Y Y D G S Y Y Y G L Y Y F D Y (SEQ ID NO: 45); (vi) HCDR1 comprising the amino acid sequence G Y T F T D Y Y M N (SEQ ID NO: 51), HCDR2 comprising the amino acid sequence D I N P N N G G A I Y N Q K F K G (SEQ ID NO: 52) and HCDR3 comprising the amino acid sequence G I I T E I A E D F (SEQ ID NO: 53); (vii) LCDR1 comprising the amino acid sequence S A S S S V S Y L Y (SEQ ID NO: 15), LCDR2 comprising the amino acid sequence R S S H R A S (SEQ ID NO: 16) and LCDR3 comprising the amino acid sequence H Q Y Q S Y P P T (SEQ ID NO: 17); (viii) LCDR1 comprising the amino acid sequence S A S S S V S Y L F (SEQ ID NO: 23), LCDR2 comprising the amino acid sequence R T S Y L A S (SEQ ID NO: 24) and LCDR3 comprising the amino acid sequence H Q Y H T Y P P T (SEQ ID NO: 25); (ix) LCDR1 comprising the amino acid sequence R A S G N I H S Y L A (SEQ ID NO: 31), LCDR2 comprising the amino acid sequence N A K T L P D (SEQ ID NO: 32) and LCDR3 comprising the amino acid sequence Q H F W N T P W T (SEQ ID NO: 33);

(x) LCDR1 comprising the amino acid sequence R A S Q D I S N Y L N (SEQ ID NO: 39), LCDR2 comprising the amino acid sequence Y S S R L H S (SEQ ID NO: 40) and LCDR3 comprising the amino acid sequence Q Q G K T L P L T (SEQ ID NO: 41);

(xi) LCDR1 comprising the amino acid sequence R S S Q S L V Y S D G N T Y L H (SEQ ID NO: 47), LCDR2 comprising the amino acid sequence R V S N R F S (SEQ ID NO: 48) and LCDR3 comprising the amino acid sequence L Q S T H F P P T (SEQ ID NO: 49); and (xii) LCDR1 comprising the amino acid sequence K A S Q N V G T N V V (SEQ ID NO: 55), LCDR2 comprising the amino acid sequence S A S Y R Y S (SEQ ID NO: 56) and LCDR3 comprising the amino acid sequence Q Q Y K T Y P Y T (SEQ ID NO: 57).

Furthermore, the present invention provides an isolated antibody or antigen-binding fragment thereof of claim xxx comprising one or more members selected from the group consisting of: (a) an immunoglobulin heavy chain comprising the amino acid sequence: E V Q L Q Q S G A E L V K P G A S V T L S C T A S G F N I K D T Y M H W V N Q R P E Q G L V W I G R I D P A N G H T E Y D P K F Q D K A T I T T D T S S N T A Y L H L S S L T S G D T A V Y Y C A R S Y F G S I F A Y W G Q G T L V T V S A (SEQ ID NO: 10); (b) an immunoglobulin light chain comprising the amino acid sequence: Q I V L T Q S P A

I M S A S P G E K V T I S C S A S S S V S Y L Y W Y Q Q K P G S S P K P W I F R S S H R A S G V P A R F S G S G S G T S Y S L T I S S M E A E D A A T Y Y C H Q Y Q S Y P P T F G G G T K L E I K R A (SEQ ID NO: 14); (c) an immunoglobulin heavy chain comprising the amino acid sequence: E V Q L Q Q S G A D L V K P G A S V K L S C T A S G F N I K D T Y I H W V K Q R P E Q G L E W I G R I D P A N G H T E Y D P K F Q G R A T L T T D T S S N T A Y L Q L F S L T S E D S A V Y F C A R S Y Y G S I F A Y W G Q G T L V T V S A (SEQ ID NO: 18); (d) an immunoglobulin light chain comprising the amino acid sequence: Q I V L T Q S P A I M S A S P G E K V T I S C S A S S S V S Y L F W Y Q Q K P G S S P K P W I F R T S Y L A S G V P A R F S G S G S G T S F S L T I S S M E A E D A A T Y Y C H Q Y H T Y P P T F G G G T K L E I K R A (SEQ ID NO: 22); (e) an immunoglobulin heavy chain comprising the amino acid sequence: E V Q L Q Q S G A E L V R S G A S V K L S C T T S G F N I K D Y Y I H W V K Q R P E Q G L E W I G W I D P E N G D T E Y A P K F Q G K A T M T A D T S S N T A Y L Q L S S L T S A D T A V Y Y C N A Y Y R Y D D G T W F P Y W G Q G T L V T V S A (SEQ ID NO: 26); (f) an immunoglobulin light chain comprising the amino acid sequence: D I Q L T Q S P A S L S A S V G E T V T I T C R A S G N I H S Y L A W Y Q Q K Q G K S P Q F L V D N A K T L P D G V P S R F S V S G S G T Q Y S L K I N S L Q P E D F G T Y Y C Q H F W N T P W T F G G G T K L E I K R A (SEQ ID NO: 30); (g) an immunoglobulin heavy chain comprising the amino acid sequence: E V L L Q Q S V A E L V R P G A S V R L S C T A S G F N I K D T Y I H W V R Q R P E Q G L E W F G W I D P A N G Y T K Y A P N F Q G K A T L T T D T S S N T A Y L H L S S L T S E D S A I Y Y C A R G Y Y R Y Y S L D Y W G Q G T S V T V S S (SEQ ID NO: 34);

(h) an immunoglobulin light chain comprising the amino acid sequence: D I Q M T Q T T S S L S A S L G D R V T I S C R A S Q D I S N Y L N W Y Q Q K P D G T V K L L I Y Y S S R L H S G V P S R F S G R G S G T D Y S L T I S T L E Q E D I A T Y F C Q Q G K T L P L T F G A G T K L E L K R A

(SEQ ID NO: 38); (i) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L V D S G G G L V Q P G R S L K L S C A A S G F T F S N H D M A W V R Q A P T K G L E W V A S I T P S G G T T Y Y R D S V E G R F T V S R D N V K S S L H L Q M D S L T S E D T A T Y Y C A R Q N Y Y D G S Y Y Y G L Y Y F D Y W G Q G V M V T V S S (SEQ ID NO: 42); (j) an immunoglobulin light chain comprising the amino acid sequence: D V L M T Q T P V S L P V S L G G Q V S I S C R S S Q S L V Y S D G N T Y L H W Y L Q K P G Q S P Q L L I Y R V S N R F S G V P D R F S G S G S G T D F T L K I S R V E P E D L G L Y Y C L Q S T H F P P T F G S G T K L E I K R A (SEQ ID NO: 46); (k) an immunoglobulin heavy chain comprising the amino acid sequence: E V Q L Q Q S G P E L V K P G A S V K I S C K V S G Y T F T D Y Y M N W V K Q S H G K S L E W I G D I N P N N G G A I Y N Q K F K G K A T L T V D K S S S I A Y M E L R S L T S E D S A V Y Y C T S G I I T E I A E D F W G Q G T T L T V S S (SEQ ID NO: 50); and (l) an immunoglobulin light chain comprising the amino acid sequence: D I V M T Q S Q K F M S T S V G D R V S V T C K A S Q N V G T N V V W Y Q Q K P G Q S P K A L I H S A S Y R Y S G V P D R F K G S G S G T D F T L T I T N V Q S E D L A G F F C Q Q Y K T Y P Y T F G G G T Q L E I K R A (SEQ ID NO: 54). Embodiments of the invention include, e.g., compositions comprising any of the antibodies or polypeptides of the present invention association with a further chemotherapeutic agent, e.g., a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme (ACE) inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor. In an embodiment of the invention, the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate, bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride or tolamolol. Embodiments of the invention also include those wherein the antibody or fragment is a humanized antibody, a

monoclonal antibody, a labeled antibody, a bivalent antibody, a polyclonal antibody, a bispecific antibody, a chimeric antibody, a recombinant antibody, an anti-idiotypic antibody, a humanized antibody, a bispecific antibody, a camelized single domain antibody, a diabody, an scfv, an scfv dimer, a dsfv, a $(dsfv)_2$, a dsFv-dsfv', a bispecific ds diabody, an Fv, an Fab, an Fab', an $F(ab')_2$, or a domain antibody. In an embodiment of the invention, the antibody or antigen-binding fragment thereof is linked to an immunoglobulin constant region, *e.g.*, a κ light chain, γ1 heavy chain, γ2 heavy chain, γ3 heavy chain or γ4 heavy chain. The present invention also provides a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof of the present invention in association with a pharmaceutically acceptable carrier.

The present invention also provides a method for reducing total cholesterol level; low density lipoprotein cholesterol level; apolipoprotein B level; total cholesterol/high density lipoprotein ratio; or low density lipoprotein/high density lipoprotein ratio; or for treating hypercholesterolemia; hyperlipidemia; hypertriglyceridaemia; sitosterolemia; atherosclerosis; arteriosclerosis; coronary heart disease; vascular inflammation; or xanthoma, in a subject, comprising administering, to said subject, a therapeutically effective amount of any polypeptide or antibody or antigen-binding fragment thereof of the present invention as set forth herein; or pharmaceutical composition thereof; optionally, in association with a further chemotherapeutic agent, *e.g.*, as set forth herein.

The present invention also provides a method for producing an antibody or antigen-binding fragment therof of claim 1 comprising introducing one or more polynucleotides into one or more host cells; which polynucleotides encodes and direct expression of said heavy and/or light chain immunoglobulins; and growing said host cells under conditions whereby said heavy and light chain immunoglobulins are expressed; *e.g.*, wherein said polynucleotide encoding and directing expression of said light chain and said polynucleotide encoding and directing expression of said heavy chain are in separate host cells.

Brief Description of the Figures

Figure 1. Mouse anti-human PCSK9 antibody mature variable region amino acid sequences. CDRs are underscored.

Detailed Description of the Invention

The present invention includes methods and compositions for an innovative method for treating cholesterol disorders. The methods and compositions of the present

invention are useful for treating cholesterol disorders by modulating the LDL receptor pathway. Specifically, the methods and compositions of the present invention antagonize the interaction between PCSK9 and LDLR and thereby lead to increased clearance of LDL from the bloodstream. In spite of formidable technical difficulties associated with blocking PPIs, the present invention provides a method for targeting and blocking this interaction, thus, leading to a beneficial effect with regard to blood cholesterol levels.

The term low density lipoprotein receptor (LDLR) includes any such receptor, *e.g.*, human LDLR along with any allelic variant thereof. In one embodiment of the invention, full-length LDLR comprises the following amino acid sequence (see *e.g.*, GenBank NM_000527.2):

MGPWGWKLRWTVALLLAAAGTAVGDRCERNEFQCQDGKCISYKWVCDGSAECQDGSDESQETCLSVTCKSGDFSCG
GRVNRCIPQFWRCDGQVDCDNGSDEQGCPPKTCSQDEFRCHDGKCISRQFVCDSDRDCLDGSDEASCPVLTCGPAS
FQCNSSTCIPQLWACDNDPDCEDGSDEWPQRCRGLYVFQGDSSPCSAFEFHCLSGECIHSSWRCDGGPDCKDKSDE
ENCAVATCRPDEFQCSDGNCIHGSRQCDREYDCKDMSDEVGCVNVTLCEGPNKFKCHSGECITLDKVCNMARDCRD
WSDEPIKECGTNECLDNNGGCSHVCNDLKIGYECLCPDGFQLVAQRRCEDIDECQDPDTCSQLCVNLEGGYKCQCE
EGFQLDPHTKACKAVGSIAYLFFTNRHEVRKMTLDRSEYTSLIPNLRNVVALDTEVASNRIYWSDLSQRMICSTQL
DRAHGVSSYDTVISRDIQAPDGLAVDWIHSNIYWTDSVLGTVSVADTKGVKRKTLFRENGSKPRAIVVDPVHGFMY
WTDWGTPAKIKKGGLNGVDIYSLVTENIQWPNGITLDLLSGRLYWVDSKLHSISSIDVNGGNRKTILEDEKRLAHP
FSLAVFEDKVFWTDIINEAIFSANRLTGSDVNLLAENLLSPEDMVLFHNLTQPRGVNWCERTTLSNGGCQYLCLPA
PQINPHSPKFTCACPDGMLLARDMRSCLTEAEAAVATQETSTVRLKVSSTAVRTQHTTTRPVPDTSRLPGATPGLT
TVEIVTMSHQALGDVAGRGNEKKPSSVRALSIVLPIVLLVFLCLGVFLLWKNWRLKNINSINFDNPVYQKTTEDEV
HICHNQDGYSYPSRQMVSLEDDVA

(SEQ ID NO: 1)

In an embodiment of the invention, a soluble LDLR fragment comprises the following amino acid sequence (Yamamoto *et al.*, Cell (1984) 39:27 – 38):

AVGDRCERNEFQCQDGKCISYKWVCDGSAECQDGSDESQETCLSVTCKSGDFSCGGRVNRCIPQFWRCDGQVDCDN
GSDEQGCPPKTCSQDEFRCHDGKCISRQFVCDSDRDCLDGSDEASCPVLTCGPASFQCNSSTCIPQLWACDNDPDC
EDGSDEWPQRCRGLYVFQGDSSPCSAFEFHCLSGECIHSSWRCDGGPDCKDKSDEENCAVATCRPDEFQCSDGNCI
HGSRQCDREYDCKDMSDEVGCVNVTLCEGPNKFKCHSGECITLDKVCNMARDCRDWSDEPIKECGTNECLDNNGGC
SHVCNDLKIGYECLCPDGFQLVAQRRCEDIDECQDPDTCSQLCVNLEGGYKCQCEEGFQLDPHTKACKAVGSIAYL
FFTNRHEVRKMTLDRSEYTSLIPNLRNVVALDTEVASNRIYWSDLSQRMICSTQLDRAHGVSSYDTVISRDIQAPD
GLAVDWIHSNIYWTDSVLGTVSVADTKGVKRKTLFRENGSKPRAIVVDPVHGFMYWTDWGTPAKIKKGGLNGVDIY
SLVTENIQWPNGITLDLLSGRLYWVDSKLHSISSIDVNGGNRKTILEDEKRLAHPFSLAVFEDKVFWTDIINEAIF
SANRLTGSDVNLLAENLLSPEDMVLFHNLTQPRGVNWCERTTLSNGGCQYLCLPAPQINPHSPKFTCACPDGMLLA
RDMRSCLTEAEAAVATQETSTVRLKVSSTAVRTQHTTTRPVPDTSRLPGATPGLTTVEIVTMSHQALGDVAGRGNE
KKPSSVR

(SEQ ID NO: 2)

In an embodiment of the invention, the EGF-A domain of LDL receptor comprises the amino acid sequence: GTNECLDNNGGCSHVCNDLKIGYECLCPDGFQLVAQRRCEDI (SEQ ID NO: 3)

The term "subject" includes any animal, *e.g.*, a mammal such as a human.

The present invention includes compositions and methods comprising antibodies and antigen-binding fragment thereof which bind specifically to PCSK9, for example, human PCSK9. In some embodiments of the invention, specific PCSK9 polypeptide sequences or antigenic fragments thereof from various species set forth below may be used as an antigen:

HUMAN gi|31317307|ref|NP_777596.2| proprotein convertase subtilisin/kexin type 9 preproprotein [*Homo sapiens*]

MGTVSSRRSWWPLPLLLLLLLLLGPAGARAQEDEDGDYEELVLALRSEEDGLAEAPEHGTTATFHRCAKD
PWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLPGFLVKMSGDLLELALKLPHV
DYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHREIEGRVMVTDFENVPE
EDGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPV
GPLVVLLPLAGGYSRVLNAACQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGT
LGTNFGRCVDLFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFSA
KDVINEAWFPEDQRVLTPNLVAALPPSTHGAGWQLFCRTVWSAHSGPTRMATAVARCAPDEELLSCSSFS
RSGKRRGERMEAQGGKLVCRAHNAFGGEGVYAIARCCLLPQANCSVHTAPPAEASMGTRVHCHQQGHVLT
GCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASCCHAPGLECKVKEHGIPAPQEQVTVACEEGW
TLTGCSALPGTSHVLGAYAVDNTCVVRSRDVSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

(SEQ ID NO: 4)

CHIMP gi|114556790|ref|XP_001154126.1| PREDICTED: proprotein convertase subtilisin/kexin type 9 [*Pan troglodytes*]

MGTVSSRRSWWPLPLLLLLLLLLGPAGARAQEDEDGLAEAPEHGTTATFHRCAKDPWRLPGTYVVVLKEE
THLSQSERTARRLQAQAARRGYLTKILHVFHGLLPGFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIP
WNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHREIEGRVMVTDFENVPEEDGTRFHRQASKCDS
HGTHLAGVVSGRDAGVAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSR
VLNAACQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVDLFAPG
EDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFSAKDVINEAWFPEDQRV
LTPNLVAALPPSTHGAGWQLFCRTVWSAHSGPTRMATAVARCAPDEELLSCSSFSRSGKRRGERMEAQGG
KLVCRAHNAFGGEGVYAIARCCLLPQANCSIHTAPPAEAGMGTRVHCHQQGHVLTGCSSHWEVEDLGTHK
PPMLRPRGQPNQCVGHREASIHASCCRAPGLECKVKEHGIPAPQEQVTVACEEGWTLTGCSALPGTSHVL
GAYAVDNTCVVRSRDVSTAGSTSEEAVAAVAICCRSRHLAQASQELQ

(SEQ ID NO: 5)

MOUSE gi|23956352|ref|NP_705793.1| proprotein convertase subtilisin/kexin type 9 [*Mus musculus*]

MGTHCSAWLRWPLLPLLPPLLLLLLLLCPTGAGAQDEDGDYEELMLALPSQEDGLADEAAHVATATFRRC
SKEAWRLPGTYIVVLMEETQRLQIEQTAHRLQTRAARRGYVIKVLHIFYDLFPGFLVKMSSDLLGLALKL

PHVEYIEEDSFVFAQSIPWNLERIIPAWHQTEEDRSPDGSSQVEVYLLDTSIQGAHREIEGRVTITDFNS
VPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGTSLHSLRVLNCQGKGTVSGTLIGLEFIRKSQLI
QPSGPLVVLLPLAGGYSRILNAACRHLARTGVVLVAAAGNFRDDACLYSPASAPEVITVGATNAQDQPVT
LGTLGTNFGRCVDLFAPGKDIIGASSDCSTCFMSQSGTSQAAAHVAGIVARMLSREPTLTLAELRQRLIH
FSTKDVINMAWFPEDQQVLTPNLVATLPPSTHETGGQLLCRTVWSAHSGPTRTATATARCAPEEELLSCS
SFSRSGRRRGDWIEAIGGQQVCKALNAFGGEGVYAVARCCLVPRANCSIHNTPAARAGLETHVHCHQKDH
VLTGCSFHWEVEDLSVRRQPALRSRRQPGQCVGHQAASVYASCCHAPGLECKIKEHGISGPSEQVTVACE
AGWTLTGCNVLPGASLTLGAYSVDNLCVARVHDTARADRTSGEATVAAAICCRSRPSAKASWVQ

(SEQ ID NO: 6)

RAT gi|77020250|ref|NP_954862.2| proprotein convertase subtilisin/kexin type 9 [*Rattus norvegicus*]

MGIRCSTWLRWPLSPQLLLLLLLCPTGSRAQDEDGDYEELMLALPSQEDSLVDEASHVATATFRRCSKEA
WRLPGTYVVVLMEETQRLQVEQTAHRLQTWAARRGYVIKVLHVFYDLFPGFLVKMSSDLLGLALKLPHVE
YIEEDSLVFAQSIPWNLERIIPAWQQTEEDSSPDGSSQVEVYLLDTSIQSGHREIEGRVTITDFNSVPEE
DGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGTSLHSLRVLNCQGKGTVSGTLIGLEFIRKSQLIQPSG
PLVVLLPLAGGYSRILNTACQRLARTGVVLVAAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTL
GTNFGRCVDLFAPGKDIIGASSDCSTCYMSQSGTSQAAAHVAGIVAMMLNRDPALTLAELRQRLILFSTK
DVINMAWFPEDQRVLTPNRVATLPPSTQETGGQLLCRTVWSAHSGPTRTATATARCAPEEELLSCSSFSR
SGRRRGDRIEAIGGQQVCKALNAFGGEGVYAVARCCLLPRVNCSIHNTPAARAGPQTPVHCHQKDHVLTG
CSFHWEVENLRAQQQPLLRSRHQPGQCVGHQEASVHASCCHAPGLECKIKEHGIAGPAEQVTVACEAGWT
LTGCNVLPGASLPLGAYSVDNVCVARIRDAGRADRTSEEATVAAAICCRSRPSAKASWVHQ

(SEQ ID NO: 7)

EGF-A

The present invention also provides methods for reducing total cholesterol level, low density lipoprotein cholesterol level, apolipoprotein B level, total cholesterol/high density lipoprotein ratio or low density lipoprotein/high density lipoprotein ratio, in a subject, by administering, to the subject, a polypeptide comprising an LDL receptor EGF-A domain optionally in association with a further chemotherapeutic agent (*e.g.*, as set forth herein) or a pharmaceutical composition thereof which comprises a pharmaceutically acceptable carrier. The EGF-A domain of LDL receptor binds to PCSK9 and, without being bound by any particular theory or mechanism, may reduce the activity of PCSK9 by competing with the full, endogenous LDL receptor for binding to PCSK9.

For example, in an embodiment of the invention, the EGF-A domain comprises or consists of the following amino acid sequence:

GTNECLDNNGGCSHVCNDLKIGYECLCPDGFQLVAQRRCEDI (SEQ ID NO: 3).

In an embodiment of the invention, human LDL receptor comprises the following amino acid sequence:

```
   1 mgpwgwklrw tvalllaaag tavgdrcern efqcqdgkci sykwvcdgsa ecqdgsdesq
  61 etclsvtcks gdfscggrvn rcipqfwrcd gqvdcdngsd eqgcppktcs qdefrchdgk
 121 cisrqfvcds drdcldgsde ascpvltcgp asfqcnsstc ipqlwacdnd pdcedgsdew
 181 pqrcrglyvf qgdsspcsaf efhclsgeci hsswrcdggp dckdksdeen cavatcrpde
 241 fqcsdgncih gsrqcdreyd ckdmsdevgc vnvtlcegpn kfkchsgeci tldkvcnmar
 301 dcrdwsdepi kec gtnecld nnggcshvcn dlkigyeclc pdgfqlvaqr rcedi decqd
 361 pdtcsqlcvn leggykcqce egfqldphtk ackavgsiay lfftnrhevr kmtldrseyt
 421 slipnlrnvv aldtevasnr iywsdlsqrm icstqldrah gvssydtvis rdiqapdgla
 481 vdwihsniyw tdsvlgtvsv adtkgvkrkt lfrengskpr aivvdpvhgf mywtdwgtpa
 541 kikkgglngv diyslvteni qwpngitldl lsgrlywvds klhsissidv nggnrktile
 601 dekrlahpfs lavfedkvfw tdiineaifs anrltgsdvn llaenllspe dmvlfhnltq
 661 prgvnwcert tlsnggcqyl clpapqinph spkftcacpd gmllardmrs clteaeaava
 721 tqetstvrlk vsstavrtqh tttrpvpdts rlpgatpglt tveivtmshq algdvagrgn
 781 ekkpssvral sivlpivllv flclgvfllw knwrlknins infdnpvyqk ttedevhich
 841 nqdgysypsr qmvsleddva
```

(SEQ ID NO: 1)

The EGF-A domain is underscored and in bold faced text. See also Genbank accession nos.: NP_000518, EAW84170, BAD92646.1 or AAF24515.1.

The present invention also comprises an isolated polypeptide comprising or consisting of an EGF-A polypeptide, for example, in a pharmaceutical composition which includes a pharmaceutically acceptable carrier. The present invention also provides such a polypeptide optionally fused to any other heterologous polypeptide which is not naturally contiguous with the other, immediately adjacent LDL receptor sequences as well as methods of treatments comprising administration of the fused polypeptide *e.g.*, as discussed herein. Any such polypeptide may be referred to herein as an "EGF-A polypeptide" and a polynucleotide encoding an EGF-A polypeptide may be referred to as an "EGF-A polynucleotide".

For example, in an embodiment of the invention, the EGF-A polynucleotide comprises the following nucleotide sequence:

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GGTACTAATGAATGTCTTGATAATAATGGTGGTTGTTCTCATGTTTGTAATGATCTTAAA
ATTGGTTATGAATGTCTTTGTCCTGATGGTTTTCAACTTGTTGCTCAACGTCGTTGTGAAGATATT
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(SEQ ID NO: 9)

As discussed above, the present invention also includes fusions which include the EGF-A polypeptides and polynucleotides of the present invention and a second polypeptide or polynucleotide moiety, which may be referred to as a "tag". The fused

polypeptides of the invention may be conveniently constructed, for example, by insertion of a polynucleotide of the invention or fragment thereof into an expression vector. The fusions of the invention may include tags which facilitate purification or detection. Such tags include glutathione-S-transferase (GST), hexahistidine (His6) tags, maltose binding protein (MBP) tags, haemagglutinin (HA) tags, cellulose binding protein (CBP) tags and myc tags. Detectable tags such as ^{32}P, ^{35}S, ^{3}H, ^{14}C, ^{18}F, ^{125}I, ^{131}I, ^{113m}In, ^{76}Br, ^{67}Ga, ^{99m}Tc, ^{123}I, ^{111}In and ^{68}Ga may also be used to label the polypeptides and polynucleotides of the invention. Methods for constructing and using such fusions are very conventional and well known in the art.

Any isolated polynucleotide encoding such an EGF-A polypeptide of the present invention also forms part of the present invention along with any vector comprising such a polynucleotide and a host cell (*e.g.*, bacterial host cell or eukaryotic cell) comprising such a vector. Such polynucleotides operably associated with an expression control sequence (*e.g.*, a promoter) also form part of the present invention.

In general, a "promoter" or "promoter sequence" is a DNA regulatory region capable of binding an RNA polymerase in a cell (*e.g.*, directly or through other promoter-bound proteins or substances) and initiating transcription of a coding sequence. A promoter sequence is, in general, bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at any level. Within the promoter sequence may be found a transcription initiation site (conveniently defined, for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. The promoter may be operably associated with other expression control sequences, including enhancer and repressor sequences or with a nucleic acid of the invention. Promoters which may be used to control gene expression include, but are not limited to, cytomegalovirus (CMV) promoter (U.S. Patent Nos. 5,385,839 and 5,168,062), the SV40 early promoter region (Benoist, *et al.*, (1981) Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., (1980) Cell 22:787-797), the herpes thymidine kinase promoter (Wagner, *et al.*, (1981) Proc. Natl. Acad. Sci. USA 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster, *et al.*, (1982) Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Komaroff, *et al.*, (1978) Proc. Natl. Acad. Sci. USA 75:3727-3731), or the tac promoter (DeBoer, *et al.*, (1983) Proc. Natl. Acad. Sci. USA 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American (1980) 242:74-94; and promoter elements from yeast or

other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter or the alkaline phosphatase promoter.

A coding sequence is "under the control of", "functionally associated with" or "operably associated with" transcriptional and translational control sequences in a cell or other expression system when the sequences direct RNA polymerase mediated transcription of the coding sequence into RNA, preferably mRNA, which then may be RNA spliced (if it contains introns) and, optionally, translated into a protein encoded by the coding sequence.

The terms "express" and "expression" mean allowing or causing the information in a gene, RNA or DNA sequence to become manifest; for example, producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene. A DNA sequence is expressed in or by a cell to form an "expression product" such as an RNA (e.g., mRNA) or a protein. The expression product itself may also be said to be "expressed" by the cell.

The term "vector" includes a vehicle (*e.g.*, a plasmid) by which a DNA or RNA sequence can be introduced into a host cell, so as to transform the host and, optionally, promote expression and/or replication of the introduced sequence.

Vectors that can be used in this invention include plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles that may facilitate introduction of the nucleic acids into the genome of the host. Plasmids are the most commonly used form of vector but all other forms of vectors which serve a similar function and which are, or become, known in the art are suitable for use herein. See, *e.g.*, Pouwels, *et al.*, Cloning Vectors: A Laboratory Manual, 1985 and Supplements, Elsevier, N.Y., and Rodriguez *et al.* (eds.), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, 1988, Buttersworth, Boston, MA.

The term "expression system" includes, for example, a host cell and compatible vector which, under suitable conditions, can express a protein or nucleic acid which is carried by the vector and introduced to the host cell. Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. A representative vector for amplifying DNA is pBR322 or many of its derivatives (*e.g.*, pUC18 or 19). Vectors that can be used to express the EGF-A polypeptides include, but are not limited to, those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters

(pOTS); or hybrid promoters such as ptac (pDR540). See Brosius *et al.*, "Expression Vectors Employing Lambda-, trp-, lac-, and lpp-derived Promoters", in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, 1988, Buttersworth, Boston, pp. 205-236. Many polypeptides can be expressed, at high levels, in an E.coli/T7 expression system as disclosed in U.S. Patent Nos. 4,952,496, 5,693,489 and 5,869,320 and in Davanloo, P., *et al.*, (1984) Proc. Natl. Acad. Sci. USA 81: 2035-2039; Studier, F. W., *et al.*, (1986) J. Mol. Biol. 189: 113-130; Rosenberg, A. H., *et al.*, (1987) Gene 56: 125-135; and Dunn, J. J., *et al.*, (1988) Gene 68: 259.

The present invention comprises methods of expression an EGF-A polypeptide of the present invention comprising introducing a vector comprising a polynucleotide encoding said polypeptide (*e.g.*, operably associated with an expression control sequence such as a promoter) into a suitable host cell and propagating (*e.g.*, growing in a suitable liquid growth medium) the host cell under conditions which are suitable to expression of the polypeptide from the polynucleotide in the vector.

Also part of the present invention is any isolated polypeptide with about 90% or more amino acid sequence similarity or identity to that of SEQ ID NO: 3. In an embodiment of the invention, such a polypeptide must bind to PCSK9 or a functional fragment thereof; or inhibit binding of PCSK9 and LDL receptor or any functional fragment of either; or must exhibit the ability to reduce total cholesterol level, low density lipoprotein cholesterol level, apolipoprotein B level, total cholesterol/high density lipoprotein ratio or low density lipoprotein/high density lipoprotein ratio in a subject, such as an acceptable animal model (*e.g.*, a mammal such as a dog, primate, rabbit, mouse or rat) or a human.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook, *et al.*, 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. (1985)); Transcription And Translation (B.D. Hames & S.J. Higgins, eds. (1984)); Animal Cell Culture (R.I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); B. Perbal, A Practical Guide To Molecular Cloning (1984); F.M. Ausubel, *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

The term "host cell" includes any cell of any organism that is selected, modified, transfected, transformed, grown, or used or manipulated in any way, for the production of a substance by the cell, for example the expression or replication, by the cell, of a gene, a DNA or RNA sequence or a protein.

The terms "isolated polynucleotide" or "isolated polypeptide" include a polynucleotide (*e.g.*, RNA or DNA molecule, or a mixed polymer) or a polypeptide, respectively, which are partially or fully separated from other components that are normally found in cells or in recombinant DNA expression systems. These components include, but are not limited to, cell membranes, cell walls, ribosomes, polymerases, serum components and extraneous genomic sequences.

An isolated polynucleotide or polypeptide will, in an embodiment of the invention, be an essentially homogeneous composition of molecules but may contain some heterogeneity.

The present invention contemplates any superficial or slight modification to the amino acid or nucleotide sequences which correspond to the EGF-A polypeptides and polynucleotides of the invention. In particular, the present invention contemplates sequence conservative variants of the nucleic acids which encode the EGF-A polypeptides of the invention. "Sequence-conservative variants" of an EGF-A polynucleotide sequence are those in which a change of one or more nucleotides in a given codon results in no alteration in the amino acid encoded at that position. Function-conservative variants of the EGF-A polypeptides of the invention are also contemplated by the present invention. "Function-conservative variants" are those in which one or more amino acid residues in an EGF-A polypeptide have been changed without altering the overall conformation and/or function of the polypeptide (*e.g.*, ability to bind to PCSK9 or inhibit binding of PCSK9 and an LDL receptor or fragment thereof), including, but, by no means limited to, replacement of an amino acid with one having similar properties. Amino acids with similar properties are well known in the art. For example, polar/hydrophilic amino acids which may be interchangeable include asparagine, glutamine, serine, cysteine, threonine, lysine, arginine, histidine, aspartic acid and glutamic acid; nonpolar/hydrophobic amino acids which may be interchangeable include glycine, alanine, valine, leucine, isoleucine, proline, tyrosine, phenylalanine, tryptophan and methionine; acidic amino acids which may be interchangeable include aspartic acid and glutamic acid and basic amino acids which may be interchangeable include histidine, lysine and arginine.

The present invention includes polynucleotides encoding EGF-A (*e.g.*, SEQ ID NO: 9) and functional fragments thereof as well as nucleic acids which hybridize to the polynucleotides. In an embodiment of the invention, the nucleic acids hybridize under low stringency conditions, under moderate stringency conditions or under high stringency conditions. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook, *et al.*, *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Typical low stringency hybridization conditions are 55°C, 5X SSC, 0.1% SDS, 0.25% milk, and no formamide at 42°C; or 30% formamide, 5X SSC, 0.5% SDS at 42°C. Typical, moderate stringency hybridization conditions are similar to the low stringency conditions except the hybridization is carried out in 40% formamide, with 5X or 6X SSC at 42°C. High stringency hybridization conditions are similar to low stringency conditions except the hybridization conditions are carried out in 50% formamide, 5X or 6X SSC and, optionally, at a higher temperature (*e.g.*, higher than 42°C: 57°C, 59°C, 60°C, 62°C, 63°C, 65°C or 68°C). In general, SSC is 0.15M NaCl and 0.015M Na-citrate. Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the higher the stringency under which the nucleic acids may hybridize. For hybrids of greater than 100 nucleotides in length, equations for calculating the melting temperature have been derived (see Sambrook, *et al.*, *supra*, 9.50-9.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook, *et al.*, *supra*). In an embodiment of the invention, such a polynucleotide encodes a polypeptide that must bind to PCSK9 or a functional fragment thereof; or inhibit binding of PCSK9 and LDL receptor or any functional fragment of either; or must exhibit the ability to reduce total cholesterol level, low density lipoprotein cholesterol level, apolipoprotein B level, total cholesterol/high density lipoprotein ratio or low density lipoprotein/high density lipoprotein ratio in a subject, such as an acceptable animal model (*e.g.*, a mammal such as a dog, primate, rabbit, mouse or rat) or a human.

Also included in the present invention are polynucleotides comprising nucleotide sequences and polypeptides comprising amino acid sequences which are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to a reference nucleotide sequence (SEQ ID NO: 9) or reference amino acid sequence (SEQ ID NO: 3), when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. Polypeptides comprising amino acid sequences which are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the reference EGF-A amino acid sequence of SEQ ID NO: 3, when the comparison is performed with a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

Sequence identity refers to exact matches between the nucleotides or amino acids of two sequences which are being compared. Sequence similarity refers to both exact matches between the amino acids of two polypeptides which are being compared in addition to matches between nonidentical, biochemically related amino acids. Biochemically related amino acids which share similar properties and may be interchangeable are discussed above.

The following references regarding the BLAST algorithm are herein incorporated by reference: **BLAST ALGORITHMS:** Altschul, S.F., *et al.*, (1990) J. Mol. Biol. 215:403-410; Gish, W., *et al.*, (1993) Nature Genet. 3:266-272; Madden, T.L., *et al.*, (1996) Meth. Enzymol. 266:131-141; Altschul, S.F., *et al.*, (1997) Nucleic Acids Res. 25:3389-3402; Zhang, J., *et al.*, (1997) Genome Res. 7:649-656; Wootton, J.C., *et al.*, (1993) Comput. Chem. 17:149-163; Hancock, J.M., *et al.*, (1994) Comput. Appl. Biosci. 10:67-70; **ALIGNMENT SCORING SYSTEMS**: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, Natl. Biomed. Res. Found., Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3." M.O. Dayhoff (ed.), pp. 353-358, Natl. Biomed. Res. Found., Washington, DC; Altschul, S.F., (1991) J. Mol. Biol. 219:555-565; States, D.J., *et al.*, (1991) Methods 3:66-70; Henikoff, S., *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89:10915-10919; Altschul, S.F., *et al.*, (1993) J. Mol. Evol. 36:290-300; **ALIGNMENT**

STATISTICS: Karlin, S., *et al.*, (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268; Karlin, S., *et al.*, (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877; Dembo, A., *et al.*, (1994) Ann. Prob. 22:2022-2039; and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in Theoretical and Computational Methods in Genome Research (S. Suhai, ed.), (1997) pp. 1-14, Plenum, New York.

Antibodies

The present invention includes methods and compositions comprising anti-PCSK9 antibodies and antigen-binding fragments thereof. The term anti-PCSK9 antibody or the like includes any antibody that binds specifically to PCSK9 (*e.g.*, human PCSK9). The anti-PCSK9 antibodies and antigen-binding fragments thereof used in the present invention include antibodies and fragments which were raised against or bind to the whole PCSK9 protein as well as antibodies raised against or bind to particular short epitopes within PCSK9, *e.g.*, the catalytic domain of PCSK9 or a portion thereof (*e.g.*, binding to an VFAQSIPWNLER epitope (SEQ ID NO: 8)) or a C-terminal domain of PCSK9 (*i.e.*, C-terminal relative to the cat domain of PCSK9)-having amino acids SRSGKRRGERMEA (amino acids 490-502 of SEQ ID NO: 4). In an embodiment of the invention, the anti-PCSK9 antibody or antigen-binding fragment thereof binds to the domain of PCSK9 which interacts with the EGF-A domain of the LDL receptor.

Specific isolated mouse anti-human PCSK9 antibody immunoglobulin sequences of the present invention are set forth below. CDR sequences for each immunoglobulin chain are underscored.

11B5 immunoglobulin heavy chain

E V Q L Q Q S G A E L V K P G A S V T L S C T A S G F N I K D T Y
M H W V N Q R P E Q G L V W I G R I D P A N G H T E Y D P K F Q D
K A T I T T D T S S N T A Y L H L S S L T S G D T A V Y Y C A R S
Y F G S I F A Y W G Q G T L V T V S A

(SEQ ID NO: 10)

HCDR1: G F N I K D T Y M H (SEQ ID NO: 11)

HCDR2: R I D P A N G H T E Y D P K F Q D (SEQ ID NO: 12)

HCDR3: S Y F G S I F A Y (SEQ ID NO: 13)

11B5 immunoglobulin light chain

Q I V L T Q S P A I M S A S P G E K V T I S C S A S S S V S Y L Y
W Y Q Q K P G S S P K P W I F R S S H R A S G V P A R F S G S G S
G T S Y S L T I S S M E A E D A A T Y Y C H Q Y Q S Y P P T F G G
G T K L E I K R A

(SEQ ID NO: 14)

LCDR1: S A S S S V S Y L Y (SEQ ID NO: 15)

LCDR2: R S S H R A S (SEQ ID NO: 16)

LCDR3: H Q Y Q S Y P P T (SEQ ID NO: 17)

75B9 immunoglobulin heavy chain

E V Q L Q Q S G A D L V K P G A S V K L S C T A S G F N I K D T Y
I H W V K Q R P E Q G L E W I G R I D P A N G H T E Y D P K F Q G
R A T L T T D T S S N T A Y L Q L F S L T S E D S A V Y F C A R S
Y Y G S I F A Y W G Q G T L V T V S A

(SEQ ID NO: 18)

HCDR1: G F N I K D T Y I H (SEQ ID NO: 19)

HCDR2: R I D P A N G H T E Y D P K F Q G (SEQ ID NO: 20)

HCDR3: S Y Y G S I F A Y (SEQ ID NO: 21)

75B9 immunoglobulin light chain

Q I V L T Q S P A I M S A S P G E K V T I S C S A S S S V S Y L F
W Y Q Q K P G S S P K P W I F R T S Y L A S G V P A R F S G S G S
G T S F S L T I S S M E A E D A A T Y Y C H Q Y H T Y P P T F G G
G T K L E I K R A

(SEQ ID NO: 22)

LCDR1: S A S S S V S Y L F (SEQ ID NO: 23)

LCDR2: R T S Y L A S (SEQ ID NO: 24)

LCDR3: H Q Y H T Y P P T (SEQ ID NO: 25)

77D10 immunoglobulin heavy chain

E V Q L Q Q S G A E L V R S G A S V K L S C T T S G F N I K D Y Y
I H W V K Q R P E Q G L E W I G W I D P E N G D T E Y A P K F Q G
K A T M T A D T S S N T A Y L Q L S S L T S A D T A V Y Y C N A
Y Y R Y D D G T W F P Y W G Q G T L V T V S A
(SEQ ID NO: 26)

HCDR1: G F N I K D Y Y I H (SEQ ID NO: 27)
HCDR2: W I D P E N G D T E Y A P K F Q G (SEQ ID NO: 28)
HCDR3: Y Y R Y D D G T W F P Y (SEQ ID NO: 29)

77D10 immunoglobulin light chain

D I Q L T Q S P A S L S A S V G E T V T I T C R A S G N I H S Y L
A W Y Q Q K Q G K S P Q F L V D N A K T L P D G V P S R F S V S G
S G T Q Y S L K I N S L Q P E D F G T Y Y C Q H F W N T P W T F G
G G T K L E I K R A
(SEQ ID NO: 30)

LCDR1: R A S G N I H S Y L A (SEQ ID NO: 31)
LCDR2: N A K T L P D (SEQ ID NO: 32)
LCDR3: Q H F W N T P W T (SEQ ID NO: 33)

29C10 immunoglobulin heavy chain

E V L L Q Q S V A E L V R P G A S V R L S C T A S G F N I K D T Y
I H W V R Q R P E Q G L E W F G W I D P A N G Y T K Y A P N F Q G
K A T L T T D T S S N T A Y L H L S S L T S E D S A I Y Y C A R G
Y Y R Y Y S L D Y W G Q G T S V T V S S
(SEQ ID NO: 34)

HCDR1: G F N I K D T Y I H (SEQ ID NO: 35)
HCDR2: W I D P A N G Y T K Y A P N F Q G (SEQ ID NO: 36)
HCDR3: G Y Y R Y Y S L D Y (SEQ ID NO: 37)

29C10 immunoglobulin light chain

D I Q M T Q T T S S L S A S L G D R V T I S C R A S Q D I S N Y L
N W Y Q Q K P D G T V K L L I Y Y S S R L H S G V P S R F S G R G
S G T D Y S L T I S T L E Q E D I A T Y F C Q Q G K T L P L T F G
A G T K L E L K R A

(SEQ ID NO: 38)

LCDR1: R A S Q D I S N Y L N (SEQ ID NO: 39)

LCDR2: Y S S R L H S (SEQ ID NO: 40)

LCDR3: Q Q G K T L P L T (SEQ ID NO: 41)

22D11 immunoglobulin heavy chain

E V Q L V D S G G G L V Q P G R S L K L S C A A S G F T F S N H D
M A W V R Q A P T K G L E W V A S I T P S G G T T Y Y R D S V E G
R F T V S R D N V K S S L H L Q M D S L T S E D T A T Y Y C A R Q
N Y Y D G S Y Y Y G L Y Y F D Y W G Q G V M V T V S S

(SEQ ID NO: 42)

HCDR1: G F T F S N H D M A (SEQ ID NO: 43)

HCDR2: S I T P S G G T T Y Y R D S V E G (SEQ ID NO: 44)

HCDR3: Q N Y Y D G S Y Y Y G L Y Y F D Y (SEQ ID NO: 45)

22D11 immunoglobulin light chain

D V L M T Q T P V S L P V S L G G Q V S I S C R S S Q S L V Y S D
G N T Y L H W Y L Q K P G Q S P Q L L I Y R V S N R F S G V P D R
F S G S G S G T D F T L K I S R V E P E D L G L Y Y C L Q S T H F
P P T F G S G T K L E I K R A

(SEQ ID NO: 46)

LCDR1: R S S Q S L V Y S D G N T Y L H (SEQ ID NO: 47)

LCDR2: R V S N R F S (SEQ ID NO: 48)

LCDR3: L Q S T H F P P T (SEQ ID NO: 49)

1F11/1G11 immunoglobulin heavy chain

E V Q L Q Q S G P E L V K P G A S V K I S C K V S G Y T F T D Y Y
M N W V K Q S H G K S L E W I G D I N P N N G G A I Y N Q K F K G
K A T L T V D K S S S I A Y M E L R S L T S E D S A V Y Y C T S G
I I T E I A E D F W G Q G T T L T V S S

(SEQ ID NO: 50)

HCDR1: G Y T F T D Y Y M N (SEQ ID NO: 51)

HCDR2: D I N P N N G G A I Y N Q K F K G (SEQ ID NO: 52)

HCDR3: G I I T E I A E D F (SEQ ID NO: 53)

1F11/1G11 immunoglobulin light chain

D I V M T Q S Q K F M S T S V G D R V S V T C K A S Q N V G T N V
V W Y Q Q K P G Q S P K A L I H S A S Y R Y S G V P D R F K G S G
S G T D F T L T I T N V Q S E D L A G F F C Q Q Y K T Y P Y T F G
G G T Q L E I K R A

(SEQ ID NO: 54)

LCDR1: K A S Q N V G T N V V (SEQ ID NO: 55)

LCDR2: S A S Y R Y S (SEQ ID NO: 56)

LCDR3: Q Q Y K T Y P Y T (SEQ ID NO: 57)

The present invention includes isolated polypeptides (*e.g.*, antibodies and antigen-binding fragments thereof) comprising one or more (*e.g.*, 3) CDRs taken from the light and/or heavy chain immunoglobulin set forth above as defined by the convention set forth in Kabat, "Sequences of Proteins of Immunological Interest" (National Institutes of Health, Bethesda, Md., 1987 and 1991) or in Chothia *et al.*, J. Mol. Biol. 196:901 (1987); Nature 342:878 (1989); and J. Mol. Biol. 186:651 (1989) (Kabat or Chothia) or Al-Lazikani *et al.*, J. Mol. Biol. 273: 927-948 (1997).

Thus, the invention includes any antibody or antigen-binding fragment thereof comprising one or more of the light chain and/or heavy chain immunoglobulin CDRs set forth above. In an embodiment of the invention, the antibody or fragment comprises all 3 light chain and/or all 3 heavy chain CDRs, *e.g.*, in the order specified above. Embodiments of the invention include anti-PCSK9 antibodies and antigen-binding fragments thereof, e.g., as set forth above wherein, the antibodies or fragments are

monoclonal antibodies, camelized single domain antibodies, polyclonal antibodies, bispecific antibodies, chimeric antibodies, recombinant antibodies, anti-idiotypic antibodies, humanized antibodies, bispecific antibodies, diabodies, single chain antibodies, disulfide Fvs (dsfv), Fvs, Fabs, Fab's, $F(ab')_2$s and domain antibodies. Thus, the term antibody covers, but is not limited to, monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies). The term antigen-binding fragment of an antibody encompasses a fragment or a derivative of an antibody, typically including at least a portion of the antigen-binding or variable regions (*e.g.*, one or more CDRs) of the parental antibody, that retains at least some of the binding specificity of the parental antibody. Examples of antibody antigen-binding fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments; dsFv; $(dsFv)_2$, ds diabodies; dsFv-dsFv'; single-chain antibody molecules, *e.g.*, sc-Fv, sc-Fv dimers (bivalent diabodies); bispecific diabodies; and multispecific antibodies formed from antibody fragments.

The present invention includes anti-PCSK9 antibodies and antigen-binding fragments thereof which binds specifically to PCSK9, for example, human PCSK9. In an embodiment of the invention an antibody or fragment that binds specifically to human PCSK9 binds preferentially to human PCSK9 as compared to that of rat, mouse or chimp PCSK9. Preferential binding to human PCSK9 means binding with an affinity which is greater than that of rat, mouse or chimp PCSK9 binding to any degree (*e.g.*, 1%, 10%, 50%, 100%, or 10X higher affinity). In an embodiment of the invention, an anti-human PCSK9 antibody binds to human PCSK9 without any detectable binding to any other species of PCSK9 (*e.g.*, no detectable binding to a mouse or rat PCSK9). Specific anti-PCSK9 binding refers to binding of the antibody to PCSK9 or an antigenic fragment thereof with a Kd at least about 100-fold higher than that of any other protein that might be bound and a minimum Kd of about 500 nM.

Any suitable method for generating antibodies may be used. For example, a recipient may be immunized with PCSK9 or an immunogenic fragment thereof. Any suitable method of immunization can be used. Such methods can include adjuvants, other immunostimulants, repeated booster immunizations, and the use of one or more immunization routes. Any suitable source of PCSK9 can be used as the immunogen for the generation of the antibodies and fragments of the compositions and methods disclosed herein. Such forms include, but are not limited whole protein, peptide(s), and epitopes generated through recombinant, synthetic, chemical or enzymatic degradation means known in the art.

Any form of the antigen can be used to generate the antibody that is sufficient to generate a biologically active antibody. Thus, the eliciting antigen may be a single epitope, multiple epitopes, or the entire protein alone or in combination with one or more immunogenicity enhancing agents known in the art. The eliciting antigen may be an isolated full-length protein, a cell surface protein (*e.g.*, immunizing with cells transfected with at least a portion of the antigen), or a soluble protein fragment.

Any suitable method can be used to elicit an antibody with the desired biologic properties to inhibit PCSK9. Monoclonal antibodies (mAbs) may be prepared from various mammalian hosts, such as mice, rats, other rodents, humans, other primates, *etc.* Description of techniques for preparing such monoclonal antibodies may be found in, *e.g.*, Stites *et al.* (eds.) BASIC AND CLINICAL IMMUNOLOGY (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) ANTIBODIES: A LABORATORY MANUAL CSH Press; Goding (1986) MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY. Thus, monoclonal antibodies may be obtained by a variety of techniques familiar to researchers skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell. See Kohler and Milstein (1976) Eur. J. Immunol. 6:511-519. Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. See, *e.g.*, Doyle *et al.* (eds. 1994 and periodic supplements) CELL AND TISSUE CULTURE: LABORATORY PROCEDURES, John Wiley and Sons, New York, NY. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences that encode a monoclonal antibody or an antigen binding fragment thereof by screening a DNA library from human B cells according, *e.g.*, to the general protocol outlined by Huse *et al.* (1989) Science 246:1275-1281.

Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. See, *e.g.*, Huse *et al. supra*; and Ward *et al.* (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature.

Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly U.S. Patent No. 4,816,567; and Queen *et al.* (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; or made in transgenic mice, see Mendez *et al.* (1997) Nature Genetics 15:146-156.

Mice which produce human immunoglobulins when immunized with an given antigen are also available in the art. See *e.g.*, Lonberg, N., *et al.*, (1994) Nature 368(6474): 856-859; Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49-101; Lonberg, N., *et al.*, (1995) Intern. Rev. Immunol. 13:65-93, and Harding, F., *et al.*, (1995) Ann. N. Y Acad. Sci 764:536-546); Taylor, L., *et al.*, (1992) Nucleic Acids Research 20:6287-6295; Chen, J., *et al.*, (1993) International Immunology 5: 647-656; Tuaillon, *et al.*, (1993) Proc. Natl. Acad. Sci USA 90:3720-3724; Choi, *et al.*, (1993) Nature Genetics 4:117-123; Chen, J., *et al.*, (1993) EMBO J. 12: 821- 830; Tuaillon, *et al.*, (1994) J Immunol. 152:2912-2920; Lonberg, *et al.*, (1994) Nature 368(6474): 856-859; Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49-101; Taylor, L., *et al.*, (1994) International Immunology 6: 579-591; Lonberg, N., *et al.*, (1995) Intern. Rev. Immunol. Vol. 13: 65-93; Harding, F., *et al.*, (1995) Ann. N.Y Acad. Sci 764:536-546; Fishwild, D., *et al.*, (1996) Nature Biotechnology 14: 845-851 and Harding, *et al.*, (1995) Annals NY Acad. Sci. 764:536-546. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874, 299; 5,770,429 and 5,545,807; and International Patent Application Publication Nos. WO 98/24884; WO 94/25585; WO 93/12227; WO 92/22645 and WO 92/03918.

A "Fab fragment" is comprised of 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.

An "Fc" region contains 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" contains one light chain and a portion of one heavy chain that contains the V_H 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 a $F(ab')_2$ 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.

"Disulfide stabilized Fv fragments" and "dsFv" include molecules having a variable heavy chain (V_H) and/or a variable light chain (V_L) which are linked by a disulfide bridge.

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

The term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the V_H and V_L chains to pair and form a binding site (*e.g.*, 5-12 residues long). For a review of scFv, see Pluckthun (1994) THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315. See also, International Patent Application Publication No. WO 88/01649 and U.S. Pat. Nos. 4,946, 778 and 5,260,203.

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 may target the same or different antigens.

A "bivalent antibody" comprises two antigen-binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific. For example, the present invention comprises scfv dimers and dsfv dimers, each of which scfv and dsfv moieties may have a common or different antigen binding specificity.

In an embodiment of the invention, a $(dsfv)_2$ comprises three peptide chains: two V_H moieties linked by a peptide linker and bound by disulfide bridges to two V_L moieties. In an embodiment of the invention, a bispecific ds diabody comprises a VH_1-VL_2 (tethered by a peptide linker) linked, by a disulfide bridge between the VH_1 and VL_1, to a VL_1-VH_2 moiety (also tethered by a peptide linker). In an embodiment of the

invention, a bispecific dsfv-dsfv' also comprises three peptide chains: a VH_1-VH_2 moiety wherein the heavy chains are linked by a peptide linker (*e.g.*, a long flexible linker) and are bound to VL_1 and VL_2 moieties, respectively, by disulfide bridges; wherein each disulfide paired heavy and light chain has a different antigen specificity. In an embodiment of the invention, an scfv dimer (a bivalent diabody) comprises a V_H-V_L moiety wherein the heavy and light chains are bound to by a peptide linker and dimerized with another such moiety such that V_Hs of one chain coordinate with the V_Ls of another chain and form two identical binding sites. In an embodiment of the invention a bispecific diabody comprises VH_1-VL_2 moiety (linked by a peptide linker) associated with a VL_1-VH_2 (linked by a peptide linker), wherein the VH_1 and VL_1 coordinate and the VH_2 and VL_2 coordinate and each coordinated set has diverse antigen specificities.

The term "monoclonal antibody", as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made recombinantly or by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256: 495, or may be made by recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature* 352: 624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597, for example. *See also* Presta (2005) *J. Allergy Clin. Immunol.* 116:731.

Monoclonal antibodies include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from

another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison *et al.*, (1984) *Proc. Natl. Acad. Sci.USA* 81: 6851-6855). For example, variable domains are obtained from an antibody from an experimental animal (the "parental antibody"), such as a mouse, and the constant domain sequences are obtained from human antibodies, so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a human subject than the parental mouse antibody.

A recombinant antibody or antigen-binding fragment thereof of the invention is, in an embodiment of the invention, an antibody which is produced recombinantly, *e.g.*, expressed from a polynucleotide which has been introduced into an organism (*e.g.*, a plasmid containing a polynucleotide encoding the antibody or fragment transformed into a bacterial cell (*e.g.*, *E.coli*) or a mammalian cell (*e.g.*, CHO cell)), followed by isolation of the antibody or fragment from the organism.

The present invention comprises methods for expressing any antibody or antigen-binding fragment theref or polypeptide of the present invention. For example, an embodiment of the present invention comprises a process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising at least the variable domains of the immunoglobulin heavy and/or light chains, in a host cell (*e.g.*, a single host cell), such as a CHO cell (*e.g.*, CHO-K1 or DXB11 cell), comprising the steps of (i) transforming said host cell with a first polynucleotide encoding at least the variable domain of the immunoglobulin heavy chain and a second polynucleotide encoding at least the variable domain of the immunoglobulin light chain, and (ii) independently expressing said first polynucleotide and said second polynucleotide so that said immunoglobulin heavy and light chains are produced as separate molecules in said transformed host cell. In an embodiment of the invention, the polynucleotides are operably linked to a promoter such as a CMV promoter. The present invention also comprises a process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising introducing transforming a first haploid yeast host cell (*e.g.*, *Pichia* such as *Pichia pastoris*) with a first polynucleotide encoding at least the variable domain of the immunoglobulin heavy chain or light chain and transforming a second haploid yeast host cell (*e.g.*, *Pichia* such as *Pichia pastoris*) with a second polynucleotide encoding the other chain, allowing the haploid cells to form polyploids, such as diploids, (*e.g.*,

via mating), selecting the polyploids from the haploids, and growing the polyploids under conditions wherein the heavy and light chains are expressed in the polyploids and, optionally, secreted into the culture medium.

In an embodiment of the invention, polynucleotides introduced into a host cell remain ectopic whereas in another embodiment of the invention, the polynucleotide is integrated into the chromosomal DNA of the host cell.

In an embodiment of the invention, a method for producing an antibody or fragment further comprises isolating the chains that are expressed from the host cell and/or culture medium.

The present invention also includes camelized single domain antibodies. *See, e.g.,* Muyldermans *et al.* (2001) *Trends Biochem. Sci.* 26:230; Reichmann *et al.* (1999) *J. Immunol. Methods* 231:25; WO 94/04678; WO 94/25591; U.S. Pat. No. 6,005,079, which are hereby incorporated by reference in their entireties). *Camelidae* (camels, dromedaries and llamas) comprise IgG antibodies in which are devoid of light chains and therefore called 'heavy-chain' IgGs or HCAb (for heavy-chain antibody). HCAbs typically have a molecular weight of ~95 kDa since they consist only of the heavy-chain variable domains. Although the HCAbs are devoid of light chains, they have an authentic antigen-binding repertoire (Hamers-Casterman *et al.*, Nature (1993) 363:446–448; Nguyen *et al.*, Adv. Immunol. (2001) 79:261–296; Nguyen *et al.*, Immunogenetics. (2002) 54:39–47). In one embodiment, the present invention provides single domain antibodies comprising two V_H domains with modifications such that single domain antibodies are formed.

As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90: 6444-6448. For a review of engineered antibody variants generally see Holliger and Hudson (2005) *Nat. Biotechnol.* 23:1126-1136.

As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from both human and non-human (*e.g.*, mouse or rat) antibodies. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the

hypervariable loops (CDRs) correspond to those of a non-human immunoglobulin, and all or substantially all of the framework (FR) regions are those of a human immunoglobulin sequence. An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called CDRs. Several public sources for human framework sequence are available including, *e.g.*, V-base (MRC Center for Protein Engineering). The humanized antibody may optionally comprise at least a portion of a human immunoglobulin constant region (Fc). For example, an embodiment of the invention includes humanized anti-PCSK9 (*e.g.*, anti-human PCSK9) antibodies comprising the specific mouse immunoglobulin CDRs and human immunoglobulin framework regions which are set forth herein, *e.g.*, fused to a human immunoglobulin constant region. The following U.S. patents are herein incorporated by reference: 5,585,089, 5,693,761, 5,693,762 and 6,180,370. For example, embodiments of the invention include those wherein any of the following groups of CDRs are included within a humanized antibody of the invention:

(i) HCDR1 comprising the amino acid sequence G F N I K D T Y M H (SEQ ID NO: 11), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q D (SEQ ID NO: 12) and HCDR3 comprising the amino acid sequence S Y F G S I F A Y (SEQ ID NO: 13);

(ii) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 19), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q G (SEQ ID NO: 20) and HCDR3 comprising the amino acid sequence S Y Y G S I F A Y (SEQ ID NO: 21);

(iii) HCDR1 comprising the amino acid sequence G F N I K D Y Y I H (SEQ ID NO: 27), HCDR2 comprising the amino acid sequence W I D P E N G D T E Y A P K F Q G (SEQ ID NO: 28) and HCDR3 comprising the amino acid sequence Y Y R Y D D G T W F P Y (SEQ ID NO: 29);

(iv) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 35), HCDR2 comprising the amino acid sequence W I D P A N G Y T K Y A P N F Q G (SEQ ID NO: 36) and HCDR3 comprising the amino acid sequence G Y Y R Y Y S L D Y (SEQ ID NO: 37);

(v) HCDR1 comprising the amino acid sequence G F T F S N H D M A (SEQ ID NO: 43), HCDR2 comprising the amino acid sequence S I T P S G G T T Y Y R D S V

E G (SEQ ID NO: 44) and HCDR3 comprising the amino acid sequence Q N Y Y D G S Y Y Y G L Y Y F D Y (SEQ ID NO: 45);

(vi) HCDR1 comprising the amino acid sequence G Y T F T D Y Y M N (SEQ ID NO: 51), HCDR2 comprising the amino acid sequence D I N P N N G G A I Y N Q K F K G (SEQ ID NO: 52) and HCDR3 comprising the amino acid sequence G I I T E I A E D F (SEQ ID NO: 53);

(vii) LCDR1 comprising the amino acid sequence S A S S S V S Y L Y (SEQ ID NO: 15), LCDR2 comprising the amino acid sequence R S S H R A S (SEQ ID NO: 16) and LCDR3 comprising the amino acid sequence H Q Y Q S Y P P T (SEQ ID NO: 17);

(viii) LCDR1 comprising the amino acid sequence S A S S S V S Y L F (SEQ ID NO: 23), LCDR2 comprising the amino acid sequence R T S Y L A S (SEQ ID NO: 24) and LCDR3 comprising the amino acid sequence H Q Y H T Y P P T (SEQ ID NO: 25);

(ix) LCDR1 comprising the amino acid sequence R A S G N I H S Y L A (SEQ ID NO: 31), LCDR2 comprising the amino acid sequence N A K T L P D (SEQ ID NO: 32) and LCDR3 comprising the amino acid sequence Q H F W N T P W T (SEQ ID NO: 33);

(x) LCDR1 comprising the amino acid sequence R A S Q D I S N Y L N (SEQ ID NO: 39), LCDR2 comprising the amino acid sequence Y S S R L H S (SEQ ID NO: 40) and LCDR3 comprising the amino acid sequence Q Q G K T L P L T (SEQ ID NO: 41);

(xi) LCDR1 comprising the amino acid sequence R S S Q S L V Y S D G N T Y L H (SEQ ID NO: 47), LCDR2 comprising the amino acid sequence R V S N R F S (SEQ ID NO: 48) and LCDR3 comprising the amino acid sequence L Q S T H F P P T (SEQ ID NO: 49); or

(xii) LCDR1 comprising the amino acid sequence K A S Q N V G T N V V (SEQ ID NO: 55), LCDR2 comprising the amino acid sequence S A S Y R Y S (SEQ ID NO: 56) and LCDR3 comprising the amino acid sequence Q Q Y K T Y P Y T (SEQ ID NO: 57).

The present invention also includes isolated polypeptides comprising the amino acid sequences of the immunoglobulin chains set forth herein along with isolated polynucleotides encoding said polypeptides, vectors comprising the polynucleotides and isolated host cells comprising the polynucleotides and vectors (*e.g.*, CHO cells, bacterial cells such as *E. coli* and fungal cells such as *S.cerevisiae* and *Pichia* such as *Pichia pastoris*). Methods for making the polypeptides are also included: comprising introducing

a polynucleotide or vector into a host cell and culturing the host cell under condition whereby the polypeptide can be expressed and, optionally, secreted and, optionally, isolating the polypeptide.

Therapeutic Methods, Administration and Pharmaceutical Formulations

The present invention provides methods for treating or preventing disorders of cholesterol or lipid homeostasis and disorders associated therewith, *e.g.*, hypercholesterolemia, hyperlipidemia, hypertriglyceridaemia, sitosterolemia, atherosclerosis, arteriosclerosis, coronary heart disease, vascular inflammation and xanthoma by administering a therapeutically effective amount of an anti-PCSK9 antibody (*e.g.*, as set forth herein) or antigen-binding fragment thereof or an EGF-A polypeptide.

The term hypercholesterolemia includes, *e.g.*, familial and non-familial hypercholesterolemia. Familial hypercholesterolemia (FHC) is an autosomal dominant disorder characterized by elevation of serum cholesterol bound to low density lipoprotein (LDL). Familial hypercholesterolemia includes both heterozygous FHC and homozygous FHC.

Hyperlipidemia is an elevation of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. Hyperlipidemia includes for example, type I, IIa, IIb, III, IV and V.

Sitosterolemia is a rare inherited plant sterol storage disease. In general, the metabolic defect in the affected patient causes hyperabsorption of sitosterol from the gastrointestinal tract, decreased hepatic secretion of sitosterol with subsequent decreased elimination, and altered cholesterol synthesis.

Atherosclerosis includes hardening of arteries associated with deposition of fatty substances, cholesterol, cellular waste products, calcium and fibrin in the inner lining of an artery. The buildup that results is called plaque.

Arteriosclerosis includes the diffuse build-up and deposition of calcium in artery walls which leads to hardening.

The present invention also provides methods for improving blood cholesterol markers associated with increased risk of heart disease. These markers include high total cholesterol, high LDL, high total cholesterol to HDL ratio and high LDL to HDL ratio.

In general, a total cholesterol of less than 200 mg/dL is considered desirable, 200-239 mg/dL is considered borderline high and 240 mg/dL and above is considered high.

In general, a blood LDL level of less than 100 mg/dL is considered optimal; 100-129 mg/dL is considered near optimal/above optimal, 130-159 mg/dL is considered

borderline high, 160-189 mg/dL is considered high and 190 mg/dL and above is considered very high.

In general, HDL levels considered normal are at least 35 - 40 mg/dL.

Another indicator of heart disease risk is the ratio of total cholesterol to HDL. In general, a very low risk of heart disease correlates with a ratio of <3.4 (men) or <3.3 (women); a low risk is associated with a ratio of 4.0 (men) or 3.8 (women), an average risk is associated with a ratio of 5.0 (men) or 4.5 (women), a moderate risk is associated with a ratio of 9.5 (men) or 7.0 (women) and a high risk is associated with a ratio of >23 (men) or >11 (women).

A further indicator of heart disease risk is the ratio of LDL to HDL. In general, a very low risk is associated with a ratio of 1 (men) or 1.5 (women), an average risk is associated with a ratio of 3.6 (men) or 3.2 (women), a moderate risk is associated with a ratio of 6.3 (men) or 5.0 (women) and a high risk is associated with a ratio of 8 (men) or 6.1 (women).

In an embodiment of the invention, anti-PCSK9 antibodies and antigen-binding fragments thereof or EGF-A polypeptides of the invention are formulated into a pharmaceutical formulation which comprises a pharmaceutically acceptable carrier. For general information concerning formulations, see, *e.g.*, Gilman, *et al.*, (eds.) (1990), The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; A. Gennaro (ed.), Remington's Pharmaceutical Sciences, 18th Edition, (1990), Mack Publishing Co., Easton, Pennsylvania.; Avis, *et al.*, (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, New York; Lieberman, *et al.*, (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, New York; and Lieberman, *et al.*, (eds.) (1990), Pharmaceutical Dosage Forms: Disperse Systems Dekker, New York, Kenneth A. Walters (ed.) (2002) Dermatological and Transdermal Formulations (Drugs and the Pharmaceutical Sciences), Vol 119, Marcel Dekker.

The anti-PCSK9 antibodies and antigen-binding fragments thereof or EGF-A polypeptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the antibody or fragment is combined in admixture with a pharmaceutically acceptable carrier. Carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides,

disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween™, Pluronics™ or PEG. The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. Therapeutic or pharmaceutical compositions or formulations herein 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.

The route of administration of the antibodies and antigen-binding fragments thereof of the invention are, in an embodiment of the invention, by a parenteral route (*e.g.*, intravenous, subcutaneous, intraarterial, intratumoral, intramuscular, intraperitoneal).

Dosages and desired anti-PCSK9 or EGF-A polypeptide concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" In Toxicokinetics and New Drug Development, Yacobi *et al.*, Eds., Pergamon Press, New York 1989, pp. 42 96.

When used to treat a disorder in a subject (*e.g.*, as discussed herein), a therapeutically effective dosage or amount of anti-PCSK9 antibody or antigen-binding fragment thereof or EGF-A polypeptides is administered to the subject. In an embodiment of the invention, a therapeutically effective dosage is a dosage sufficient to decrease total serum cholesterol, decrease blood LDL levels or increase blood HDL levels to any degree whatsoever. In an embodiment of the invention, a therapeutically effective dosage of anti-PCSK9 antibody or antigen-binding fragment thereof (*e.g.*, as set forth herein) for treatment of hypercholesterolemia, hyperlipidemia, hypertriglyceridaemia, sitosterolemia, atherosclerosis, arteriosclerosis, coronary heart disease, vascular inflammation or xanthoma or for treatment of any blood marker of heart disease risk (*e.g.*, as discussed herein) is about 0.1 mg/kg (body weight)/week to about 1.0 mg/kg/week. A therapeutically effective dosage of soluble PCSK9 EGF-A polypeptide is, in an embodiment of the invention, about 0.25 mg/kg/week to about 25 mg/kg/week.

When possible, administration and dosage of an agent (*e.g.*, further therapeutic agents discussed herein) is done according to the schedule listed in the product information sheet of the agents, in the Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002), as well as therapeutic protocols well known in the art.

A physician or clinician may monitor the blood cholesterol levels in a subject being treated or about to be treated with an anti-PCSK9 antibody or antigen-binding fragment thereof or EGF-A polypeptide and make adjustments to the subject's treatment regimen as needed to reach a positive medical outcome.

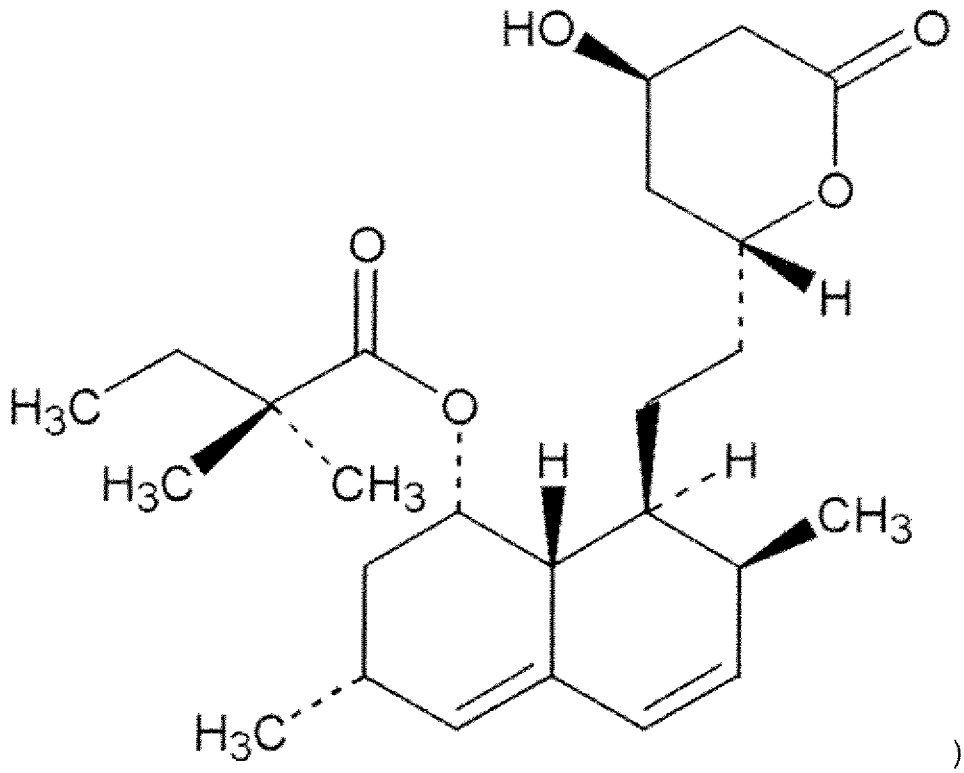
Further chemotherapeutic agents

The present invention provides methods and compositions for treating disorders of lipid and cholesterol metabolism (*e.g.*, any set forth herein) by administration of an anti-PCSK9 antibody or antigen-binding fragment thereof or EGF-A polypeptide. The antibodies may, in an embodiment of the invention, be provided or administered in association with any additional or further chemotherapeutic agent. In an embodiment of the invention, the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme (ACE) inhibitor, a coronary vasodilator, a diuretic or an adrenergic stimulant. In an embodiment of the invention, the further therapeutic agent is a cholesterol lowering medication such as an HMG-CoA reductase inhibitor.

Cardiovascular agents which may used in connection with the present invention include those for treatment or prevention of lipid and/or cholesterol disorders or hypertension and other cardiovascular disorders and diseases. Disorders of lipid or cholesterol metabolism may be caused or aggravated by hypertension. Hypertension is defined as persistently high blood pressure. Generally, adults are classified as being hypertensive when systolic blood pressure is persistently above 140 mmHg or when diastolic blood pressure is above 90 mmHg. Long-term risks for cardiovascular mortality increase in a direct relationship with persistent blood pressure. Examples of antihypertensive agents which may be used in the present invention include *e.g.*, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-II receptor antagonists, diuretics, adrenergic blockers including beta-adrenergic receptor blockers and alpha-adrenergic receptor blockers or diuretics.

Other cardiac drugs that may be provided in association with an anti-PCSK9 antibody or antigen-binding fragment thereof includes anti-anginal agents, such as adrenergic stimulants or coronary vasodilators and HMG-CoA reductase inhibitors.

HMG-CoA reductase inhibitors inhibit the HMG-CoA reductase enzyme and, thus, reduce production of cholesterol in the body of a subject. HMG-CoA reductase inhibitors include, *e.g.*, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin and simvastatin



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Adrenergic blockers include those compounds which are β-receptor inhibitors and/or α-receptor inhibitors. Adrenergic blockers which are β-receptor inhibitors include a class of drugs that antagonize the cardiovascular effects of catecholamines in hypertension, angina pectoris, and cardiac arrhythmias. β-adrenergic receptor blockers include, but are not limited to, bunolol hydrochloride (1(2H)-Naphthalenone, 5-[3-(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-,hydrochloride, CAS RN 31969-05-8 which can be obtained from Parke-Davis); acebutolol (±N-[3-Acetyl-4-[2-hydroxy-3-[(1 methylethyl)amino]propoxy]phenyl]-butanamide, or (±)-3'-Acetyl-4'-[2-hydroxy -3- (isopropylamino) propoxy] butyranilide); acebutolol hydrochloride (such as N-[3-acetyl-4-[2-hydroxy-3-[1-methyl-ethyle)amino]propoxy]phenyl]-,monohydrocochloride, (±-;-3'-Acetyl-4'-[2-hydroxy-3-(isopropylamino)propoxy]butyranilide monohydrochloride, for example, SECTRAL®

Capsules available from Wyeth-Ayerst); alprenolol hydrochloride (2-Propanol, 1-[(1-methylethyl)amino]-3-[2-(2-propenyl)phenoxy]-,hydrochloride, CAS RN 13707-88-5 see Netherlands Patent Application No. 6,605,692); atenolol (such as benzeneacetamide 4-[2'-hydroxy-3'-[(1-methylethyl)amino]propoxy]- , for example, TENORMIN® I.V. Injection available from AstraZeneca); carteolol hydrochloride (such as 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-2(1H)-quinolinone monohydrochloride, for example, Cartrol® Filmtab® Tablets available from Abbott); Celiprolol hydrochloride (3-[3-Acetyl-4-[3-(*tert*-butylamino)-2-hydroxypropoxyl]phenyl]-1,1-diethylurea monohydrochloride, CAS RN 57470-78-7, also see in US Patent No. 4,034,009); cetamolol hydrochloride (Acetamide, 2-[2-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-phenoxy]-N-methyl-,monohydrochloride, CAS RN 77590-95-5, see also US Patent No. 4,059,622); labetalol hydrochloride (such as 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl]salicylamide monohydrochloride, for example, NORMODYNE® Tablets available from Schering; esmolol hydrochloride ((±)-Methyl p-[2-hydroxy-3-(isopropylamino) propoxy] hydrocinnamate hydrochloride, for example, BREVIBLOC® Injection available from Baxter); levobetaxolol hydrochloride (such as (S)-1-[p-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride, for example, BETAXON™ Ophthalmic Suspension available from Alcon); levobunolol hydrochloride (such as (-)-5-[3-(tert-Butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2H)-naphthalenone hydrochloride, for example, BETAGAN® Liquifilm® with C CAP® Compliance Cap available from Allergan); nadolol (such as 1-(tert-butylamino)-3-[(5,6,7,8-tetrahydro-cis-6,7-dihydroxy-1-naphthyl)oxy]-2-propanol, for example, Nadolol Tablets available from Mylan); practolol (Acetamide, N-[4-[2-hydroxy-3-[1-methylethyl)amino]-propoxy]phenyl]-, CAS RN 6673-35-4, see also US Patent No3,408,387); propranolol hydrochloride (1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride CAS RN 318-98-9); sotalol hydrochloride (such as d,l-N-[4-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]-phenyl]methane-sulfonamide monohydrochloride, for example, BETAPACE AF™ Tablets available from Berlex);timolol (2-Propanol, 1-[(1,1-dimethylethyl)amino]-3-[[4-4(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-,hemihydrate, (S)-, CAS RN 91524-16-2); timolol maleate (S)-1-[(1, 1-dimethylethyl) amino]-3-[[4- (4-morpholinyl)-1, 2, 5-thiadiazol -3- yl] oxy]-2-propanol (Z)-2-butenedioate (1:1) salt, CAS RN 26921-17-5); bisoprolol (2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]-methyl]phenoxyl]-3-[(1-methylethyl)amino]-, (±), CAS RN 66722-44-9); bisoprolol fumarate (such as (±)-1-[4-[[2-(1-Methylethoxy) ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol (E) -2-butenedioate (2:1) (salt), for example, ZEBETA™ Tablets available from

Lederle Consumer); nebivalol (2H-1-Benzopyran-2-methanol, $\alpha\alpha'$-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-, CAS RN 99200-09-6 see also US Patent No. 4,654,362); cicloprolol hydrochloride, such 2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-[1-methylethyl)amino]-,hydrochloride, A.A.S. RN 63686-79-3); and dexpropranolol hydrochloride (2-Propanol, 1-[1-methylethyl)-amino]-3-(1-naphthalenyloxy)-hydrochloride (CAS RN 13071-11-9); diacetolol hydrochloride (Acetamide, N-[3-acetyl-4-[2-hydroxy-3-[(1-methyl-ethyl)amino]propoxy][phenyl]-,monohydrochloride CAS RN 69796-04-9);dilevalol hydrochloride (Benzamide, 2-hydroxy-5-[1-hydroxy-2-[1-methyl-3-phenylpropyl)amino]ethyl]-,monohydrochloride, CAS RN 75659-08-4); exaprolol hydrochloride (2-Propanol, 1-(2-cyclohexylphenoxy)-3-[(1-methylethyl)amino]-,hydrochloride CAS RN 59333-90-3); flestolol sulfate (Benzoic acid, 2-fluro-,3-[[2-[aminocarbonyl)amino]-1-dimethylethyl]amino]-2-hydroxypropyl ester, (±)-sulfate (1:1) (salt), CAS RN 88844-73-9; metalol hydrochloride (Methanesulfonamide, N-[4-[1-hydroxy-2-(methylamino)propyl]phenyl]-,monohydrochloride CAS RN 7701-65-7);metoprolol 2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[1-methylethyl)amino]-; CAS RN 37350-58-6);metoprolol tartrate (such as 2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, for example, LOPRESSOR® available from Novartis); pamatolol sulfate (Carbamic acid, [2-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxyl]phenyl]-ethyl]-,methyl ester, (±) sulfate (salt) (2:1), CAS RN 59954-01-7); penbutolol sulfate (2-Propanol, 1-(2-cyclopentylphenoxy)-3-[1,1-dimethylethyl)amino]1, (S)-, sulfate (2:1) (salt), CAS RN 38363-32-5); practolol (Acetamide, N-[4-[2-hydroxy-3-[(1-methylethyl)amino]-propoxy]phenyl]-, CAS RN 6673-35-4;) tiprenolol hydrochloride (Propanol, 1-[(1-methylethyl)amino]-3-[2-(methylthio)-phenoxy]-, hydrochloride, (±), CAS RN 39832-43-4); tolamolol (Benzamide, 4-[2-[[2-hydroxy-3-(2-methylphenoxy)-propyl]amino]ethoxyl]-, CAS RN 38103-61-6).

Adrenergic receptors which are α-receptor inhibitors act to block vasoconstriction induced by endogenous catecholamines. The resulting fall in peripheral resistance leads to a fall in mean blood pressure. The magnitude of this effect is dependent upon the degree of sympathetic tone at the time the antagonist is administered.

Suitable adrenergic receptors which are α-receptor inhibitors include, but are not limited to, fenspiride hydrochloride (which may be prepared as disclosed in US Patent No. 3,399,192 herein incorporated by reference); proroxan (CAS RN 33743-96-3); alfuzosin hydrochloride (CAS RN : 81403-68-1); and labetalol hydrochloride as described above or combinations thereof.

Adrenergic blockers with α and β receptor inhibitor activity which may be used with the present invention include, but are not limited to, bretylium tosylate (CAS RN : 61-75-6); dihydroergtamine mesylate (such as ergotaman-3', 6',18-trione,9,-10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-,(5'(alpha))-, monomethanesulfonate, for example, DHE 45® Injection available from Novartis); carvedilol (such as (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol, for example, COREG® Tablets available from SmithKline Beecham);. labetalol (such as 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl]salicylamide monohydrochloride, for example, NORMODYNE® Tablets available from Schering); bretylium tosylate (Benzenemethanaminium, 2-bromo-N-ethyl-N,N-dimethyl-, salt with 4-methylbenzenesulfonic acid (1:1) CAS RN 61-75-6); phentolamine mesylate (Phenol, 3-[[(4,5-dihydro-1H-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt) CAS RN 65-28-1); solypertine tartrate (5H-1,3-Dioxolo[4,5-f]indole, 7-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-, (2R,3R)-2,3-dihydroxybutanedioate (1:1) CAS RN 5591-43-5); zolertine hydrochloride (Piperazine, 1-phenyl-4-[2-(1H-tetrazol-5-yl)ethyl]-, monohydrochloride (8CI, 9CI) CAS RN 7241-94-3)

An angiotensin system inhibitor is an agent that interferes with the function, synthesis or catabolism of angiotensin II. These agents which may be used in the present invention include but are not limited to angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II and agents that prevent the synthesis of angiotensin I from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal profusion, or the concentration of Na+ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function. Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on angiotensinogen, a pseudoglobulin in blood plasma, to produce the cecapeptide angiotensin I. Angiotensin I is converted by angiotensin-converting enzyme (ACE) to angiotensin II. The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species.

Angiotensin II receptor antagonists are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II receptor antagonists which may be used in the present invention are well known and include peptide compounds and non-peptide compounds. Non-limiting

examples of angiotensin II receptor antagonists include: candesartan cilexetil (1H-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[[(cyclohexyloxy)carbonyl]oxy]ethyl ester) CAS RN 145040-37-5); telmisartan([1,1'-Biphenyl]-2-carboxylic acid, 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]- CAS RN 144701-48-4); candesartan (1H-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- CAS RN 139481-59-7); losartan potassium (1H-Imidazole-5-methanol, 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, monopotassium Irbesartan1,3-Diazaspiro[4.4]non-1-en-4-one, 2-butyl-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- CAS RN 138402-11-6).

Angiotensin-converting enzyme (ACE), is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors which may be used in the present invention include amino acids and derivatives thereof, peptides, including di and tri peptides and antibodies to ACE which intervene renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Suitable ACE inhibitors include, but are not limited to, benazepril hydrochloride (such as 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl]amino}-2,3,4,5-tetrahydro-2-oxo-1 H -1-(3S)-benzazepine-1-acetic acid monohydrochloride, for example, LOTREL® Capsules available from Novartis); captopril (such as 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline, for example, CAPTOPRIL Tablets available from Mylan); fosinopril (such as L-proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy) propoxy] (4-phenylbutyl) phosphinyl] acetyl]-, sodium salt, trans -.,for example, MONOPRIL® Tablets available from Bristol-Myers Squibb); moexipril hydrochloride (such as [3S-[2[R*(R*)],3R*]]-2-[2-[[1- (Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid, monohydrochloride, for example, UNIRETIC® Tablets available from Schwarz); perindopril erbumine (such as 2S,3aS,7aS)-1-[(S)-N-[(S)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), for example, ACEON® Tablets available from Solvay); quinapril (such as [3S-[2[R*(R*)],3R*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, monohydrochloride, for example, ACCURETIC® Tablets available from Parke-Davis); ramipril (such as 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative, for example, ALTACE® Capsules available from Monarch); enalapril maleate (such as (S)-1-[N -[1-(ethoxycarbonyl)-3-

phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1)., for example, VASOTEC® Tablets available from Merck); lisinopril (such as (S)-1-[N 2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, for example, PRINZIDE® Tablets available from Merck); delapril (which may be prepared as disclosed in US Patent No. 4,385,051); and spirapril (which may be prepared as disclosed in US Patent No. 4,470,972); benazeprilat (1H-1-Benzazepine-1-acetic acid, 3-[[(1S)-1-carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-, (3S)- CAS RN 86541-78-8); delapril hydrochloride (Glycine, N-[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-N-(2,3-dihydro-1H-inden-2-yl)-, monohydrochloride CAS RN 83435-67-0); fosinopril sodium (L-Proline, 4-cyclohexyl-1-[[(R)-[(1S)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]acetyl]-, sodium salt, (4S)- CAS RN 88889-14-9); libenzapril (1H-1-Benzazepine-1-acetic acid, 3-[[(1S)-5-amino-1-carboxypentyl]amino]-2,3,4,5-tetrahydro-2-oxo-, (3S)- CAS RN 109214-55-3); pentopril (1H-Indole-1-pentanoic acid, 2-carboxy-2,3-dihydro-.alpha.,.gamma.-dimethyl-.delta.-oxo-, .alpha.-ethyl ester, (.alpha.R,.gamma.R,2S)- CAS RN 82924-03-6); perindopril 1H-Indole-2-carboxylic acid, 1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)butyl]amino]-1-oxopropyl]octahydro-, (2S,3aS,7aS)- CAS RN 82834-16-0); quinapril hydrochloride (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, (3S)- CAS RN 82586-55-); quinaprilat (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[(1S)-1-carboxy-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, (3S)- CAS RN 82768-85-2); spirapril hydrochloride (1,4-Dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, 7-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-, monohydrochloride, (8S)- CAS RN 94841-17-5); spiraprilat 1(,4-Dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, 7-[(2S)-2-[[(1S)-1-carboxy-3-phenylpropyl]amino]-1-oxopropyl]-, (8S)- CAS RN 83602-05-5); teprotide (Bradykinin potentiator BPP9a CAS RN 35115-60-7); lisinopril (L-Proline, N2-[(1S)-1-carboxy-3-phenylpropyl]-L-lysyl- CAS RN 76547-98-3); zofenopril (L-Proline, 1-[(2S)-3-(benzoylthio)-2-methyl-1-oxopropyl]-4-(phenylthio)-, calcium salt (2:1), (4S)- CAS RN 81938-43-4).

"Calcium channel blockers" are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, *Cir. Res. V.* 52 (suppl. 1), p. 13-16 (1983); Fleckenstein, *Experimental Facts and Therapeutic Prospects*, John Wiley, New York (1983); McCall, D., *Curr. Pract Cardiol.*, v. 10, p. 1-11 (1985)). Calcium channel blockers are a heterogeneous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium

channels (Remington, *The Science and Practice of Pharmacy,* Nineteenth Edition, Mack Publishing Company, Eaton, PA, p. 963 (1995)). Calcium channel blockers useful in the present invention include but are not limited to, the besylate salt of amlodipine (such as 3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulphonate, for example, NORVASC® available from Pfizer); clentiazem maleate (1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-(2S-*cis*)-, (Z)-2-butenedioate (1:1), see also U.S. Patent No. 4,567,195); isradipine (3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-,methyl 1-methylethyl ester, (±)-4(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, see also US Patent 4,466,972); nimodipine (such as is isopropyl (2 - methoxyethyl) 1, 4- dihydro - 2, 6 - dimethyl - 4 - (3 - nitrophenyl) - 3, 5 - pyridine – dicarboxylate, for example, NIMOTOP® available from Bayer); felodipine (such as ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, for example, PLENDIL® Extended-Release Tablets available from AstraZeneca LP); nilvadipine (3,5-Pyridinedicarboxylic acid, 2-cyano-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-,3-methyl 5-(1-methylethyl) ester, also see US Patent No.3,799,934); nifedipine (such as 3,5-pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester, for example, PROCARDIA XL® Extended Release Tablets available from Pfizer); diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one,3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,-3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis., for example, TIAZAC® Capsules available from Forest); verapamil hydrochloride (such as benzeneacetronitrile, (alpha)-[[3-[[2-(3,4-dimethoxyphenyl) ethyl] methylamino] propyl]-3,4-dimethoxy-(alpha)-(1-methylethyl) hydrochloride, for example, ISOPTIN® SR Tablets available from Knoll Labs); teludipine hydrochloride (3,5-Pyridinedicarboxylic acid, 2-[(dimethylamino)methyl]-4-[2-[(1E)-3-(1,1-dimethylethoxy)-3-oxo-1-propenyl]phenyl]-1,4-dihydro-6-methyl-, diethyl ester, monohydrochloride) CAS RN 108700-03-4); belfosdil (Phosphonic acid, [2-(2-phenoxyethyl)-1,3-propanediyl]bis-, tetrabutyl ester CAS RN 103486-79-9); fostedil (Phosphonic acid, [[4-(2-benzothiazolyl)phenyl]methyl]-, diethyl ester CAS RN 75889-62-2).

Cardiovascular agents of the present invention which also act as "anti-anginal agents" are useful in the present invention. Angina includes those symptoms that occur when myocardial oxygen availability is insufficient to meet myocardial oxygen demand. Non-limiting examples of these agents include: ranolazine (hydrochloride1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-

methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); betaxolol hydrochloride (2-Propanol, 1-[4-[2 (cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride CAS RN 63659-19-8); butoprozine hydrochloride (Methanone, [4-[3(dibutylamino)propoxy]phenyl](2-ethyl-3-indolizinyl)-, monohydrochloride CAS RN 62134-34-3); cinepazet maleate1-Piperazineacetic acid, 4-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-, ethyl ester, (2Z)-2-butenedioate (1:1) CAS RN 50679-07-7); tosifen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-18-4); verapamilhydrochloride (Benzeneacetonitrile, .alpha.-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-.alpha.-(1-methylethyl)-, monohydrochloride CAS RN 152-11-4); molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0); ranolazine hydrochloride (1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); tosifen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-18-4).

"Coronary vasodilators" may act to reduce angina systems by increasing the oxygen supply to the heart. Coronary vasodilators useful in the present invention include, but are not limited to, diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one,3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,-3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis, for example, TIAZAC® Capsules available from Forest); isosorbide dinitrate (such as 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate, for example, ISORDIL® TITRADOSE® Tablets available from Wyeth-Ayerst); sosorbide mononitrate (such as 1,4:3,6-dianhydro-D-glucitol,5-nitrate, an organic nitrate, for example, Ismo® Tablets available from Wyeth-Ayerst); nitroglycerin (such as 2,3 propanetriol trinitrate, for example, NITROSTAT® Tablets available from Parke-Davis); verapamil hydrochloride (such as benzeneacetonitrile, (±)-(alpha)[3[[2-(3,4 dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-(alpha)- (1-methylethyl) hydrochloride, for example, COVERA HS® Extended-Release Tablets available from Searle); chromonar (which may be prepared as disclosed in US Patent No. 3,282,938); clonitate (*Annalen* 1870 155); droprenilamine (which may be prepared as disclosed in German Patent No. 2,521,113); lidoflazine (which may be prepared as disclosed in US Patent No. 3,267,104); prenylamine (which may be prepared as disclosed in US Patent No. 3152173); propatyl nitrate (which may be prepared as disclosed in French Patent No. 1,103,113); mioflazine hydrochloride (1-

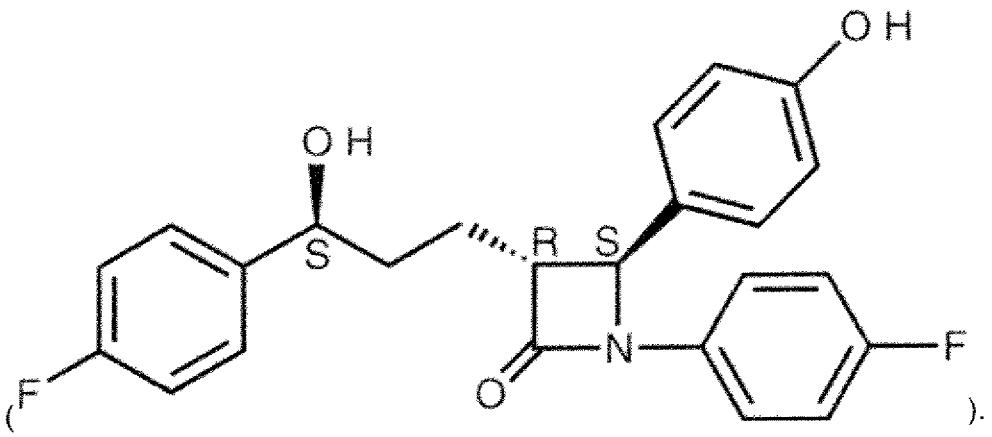
Piperazineacetamide, 3-(aminocarbonyl)-4-[4,4-bis(4-fluorophenyl)butyl]-N-(2,6-dichlorophenyl)-, dihydrochloride CAS RN 83898-67-3); mixidine (Benzeneethanamine, 3,4-dimethoxy-N-(1-methyl-2-pyrrolidinylidene)- Pyrrolidine, 2-[(3,4-dimethoxyphenethyl)imino]-1-methyl- 1-Methyl-2-[(3,4-dimethoxyphenethyl)imino]pyrrolidine CAS RN 27737-38-8); molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0); isosorbide mononitrate (D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate CAS RN 16051-77-7); erythrityl tetranitrate (1,2,3,4-Butanetetrol, tetranitrate, (2R,3S)-rel- CAS RN 7297-25-8); clonitrate(1,2-Propanediol, 3-chloro-, dinitrate (7CI, 8CI, 9CI) CAS RN 2612-33-1); dipyridamole Ethanol, 2,2',2'',2'''-[(4,8-di-1-piperidinylpyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]tetrakis- CAS RN 58-32-2); nicorandil (CAS RN 65141-46-0 3-); pyridinecarboxamide (N-[2-(nitrooxy)ethyl]- Nisoldipine3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester CAS RN 63675-72-9); nifedipine3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester CAS RN 21829-25-4); perhexiline maleate (Piperidine, 2-(2,2-dicyclohexylethyl)-, (2Z)-2-butenedioate (1:1) CAS RN 6724-53-4); oxprenolol hydrochloride2-Propanol, 1-[(1-methylethyl)amino]-3-[2-(2-propenyloxy)phenoxy]-, hydrochloride CAS RN 6452-73-9); pentrinitrol (1,3-Propanediol, 2,2-bis[(nitrooxy)methyl]-, mononitrate (ester) CAS RN 1607-17-6); verapamil (Benzeneacetonitrile, .alpha.-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-.alpha.-(1-methylethyl)- CAS RN 52-53-9).

The term "diuretic" includes compounds that increase the excretion of solutes (mainly NaCl) and water. In general, the primary goal of diuretic therapy is to reduce extracellular fluid volume in order to lower blood pressure or rid the body of excess interstitial fluid (edema). Non-limiting examples of diuretics which may be used within the scope of this invention include althiazide (which may be prepared as disclosed in British Patent No. 902,658); benzthiazide (which may be prepared as disclosed in U.S. Patent No. 3,108,097); buthiazide (which may be prepared as disclosed in British Patent Nos. 861,367); chlorothiazide (which may be prepared as disclosed in U.S. 2,809,194); spironolactone (CAS Number 52-01-7); and triamterene (CAS Number 396-01-0).

"Adrenergic stimulants" useful as cardiovascular agents in the present invention include, but are not limited to, guanfacine hydrochloride (such as N-amidino-2-(2,6-dichlorophenyl) acetamide hydrochloride, for example, TENEX® Tablets available from

Robins); methyldopa-hydrochlorothiazide (such as levo-3-(3,4-dihydroxyphenyl)-2-methylalanine) combined with Hydrochlorothiazide (such as 6-chloro-3,4-dihydro-2 H -1,2,4-benzothiadiazine-7- sulfonamide 1,1-dioxide, for example, the combination as, for example, ALDORIL® Tablets available from Merck); methyldopa-chlorothiazide (such as 6-chloro-2 H -1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide and methyldopa as described above, for example, ALDOCLORr® Tablets available from Merck) ; clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride and chlorthalidone (such as 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide), for example, COMBIPRES® Tablets available from Boehringer Ingelheim); clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, for example, CATAPRES® Tablets available from Boehringer Ingelheim); clonidine (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)-4,5-dihydro- CAS RN 4205-90-7).

The anti-PCSK9 antibodies and antigen-binding fragments thereof and EGF-A polypeptides may also be administered in association with any azetidinone which inhibits intestinal cholesterol absorption. Such azetidinones include ezetimibe



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Further chemotherapeutic agents that may be administered in association with an anti-PCSK9 antibody or antigen-binding fragment thereof or EGF-A polypeptide include fish oil, eicosaepenanoic acid, docosahexanoic acid, linoleic acid, niacin, fibrates such as fenofibrate, gemfibrozil and bile acid sequestrants such as cholestyramine, colestipol and colesevelam.

Other chemotherapeutic agents include althiazide (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-3-[(2-propenylthio)methyl]-, 1,1-dioxide CAS RN 5588-16-9); benzthiazide (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3-[[(phenylmethyl)thio]methyl]-, 1,1-dioxide CAS RN 91-33-8); captopril (L-Proline, 1-[(2S)-3-mercapto-2-methyl-1-oxopropyl]- CAS RN 62571-86-2); carvedilol (2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]- CAS RN 72956-09-3), chlorothiazide (sodium 2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-

methoxyphenoxy)ethyl]amino]- CAS RN 72956-09-3); clonidine hydrochloride (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)-4,5-dihydro-, monohydrochloride CAS RN 4205-91-8); cyclothiazide (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3-bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-, 1,1-dioxide CAS RN 2259-96-3); delapril hydrochloride (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3-bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-, 1,1-dioxide CAS RN 2259-96-3); dilevalol hydrochloride (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3-bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-, 1,1-dioxide CAS RN 2259-96-3); delapril hydrochloride (Glycine, N-[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-N-(2,3-dihydro-1H-inden-2-yl)-, monohydrochloride CAS RN 83435-67-0); doxazosin mesylate (Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl)carbonyl]-, monomethanesulfonate CAS RN 77883-43-3); fosinopril sodium (L-Proline, 4-cyclohexyl-1-[[(R)-[(1S)-2-methyl-1-(1-oxopropoxy)propox); moexipril hydrochloride (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-, monohydrochloride, (3S)- CAS RN 82586-52-5); monatepil maleate (1-Piperazinebutanamide, N-(6,11-dihydrodibenzo(b,e)thiepin-11-yl)-4-(4-fluorophenyl)-, (±)-, (Z)-2-butenedioate (1:1) (±)-N-(6,11-Dihydrodibenzo(b,e)thiepin-11-yl)-4-(p-fluorophenyl)-1-piperazinebutyramide maleate (1:1) CAS RN 132046-06-1), Metoprolol succinate (Butanedioic acid, compd. with 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol (1:2) CAS RN 98418-47-4); guanfacine hydrochloride (Benzeneacetamide, N-(aminoiminomethyl)-2,6-dichloro-, monohydrochloride CAS RN 29110-48-3; methyldopa (L-Tyrosine, 3-hydroxy-.alpha.-methyl- CAS RN 555-30-6); quinaprilat (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[(1S)-1-carboxy-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, (3S)- CAS RN 82768-85-2); quinapril hydrochloride (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, (3S)- CAS RN 82586-55-8); Primidolol (2,4(1H,3H)-Pyrimidinedione, 1-[2-[[2-hydroxy-3-(2-methylphenoxy)propyl]amino]ethyl]-5-methyl- CAS RN 67227-55-8); prazosin hydrochloride (Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)-, monohydrochloride CAS RN 19237-84-4); pelanserin hydrochloride 2,4(1H,3H)-Quinazolinedione, 3-[3-(4-phenyl-1-piperazinyl)propyl]-, monohydrochloride CAS RN 42877-18-9); phenoxybenzamine hydrochloride (Benzenemethanamine, N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)-, hydrochloride CAS RN 63-92-3); candesartan cilexetil (1H-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[[(cyclohexyloxy)carbonyl]oxy]ethyl ester CAS RN 145040-37-

5); telmisartan (1,1'-Biphenyl]-2-carboxylic acid, 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]- CAS RN 144701-48-4); candesartan1H-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- CAS RN 139481-59-7); amlodipine besylate3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, monobenzenesulfonate CAS RN 111470-99-6 Amlodipine maleate 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (2Z)-2-butenedioate (1:1) CAS RN 88150-47-4); terazosin hydrochloride (Piperazine, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)carbonyl]-, monohydrochloride CAS RN 63074-08-8); bevantolol hydrochloride (2-Propanol, 1-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-3-(3-methylphenoxy)-, hydrochloride CAS RN 42864-78-8); ramipril (Cyclopenta[b]pyrrole-2-carboxylic acid, 1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-, (2S,3aS,6aS)- CAS RN 87333-19-5).

Screening Assays

The present invention further provides a method for identifying a substance which is a PCSK9 inhibitor or an inhibitor of PCSK9/LDL receptor binding or an inhibitor of PCSK9/EGF-A domain binding; or a substance which reduces total cholesterol level, low density lipoprotein cholesterol level, apolipoprotein B level, total cholesterol/high density lipoprotein ratio or low density lipoprotein/high density lipoprotein ratio; or a substance which treats or prevents hypercholesterolemia, hyperlipidemia, hypertriglyceridaemia, sitosterolemia, atherosclerosis, arteriosclerosis, coronary heart disease, vascular inflammation or xanthoma in a subject comprising contacting PCSK9 polypeptide or a functional fragment thereof and a polypeptide comprising LDL receptor EGF-A domain (*e.g.*, SEQ ID NO: 3) and a sample to be tested for the presence of said substance; wherein said substance is identified if less binding of PCSK9 and the polypeptide comprising LDL receptor EGF-A domain is observed in the presence of the sample than in the absence of the sample. Any such substance identified in such an assay also forms part of the present invention.

An embodiment of the invention further comprises a negative-control assay wherein said PCSK9 and EGF-A polypeptides are contacted with a substance known not to inhibit binding of PCSK9 and LDL receptor or EGF-A domain thereof wherein the assay is determined to be operating properly if more binding of the PCSK9 and LDL receptor or EGF-A domain thereof is observed than in the present of an inhibitor of said binding.

An embodiment of the invention further comprises a positive-control assay wherein said PCSK9 and EGF-A polypeptides are contacted with a substance known to inhibit binding of PCSK9 and LDL receptor or EGF-A domain thereof wherein the assay is determined to be operating properly if less binding of the PCSK9 and LDL receptor or EGF-A domain thereof is observed than in the present of an inhibitor of said binding.

Binding of the substance can be determined using any of several methods known in the art.

Examples

The following information is provided for more clearly describing the present invention and should not be construed to limit the present invention. Any and all of the compositions and methods described below fall within the scope of the present invention.

Example 1: Soluble EGF-A domain of the LDL receptor or anti-PCSK9 antibody blocks the LDLR-PCSK9 interaction.

In this example, a soluble peptide encoding the EGF-A domain of the human LDL receptor as well as various anti-PCSK9 antibodies are shown to block interactions between PCSK9 and the receptor.

The following are the AlphaScreen methods and materials for the antibody and EGF-A inhibition of PCSK9-LDLR interaction experiments:

Purified PCSK9 carrying a C-terminal FLAG tag was purified from HEK293 cells stably expressing PCSK9. Purity was estimated, by silver staining, at greater than >90%. Anti-PCSK9-catalytic domain antibody was purchased from Cayman Chemical (Rabbit anti-murine-PCSK9 polyclonal antibody, Cayman Chemical; Ann Arbor, MI; Cat.# 10008811). This rabbit anti-mouse/human PCSK9-catalytic domain antibody recognizes an epitope with the following amino acid sequence: VFAQSIPWNLER (SEQ ID NO: 8). A rabbit anti- human PCSK9 C-terminal domain antibody (which binds specifically to SRSGKRRGERMEA (amino acids 490-502 of SEQ ID NO: 4)) and a rabbit anti-human PCSK9 whole protein antibody were also used.

Soluble EGF-A domain peptide was synthetically generated using standard methods. 2.5 ng of purified, C-terminally FLAG tagged PCSK9 (2.5 ng/2.5 ul/well of a ProxiPlate-384 Plus (Perkin-Elmer; Waltham, Mass) in 2.5 microliters of buffer (25 mM HEPES, 0.1M NaCl, 0.1% BSA, pH 7.5) was pre-incubated with 2.5 microliters of either anti-PCSK9 antibody or EGF-A domain peptide at the indicated concentrations for 30 minutes at room temperature. Next, 5 ul buffer containing 2.5 ng purified HIS-tagged

soluble LDLR (R&D Systems; Minneapolis, MN; Cat.# 2148-LD/CF; SEQ ID NO: 2) and 5 ng biotinylated anti-FLAG antibody (BioM2 monoclonal antibody, Sigma; St. Louis, MO; Cat.# F 9291), and the mixture, was incubated at room temperature for a further 30 minutes. Finally, under low light conditions, 5 ul of a donor/acceptor bead mixture (AlphaScreen Histidine Detection Kit, Perkin-Elmer; Waltham, Mass.; Cat.#6760619C) was added and alphaScreen signal was detected using the 2103 EnVision Multilabel Plate Reader (Perkin-Elmer; Waltham, Mass). The donor/acceptor bead mixture was prepared in dark conditions by adding 10 ul Nickel-Acceptor bead and 10 ul SA-Donor bead to 2.2 ml buffer for one 384-well plate and incubated in dark at room temperature for at least 2 hours.

The data generated in these experiments is set forth below in Tables 1-3.

Table 1. Inhibition of PCSK9-LDLR interaction by soluble LDLR-EGF-A domain in the presence or absence of added calcium*. Values are the mean of three replicates.

log[EGFA]uM	No calcium (mean counts)	2mM Calcium (mean counts)
2.30103	8476	4098.333
1.823909	25291	6320.667
1.346787	74404.33	27519
0.869666	132192.7	80542.67
0.392545	182225.3	143204.7
-0.08458	205094.7	202404.3
-0.5617	214258	234140.3
-1.03882	219281	246093.7
-1.51594	221429	240820.7
-1.99306	220878	257794
-2.47018	206909.3	254980.3

*A lower number of counts represents less observed PCSK9/LDL receptor binding.

These data indicate that the PCSK9-LDLR interaction is inhibited by soluble EGF-A domain in the presence or absence of calcium. A slightly greater level of inhibition was observed in the presence of calcium than in the absence of calcium. In the presence of calcium, the IC50 was about 3 micromolar and in the absence of calcium the IC50 was about 12 micromolar.

Table 2. Inhibition of PCSK9-LDLR interaction by anti-PCSK9 antibodies (catalytic domain (Cat) and C-terminal domain (CT))

log[antibody]ug/ml	Control IgG	Anti-PCSK9 (Cat)	Anti-PCSK9 (CT)
1.826075	113607.7	6840	37878
1.348954	135127.7	25815	87575.33
0.871832	139744.7	55616	108605.7
0.394711	135829.3	93545.67	119019.3
-0.08241	140328.3	94768	127197
-0.55953	139224.7	128021.3	127770.3
-1.03665	127405.7	124136.7	129751.7
-1.51377	133565.3	126417.7	131044.3
-1.9909	135194.7	129937.3	131160.7
-2.46802	140051.7	131985.3	128822.3

These data demonstrate that an anti-PCSK9 catalytic domain antibody and an anti-PCSK9 C-terminal domain antibody inhibits the interaction between PCSK9 and LDL receptor. The anti-PCSK9 raised against a C-terminal domain was less effective at inhibiting the interaction. A non-specific IgG control was ineffective at inhibiting the interaction and is shown for comparison. Values in this table are the mean of three replicates.

Table 3. Inhibition of PCSK9-LDLR interaction by anti-PCSK9 antibodies (catalytic domain (Cat) and whole protein (whole))

log[antibody]ug/ml	Control IgG	Anti-PCSK9 (Cat)	Anti-PCSK9 (whole)
1.826075	61920.67	2101.33	1005.33
1.348954	74394.33	12259.00	1435.00
0.871832	79625.67	33347.33	14236.67
0.394711	79552.00	60074.00	52994.00
-0.08241	82751.00	70040.00	75020.33
-0.55953	88110.00	77438.00	85241.00
-1.03665	84421.00	79789.00	82679.00
-1.51377	83144.67	85603.67	87349.33
-1.9909	80747.33	81462.67	80731.67
-2.46802	78151.33	80556.33	83171.00
-2.94514	82101.00	85405.33	80429.00

These data demonstrate that the PCSK9-LDLR interaction is inhibited by antibodies that bind the PCSK9 catalytic domain peptide and antibodies raised against the whole PCSK9 protein. A non-specific IgG control was ineffective at inhibiting the interaction and is shown for comparison. These values are mean of three replicates.

Example 2: Anti-PCSK9 antibody treatment of cells leads to enhanced LDL uptake.

Incubation of cells with soluble PCSK9 leads to a decrease in the ability of the cells to absorb and clear low density lipoprotein (LDL) from the medium. This example demonstrates that inhibition of PCSK9 with an anti-PCSK9 antibody antagonizes this effect of PCSK9 and leads to increased levels of LDL uptake and clearance.

The uptake assays were performed as follows: HepG2 cells at 10,000 cells/well in 384 Collagen I coated plate were seeded and grown overnight. On the next day, a serial dilution of bacu-PCSK9-WT in MEM-1% BSA was made, mixing with a fixed concentration of anti-mouse-cat domain PCSK9 polyclonal antibody (Cayman Chemical; Ann Arbor, MI; cat# 10008811; raised against mouse PCSK9 antigen VFAQSIPWNLER (SEQ ID NO: 8) and cross reacts with human PCSK9) or anti-human PCSK9 polyclonal antibody (R&D Systems, Minneapolis, MN; cat# AF3888) at a final concentration of 100 ug/ml respectively. These mixtures were incubated for 1 hour at 4°C, then medium was aspirated out from the HepG2 cells and 25 ul/well of the mixtures was added to HepG2 cells in 384 plate and incubated for 6 hours and 18 hours, respectively. After the indicated incubation time, the mixture was removed from the HepG2 cells, washed 1X with PBS, then Dil-LDL (Dil label is 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) was added at 10 ug/ml in MEM-1% BSA to the cells, then incubation followed at room temperature for 90 minutes. Dil-LDL was removed afterwards, the cells were fixed with Prefer fixative (glyoxal, ethanol, buffer; Anatech LTD.; Hayward, CA; cat# 414) for 20 minutes, then the cells were washed with PBS two times and Dil-LDL uptake into the cells was read using a fluorescence intensity reader Analyst. The data generated in these assays are set forth below in Table 4.

Table 4. LDL uptake in the presence of PCSK9 and anti-PCSK9 antibodies.

6 hour incubation

[PCSK9] ug/ml	control	anti-CAT	anti-all
15	20675218	22419420	18606777
5	21873226	25617426	31744993
1.67	23647886	29483684	30994715
0	29824887	28736616	30124921

18 hour incubation

[PCSK9] ug/ml	control	anti-CAT	anti-all
15	20163100	21843043	17106209
5	24214957	25921384	27741479
1.67	27777510	32019111	29054521
0	31232393	32526668	28127286

Table 4 shows uptake of dil-labeled LDL by human HepG2 liver cells treated with the indicated amounts of purified, recombinant PCSK9 (control) or PCSK9 in the presence

of either polyclonal antibody raised against the catalytic domain peptide (anti-CAT) or antibody raised against the whole protein (anti-all). PCSK9-dependent uptake of LDL by the cells increased in a manner dependent upon the presence of anti-PCSK9 antibody in the mixture. Higher numbers indicated greater LDL uptake and preservation of LDLR despite the presence of PCSK9.

Example 3: Characterization of anti-PCSK9 antibodies.

The 75B9, 77D10, 11B5, 22D11, 1F11/1G11 and 29C10 anti-PCSK9 antibodies were characterized. The antibodies were found to effectively inhibit interaction between PCSK9 and the LDL receptor as well as inhibit PCSK9-mediated LDL receptor degradation. *In vivo*, the antibodies were found to inhibit PCSK9 and, thereby increase the levels of LDL receptor present. Moreover, the antibodies neutralized the *in vivo* cholesterol increase observed when exogenous PCSK9 was added.

Inhibition of PCSK9-mediated LDLR degradation with anti-PCSK9 antibodies.

The 75B9, 77D10, 11B5, 22D11, 1F11/1G11 and 29C10 antibodies were evaluated in an in-cell western assay for their ability to inhibit the degradation of the LDL receptor by PCSK9 in HepG2 cells.

In-cell western was performed to quantitate anti-PCSK9 antibody inhibition of PCSK9-mediated LDLR degradation. HepG2 cells were seeded in 384-well collagen I coated plates and treated with anti-PCSK9 antibody and/or PCSK9 (100 nM) for 18 hours. Detection of LDLR and β-actin was performed according to the manufacturer's protocol (Li-Cor Biosciences; Lincoln, Nebraska) using the antibodies described above in conjunction with IRDye 800CW goat anti-rabbit (Li-Cor) and IRDye 680 goat anti-mouse (Li-Cor). The assay was read on an Odyssey infrared imaging system (Li-Cor) and the signal for LDLR protein in each well normalized to β-actin content. The data from these experiments are set forth below in Table 5.

Table 5. Level of inhibition of each anti-PCSK9 antibody observed in in-cell western assay

Treatment	Relative level of inhibition of LDLR degradation observed*
75B9 anti-PCSK9 antibody (IgG2a)	++
77D10 anti-PCSK9 antibody (IgG2b)	++
11B5 anti-PCSK9 antibody (IgG2a)	++
22D11 anti-PCSK9 antibody (IgG1)	+
1F11/1G11 anti-PCSK9 antibody (IgG2a)	+++
29C10 anti-PCSK9 antibody (IgG2a)	+
No PCSK9 control	100%
+ PCSK9/no antibody control	0%

* Greater number of + symbols indicates a greater level of inhibition relative to other treatments.

Relative to the "+PCSK9/no antibody" control, the antibodies tested inhibited PCSK9-mediated degradation of LDL receptor with various potencies in the HepG2 cells.

Alphascreen binding assays

An Amplified Luminescent Proximity Homogeneous Assay (ALPHA, Perkin-Elmer; Waltham, Massachusetts) capable of directly determining the interaction between PCSK9-FLAG and a putative binding partner was used to determine the effect of anti-PCSK9 antibodies on this interaction. This technique required that "donor" and "acceptor" beads be brought into proximity via protein–protein interaction, resulting in increased luminescence (Ullman *et al.*, Proc. Nat. Acad. Sci. (1994) 91:5426-5430). In the basic assay, LDL receptor binding to PCSK9 was determined as follows: 5 µl of recombinant receptor at the appropriate concentrations was incubated with 2.5 µl PCSK9-FLAG (1.4 µg/ml, 30 min). About 2.5 µl of biotinylated anti-Flag-M2 antibody (1.8 µg/ml) was added and the mixture incubated for 1 hour. Afterward 5 µl of streptavidin donor bead and nickel chelate acceptor bead (1:1 mixture) was added and the assay incubated overnight. AlphaScreen signal (counts per second) was analyzed using an EnVision microplate reader (Perkin-Elmer). All data points were determined in triplicate. Assays were carried out at 23°C in buffer containing 25 mM HEPES, 0.1 M NaCl, pH 7.4, 0.1% BSA. The inhibition assays were determined similarly with slight adjustments to assay volumes and protein concentrations. Briefly, 5 µl of 1.25 µg/ml of PCSK9-Flag and 1.25 µg/ml of His-

tagged LDL receptor was incubated with 2.5 µl of antibody at the appropriate concentrations for 30 minutes followed by the addition of 2.5 µl of anti-Flag-BioM2 (1.8 µg/ml) and a 1 hour incubation. The data from these assays are set forth below in Table 6.

Table 6. Level of anti-PCSK9 antibody mediated inhibition of PCSK9/LDLR interaction

Treatment	IC_{50} (nM)	% inhibition at 450 nM of antibody
75B9 anti-PCSK9 antibody (IgG2a)	0.5	100
77D10 anti-PCSK9 antibody (IgG2b)	38	62
11B5 anti-PCSK9 antibody (IgG2a)	0.4	100
22D11 anti-PCSK9 antibody (IgG1)	ND	ND
1F11/1G11 anti-PCSK9 antibody (IgG2a)	0.049*	88*
29C10 anti-PCSK9 antibody (IgG2a)	13	71

* Average of two measurements.

The antibodies tested inhibited PCSK9/LDL receptor interaction with various potencies (expressed in terms of IC_{50} and % inhibition relative to the assay performed with no antibody added).

Effect of anti-PCSK9 on *in vivo* inhibition of LDLR degradation and plasma cholesterol

The ability of an anti-PCSK9 antibody (1F11/1G11) to inhibit LDL receptor degration in mice was evaluated in these assays along with the ability of the antibodies to modulate cholesterol levels.

In vivo Procedure:

Time-0: Male C57BL/6j mice were injected, intraperitoneal, with 400µg of anti-human PCSK9 Antibody, 1F11/1G11.

Time-2 hours: Mice were then injected, intravenously, with 10µg of human PCSK9 Protein.

Time-4 hours: Mice were terminated and plasma and liver samples were collected.

Analysis:

Plasma lipoprotein profiles of individual mice (0.1 mL plasma) were determined by fast protein liquid chromatography (FPLC) with a Pharmacia Superose 6 column. FPLC fraction cholesterol levels were determined using Wako Cholesterol Enzymatic colorimetric method.

Protein levels of Liver LDL Receptor were determined by Western Blot. 40µg of each liver homogenate was separated on an Invitrogen 4-12% NuPAGE gel, transferred to PVDF membrane and incubated with goat anti-mouse LDL receptor antibody (rabbit anti-GAPDH antibody was used as a control for equal loading). Membranes were then incubated with corresponding HRP-linked antibodies and visualization was accomplished with high performance chemiluminescence film. Band intensities were quantified using ImageQuant 5.2 software.

LDL receptor levels were expressed in Arbitrary Units (AU) as a ratio of LDL receptor band intensity to GAPDH band intensity. The results of the LDL receptor measurements are set forth in tables 7 and 8 below. Two separate analyses of the data are presented.

Table 7. Lightest exposure of both LDLR panel vs. GAPDH panel

Mouse	LDLR	GAPDH	LDLR/GAPDH	Mean	% Change
107	13714	50469	0.272	0.235 (Antibody and PCSK9)	-48.0
108	13766	49467	0.278		
109	12508	47790	0.262		
110	5977	46744	0.128		
111	1724	46456	0.037	0.081 (Saline and PCSK9)	-82.0
112	912	44130	0.021		
113	2631	51367	0.051		
114	10217	47345	0.216		
115	23237	48860	0.476	0.452 (Saline only)	Control
116	18589	47694	0.390		
117	16577	47554	0.349		
118	27116	45755	0.593		

Table 8. Darker exposure of LDLR panel vs. lightest GAPDH panel.

Mouse	LDLR	GAPDH	LDLR/GAPDH	Mean	% Change
107	19883	45242	0.439	0.377 (Antibody and PCSK9)	-35.2
108	18057	45634	0.396		
109	18642	43767	0.426		
110	10669	42898	0.249		
111	2817	43967	0.064	0.123 (Saline and PCSK9)	-78.8
112	1204	43206	0.028		
113	4252	47846	0.089		
114	13848	44288	0.313		
115	27675	44510	0.622	0.582 (Saline only)	Control
116	23016	45090	0.510		
117	19837	44526	0.446		
118	32897	43792	0.751		

The 1F11/1G11 antibody reduced the levels of PCSK9 mediated LDL receptor degration in the mice tested.

Table 9. Plasma cholesterol levels in mice tested under indicated conditions.

Treatment	Plasma total cholesterol level (mg/dl)
Anti-PCSK9 and PCSK9	72.05
Saline and PCSK9	83.28
Saline only	74.88

The 1F11/1G11 antibody reduced the level of PCSK9-mediated cholesterol increase in the mice tested.

****

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

We claim:

1. An isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:

(i) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 11B5 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 10;

(ii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 75B9 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 18;

(iii) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 77D10 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 26;

(iv) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 29C10 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 34;

(v) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 22D11 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 42;

(vi) CDR-H1, CDR-H2 and CDR-H3 of the variable region the 1F11/1G11 heavy chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 50;

(vii) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 11B5 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 14;

(viii) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 75B9 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 22;

(ix) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 77D10 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 30;

(x) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 29C10 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 38;

(xi) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 22D11 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 46; and

(xii) CDR-L1, CDR-L2 and CDR-L3 of the variable region the 1F11/1G11 light chain immunoglobulin which comprises the amino acid sequence of SEQ ID NO: 54.

2. A composition comprising the antibody or antigen-binding fragment thereof of claim 1 in association with a further chemotherapeutic agent.

3. The composition of claim 2 wherein the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin

system inhibitor, an angiotensin-converting enzyme inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor.

4. The composition of claim 2 wherein the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate, bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride or tolamolol.

5. The composition of claim 4 wherein the further chemotherapeutic agent is ezetimibe optionally in association with simvastatin.

6. The antibody or antigen-binding fragment thereof of claim 1, which is a humanized antibody.

7. The antibody or antigen-binding fragment thereof of claim 1, which is an antibody which is a monoclonal antibody, a labeled antibody, a bivalent antibody, a polyclonal antibody, a bispecific antibody, a chimeric antibody, a recombinant antibody, an anti-idiotypic antibody, a humanized antibody or a bispecific antibody.

8. The antibody or antigen-binding fragment thereof of claim 1, which is an antigen-binding fragment which is a camelized single domain antibody, a diabody, an scfv, an scfv dimer, a dsfv, a $(dsfv)_2$, a dsFv-dsfv', a bispecific ds diabody, an Fv, an Fab, an Fab', an $F(ab')_2$, or a domain antibody.

9. The antibody or antigen-binding fragment thereof of claim 1 which is linked to an immunoglobulin constant region.

10. The antibody or antigen-binding fragment thereof of claim 9 wherein the constant region is a κ light chain, γ1 heavy chain, γ2 heavy chain, γ3 heavy chain or γ4 heavy chain.

11. A pharmaceutical composition comprising the antibody or fragment of claim 1 in association with a pharmaceutically acceptable carrier.

12. An isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:

(i) HCDR1 comprising the amino acid sequence G F N I K D T Y M H (SEQ ID NO: 11), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q D (SEQ ID NO: 12) and HCDR3 comprising the amino acid sequence S Y F G S I F A Y (SEQ ID NO: 13);

(ii) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 19), HCDR2 comprising the amino acid sequence R I D P A N G H T E Y D P K F Q G (SEQ ID NO: 20) and HCDR3 comprising the amino acid sequence S Y Y G S I F A Y (SEQ ID NO: 21);

(iii) HCDR1 comprising the amino acid sequence G F N I K D Y Y I H (SEQ ID NO: 27), HCDR2 comprising the amino acid sequence W I D P E N G D T E Y A P K F Q G (SEQ ID NO: 28) and HCDR3 comprising the amino acid sequence Y Y R Y D D G T W F P Y (SEQ ID NO: 29);

(iv) HCDR1 comprising the amino acid sequence G F N I K D T Y I H (SEQ ID NO: 35), HCDR2 comprising the amino acid sequence W I D P A N G Y T K Y A P N F Q G (SEQ ID NO: 36) and HCDR3 comprising the amino acid sequence G Y Y R Y Y S L D Y (SEQ ID NO: 37);

(v) HCDR1 comprising the amino acid sequence G F T F S N H D M A (SEQ ID NO: 43), HCDR2 comprising the amino acid sequence S I T P S G G T T Y Y R D S V E G (SEQ ID NO: 44) and HCDR3 comprising the amino acid sequence Q N Y Y D G S Y Y Y G L Y Y F D Y (SEQ ID NO: 45);

(vi) HCDR1 comprising the amino acid sequence G Y T F T D Y Y M N (SEQ ID NO: 51), HCDR2 comprising the amino acid sequence D I N P N N G G A I Y N Q K F K G (SEQ ID NO: 52) and HCDR3 comprising the amino acid sequence G I I T E I A E D F (SEQ ID NO: 53);

(vii) LCDR1 comprising the amino acid sequence S A S S S V S Y L Y (SEQ ID NO: 15), LCDR2 comprising the amino acid sequence R S S H R A S (SEQ ID NO: 16) and LCDR3 comprising the amino acid sequence H Q Y Q S Y P P T (SEQ ID NO: 17);

(viii) LCDR1 comprising the amino acid sequence S A S S S V S Y L F (SEQ ID NO: 23), LCDR2 comprising the amino acid sequence R T S Y L A S (SEQ ID NO: 24) and LCDR3 comprising the amino acid sequence H Q Y H T Y P P T (SEQ ID NO: 25);
(ix) LCDR1 comprising the amino acid sequence R A S G N I H S Y L A (SEQ ID NO: 31), LCDR2 comprising the amino acid sequence N A K T L P D (SEQ ID NO: 32) and LCDR3 comprising the amino acid sequence Q H F W N T P W T (SEQ ID NO: 33);
(x) LCDR1 comprising the amino acid sequence R A S Q D I S N Y L N (SEQ ID NO: 39), LCDR2 comprising the amino acid sequence Y S S R L H S (SEQ ID NO: 40) and LCDR3 comprising the amino acid sequence Q Q G K T L P L T (SEQ ID NO: 41);
(xi) LCDR1 comprising the amino acid sequence R S S Q S L V Y S D G N T Y L H (SEQ ID NO: 47), LCDR2 comprising the amino acid sequence R V S N R F S (SEQ ID NO: 48) and LCDR3 comprising the amino acid sequence L Q S T H F P P T (SEQ ID NO: 49); and
(xii) LCDR1 comprising the amino acid sequence K A S Q N V G T N V V (SEQ ID NO: 55), LCDR2 comprising the amino acid sequence S A S Y R Y S (SEQ ID NO: 56) and LCDR3 comprising the amino acid sequence Q Q Y K T Y P Y T (SEQ ID NO: 57).

13. A composition comprising the antibody or antigen-binding fragment thereof of claim 12 in association with a further chemotherapeutic agent.

14. The composition of claim 13 wherein the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor.

15. The composition of claim 13 wherein the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate,

bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride, tolamolol fenspiride hydrochloride, proroxan, alfuzosin hydrochloride, labetalol hydrochloride, bretylium tosylate, dihydroergtamine mesylate, carvedilol, labetalol, bretylium tosylate, phentolamine mesylate, solypertine tartrate, zolertine hydrochloride, candesartan cilexetil, telmisartan, candesartan; losartan potassium, benazepril hydrochloride, captopril, fosinopril, moexipril hydrochloride, perindopril erbumine, quinapril, ramipril, enalapril maleate, lisinopril, delapril, spirapril, delapril hydrochloride, libenzapril, pentopril, perindopril 1H-Indole-2-carboxylic acid, quinaprilat, spirapril hydrochloride, spiraprilat, teprotide, zofenopril, amlodipine besylate, clentiazem maleate, isradipine, nimodipine, felodipine, nilvadipine; diltiazem hydrochloride, verapamil hydrochloride, teludipine hydrochloride, belfosdil, fostedil, ranolazine, butoprozine hydrochloride, tosifen, molsidomine, ranolazine hydrochloride, tosifen, diltiazem hydrochloride, isosorbide dinitrate, sosorbide mononitrate, nitroglycerin, verapamil hydrochloride, chromonar, clonitate, droprenilamine, lidoflazine, prenylamine, propatyl nitrate, mioflazine hydrochloride, mixidine, molsidomine, isosorbide mononitrate, erythrityl tetranitrate, clonitrate, dipyridamole; nicorandil, pyridinecarboxamide, nifedipine; perhexiline maleate; oxprenolol, pentrinitrol, verapamil, althiazide, benzthiazide, buthiazide, chlorothiazide, spironolactone, triamterene, guanfacine hydrochloride, methyldopa-hydrochlorothiazide combined with Hydrochlorothiazide, methyldopa-chlorothiazide, clonidine hydrochloride, chlorthalidone, clonidine hydrochloride, clonidine, althiazide, benzthiazide, captopril, carvedilol, chlorothiazide, clonidine hydrochloride, cyclothiazide, delapril hydrochloride, dilevalol hydrochloride, delapril hydrochloride, doxazosin mesylate, fosinopril sodium, moexipril hydrochloride, monatepil maleate, metoprolol succinate, guanfacine hydrochloride, methyldopa, quinaprilat, quinapril hydrochloride, primidolol, prazosin hydrochloride, pelanserin hydrochloride, phenoxybenzamine hydrochloride, candesartan cilexetil, telmisartan, candesartan, amlodipine maleate, terazosin hydrochloride, bevantolol hydrochloride or ramipril.

16. The composition of claim 15 wherein the further chemotherapeutic agent is ezetimibe optionally in association with simvastatin.

17. The antibody or antigen-binding fragment thereof of claim 12, which is a humanized antibody.

18. The antibody or antigen-binding fragment thereof of claim 12, which is a humanized antibody.

19. The antibody or antigen-binding fragment thereof of claim 12, which is an antibody which is a monoclonal antibody, a labeled antibody, a bivalent antibody, a polyclonal antibody, a bispecific antibody, a chimeric antibody, a recombinant antibody, an anti-idiotypic antibody, a humanized antibody or a bispecific antibody.

20. The antibody or antigen-binding fragment thereof of claim 12, which is an antigen-binding fragment which is a camelized single domain antibody, a diabody, an scfv, an scfv dimer, a dsfv, a $(dsfv)_2$, a dsFv-dsfv', a bispecific ds diabody, an Fv, an Fab, an Fab', an $F(ab')_2$, or a domain antibody.

21. The antibody or antigen-binding fragment thereof of claim 12 which is linked to an immunoglobulin constant region.

22. The antibody or antigen-binding fragment thereof of claim 21 wherein the constant region is a κ light chain, γ1 heavy chain, γ2 heavy chain, γ3 heavy chain or γ4 heavy chain.

23. A pharmaceutical composition comprising the antibody or fragment of claim 12 in association with a pharmaceutically acceptable carrier.

24. The isolated antibody or antigen-binding fragment thereof of claim 12 comprising one or more members selected from the group consisting of:

(a) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L Q Q S G A E L V K P G A S V T L S C T A S G F N I K D T Y
M H W V N Q R P E Q G L V W I G R I D P A N G H T E Y D P K F Q D
K A T I T T D T S S N T A Y L H L S S L T S G D T A V Y Y C A R S
Y F G S I F A Y W G Q G T L V T V S A

(SEQ ID NO: 10);

(b) an immunoglobulin light chain comprising the amino acid sequence:

Q I V L T Q S P A I M S A S P G E K V T I S C S A S S S V S Y L Y
W Y Q Q K P G S S P K P W I F R S S H R A S G V P A R F S G S G S
G T S Y S L T I S S M E A E D A A T Y Y C H Q Y Q S Y P P T F G G
G T K L E I K R A

(SEQ ID NO: 14);

(c) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L Q Q S G A D L V K P G A S V K L S C T A S G F N I K D T Y
I H W V K Q R P E Q G L E W I G R I D P A N G H T E Y D P K F Q G
R A T L T T D T S S N T A Y L Q L F S L T S E D S A V Y F C A R S
Y Y G S I F A Y W G Q G T L V T V S A

(SEQ ID NO: 18);

(d) an immunoglobulin light chain comprising the amino acid sequence:

Q I V L T Q S P A I M S A S P G E K V T I S C S A S S S V S Y L F
W Y Q Q K P G S S P K P W I F R T S Y L A S G V P A R F S G S G S
G T S F S L T I S S M E A E D A A T Y Y C H Q Y H T Y P P T F G G
G T K L E I K R A

(SEQ ID NO: 22);

(e) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L Q Q S G A E L V R S G A S V K L S C T T S G F N I K D Y Y
I H W V K Q R P E Q G L E W I G W I D P E N G D T E Y A P K F Q G
K A T M T A D T S S N T A Y L Q L S S L T S A D T A V Y Y C N A
Y Y R Y D D G T W F P Y W G Q G T L V T V S A

(SEQ ID NO: 26);

(f) an immunoglobulin light chain comprising the amino acid sequence:

D I Q L T Q S P A S L S A S V G E T V T I T C R A S G N I H S Y L
A W Y Q Q K Q G K S P Q F L V D N A K T L P D G V P S R F S V S G
S G T Q Y S L K I N S L Q P E D F G T Y Y C Q H F W N T P W T F G
G G T K L E I K R A

(SEQ ID NO: 30);

(g) an immunoglobulin heavy chain comprising the amino acid sequence:

E V L L Q Q S V A E L V R P G A S V R L S C T A S G F N I K D T Y
I H W V R Q R P E Q G L E W F G W I D P A N G Y T K Y A P N F Q G
K A T L T T D T S S N T A Y L H L S S L T S E D S A I Y Y C A R G
Y Y R Y Y S L D Y W G Q G T S V T V S S

(SEQ ID NO: 34);

(h) an immunoglobulin light chain comprising the amino acid sequence:

D I Q M T Q T T S S L S A S L G D R V T I S C R A S Q D I S N Y L
N W Y Q Q K P D G T V K L L I Y Y S S R L H S G V P S R F S G R G
S G T D Y S L T I S T L E Q E D I A T Y F C Q Q G K T L P L T F G
A G T K L E L K R A

(SEQ ID NO: 38);

(i) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L V D S G G G L V Q P G R S L K L S C A A S G F T F S N H D
M A W V R Q A P T K G L E W V A S I T P S G G T T Y Y R D S V E G
R F T V S R D N V K S S L H L Q M D S L T S E D T A T Y Y C A R Q
N Y Y D G S Y Y Y G L Y Y F D Y W G Q G V M V T V S S

(SEQ ID NO: 42);

(j) an immunoglobulin light chain comprising the amino acid sequence:

D V L M T Q T P V S L P V S L G G Q V S I S C R S S Q S L V Y S D
G N T Y L H W Y L Q K P G Q S P Q L L I Y R V S N R F S G V P D R
F S G S G S G T D F T L K I S R V E P E D L G L Y Y C L Q S T H F
P P T F G S G T K L E I K R A

(SEQ ID NO: 46);

(k) an immunoglobulin heavy chain comprising the amino acid sequence:

E V Q L Q Q S G P E L V K P G A S V K I S C K V S G Y T F T D Y Y
M N W V K Q S H G K S L E W I G D I N P N N G G A I Y N Q K F K G
K A T L T V D K S S S I A Y M E L R S L T S E D S A V Y Y C T S G
I I T E I A E D F W G Q G T T L T V S S

(SEQ ID NO: 50); and

(l) an immunoglobulin light chain comprising the amino acid sequence:

D I V M T Q S Q K F M S T S V G D R V S V T C K A S Q N V G T N V
V W Y Q Q K P G Q S P K A L I H S A S Y R Y S G V P D R F K G S G
S G T D F T L T I T N V Q S E D L A G F F C Q Q Y K T Y P Y T F G
G G T Q L E I K R A

(SEQ ID NO: 54).

25. A composition comprising the antibody or antigen-binding fragment thereof of claim 24 in association with a further chemotherapeutic agent.

26. The composition of claim 25 wherein the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor.

27. The composition of claim 25 wherein the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate, bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride, tolamolol fenspiride hydrochloride, proroxan, alfuzosin hydrochloride, labetalol hydrochloride, bretylium tosylate, dihydroergtamine mesylate, carvedilol, labetalol, bretylium tosylate, phentolamine mesylate, solypertine tartrate, zolertine hydrochloride, candesartan cilexetil, telmisartan, candesartan; losartan potassium, benazepril hydrochloride, captopril, fosinopril, moexipril hydrochloride, perindopril erbumine, quinapril, ramipril, enalapril maleate, lisinopril, delapril, spirapril, delapril hydrochloride, libenzapril, pentopril, perindopril 1H-Indole-2-carboxylic acid, quinaprilat, spirapril hydrochloride, spiraprilat, teprotide, zofenopril, amlodipine besylate, clentiazem maleate, isradipine, nimodipine, felodipine, nilvadipine; diltiazem hydrochloride, verapamil hydrochloride, teludipine hydrochloride, belfosdil, fostedil, ranolazine, butoprozine hydrochloride, tosifen, molsidomine, ranolazine hydrochloride, tosifen, diltiazem hydrochloride, isosorbide dinitrate, sosorbide mononitrate, nitroglycerin, verapamil hydrochloride, chromonar, clonitate, droprenilamine, lidoflazine, prenylamine, propatyl nitrate, mioflazine hydrochloride, mixidine, molsidomine, isosorbide mononitrate, erythrityl tetranitrate, clonitrate, dipyridamole; nicorandil, pyridinecarboxamide, nifedipine; perhexiline maleate; oxprenolol, pentrinitrol, verapamil, althiazide, benzthiazide, buthiazide, chlorothiazide, spironolactone, triamterene, guanfacine hydrochloride, methyldopa-hydrochlorothiazide combined with Hydrochlorothiazide, methyldopa-chlorothiazide, clonidine hydrochloride, chlorthalidone, clonidine hydrochloride, clonidine, althiazide, benzthiazide, captopril, carvedilol, chlorothiazide, clonidine hydrochloride, cyclothiazide, delapril hydrochloride,

dilevalol hydrochloride, delapril hydrochloride, doxazosin mesylate, fosinopril sodium, moexipril hydrochloride, monatepil maleate, metoprolol succinate, guanfacine hydrochloride, methyldopa, quinaprilat, quinapril hydrochloride, primidolol, prazosin hydrochloride, pelanserin hydrochloride, phenoxybenzamine hydrochloride, candesartan cilexetil, telmisartan, candesartan, amlodipine maleate, terazosin hydrochloride, bevantolol hydrochloride or ramipril.

28. The composition of claim 27 wherein the further chemotherapeutic agent is ezetimibe optionally in association with simvastatin.

29. The antibody or antigen-binding fragment thereof of claim 24, which is a humanized antibody.

30. The antibody or antigen-binding fragment thereof of claim 24, which is a humanized antibody.

31. The antibody or antigen-binding fragment thereof of claim 24, which is an antibody which is a monoclonal antibody, a labeled antibody, a bivalent antibody, a polyclonal antibody, a bispecific antibody, a chimeric antibody, a recombinant antibody, an anti-idiotypic antibody, a humanized antibody or a bispecific antibody.

32. The antibody or antigen-binding fragment thereof of claim 24, which is an antigen-binding fragment which is a camelized single domain antibody, a diabody, an scfv, an scfv dimer, a dsfv, a $(dsfv)_2$, a dsFv-dsfv', a bispecific ds diabody, an Fv, an Fab, an Fab', an $F(ab')_2$, or a domain antibody.

33. The antibody or antigen-binding fragment thereof of claim 24 which is linked to an immunoglobulin constant region.

34. The antibody or antigen-binding fragment thereof of claim 33 wherein the constant region is a κ light chain, γ1 heavy chain, γ2 heavy chain, γ3 heavy chain or γ4 heavy chain.

35. A pharmaceutical composition comprising the antibody or fragment of claim 24 in association with a pharmaceutically acceptable carrier.

36. A pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof or isolated EGF-A polypeptide which binds specifically to PCSK9, which antibody or fragment or polypeptide inhibits binding between PCSK9 and LDL receptor; and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36 wherein the antibody binds specifically to a PCSK9 catalytic domain or to a domain of PCSK9 which interacts with an LDL receptor EGF-A domain.

38. An isolated polypeptide comprising an amino acid sequence comprising about 90% or more amino acid sequence similarity to a fragment of the human LDL receptor which fragment consists of amino acids beginning at about amino acid position 314 and ending at about amino acid position 355 of said receptor; wherein said polypeptide optionally comprises one or more properties selected from the group consisting of:
(i) binds to PCSK9;
(ii) competes with LDL receptor or an anti-PCSK9 antibody or antigen-binding fragment thereof for binding to PCSK9;
(iii) reduces total cholesterol level when administered to an animal;
(iv) reduces low density lipoprotein cholesterol level when administered to an animal;
(v) reduces apolipoprotein B level when administered to an animal;
(vi) reduces total cholesterol/high density lipoprotein ratio when administered to an animal; and
(vii) reduces low density lipoprotein/high density lipoprotein ratio when administered to an animal;
or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier.

39. The polypeptide of claim 38 which consists of the amino acid sequence of SEQ ID NO: 3; or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier.

40. A method for reducing:
total cholesterol level;
low density lipoprotein cholesterol level;
apolipoprotein B level;

total cholesterol/high density lipoprotein ratio; or
low density lipoprotein/high density lipoprotein ratio;
in a subject, comprising administering, to said subject, a therapeutically effective amount of a PCSK9 antagonist EGF-A polypeptide; or an antibody or antigen-binding fragment thereof that binds specifically to PCSK9; which antibody or fragment or EGF-A polypeptide inhibits binding between PCSK9 and LDL receptor; optionally in association with a further chemotherapeutic agent.

41. The method of claim 40 wherein the antibody or fragment binds specifically to a PCSK9 catalytic domain or to a domain of PCSK9 which interacts with an LDL receptor EGF-A domain.

42. A method for treating or preventing hypercholesterolemia, hyperlipidemia, hypertriglyceridaemia, sitosterolemia, atherosclerosis, arteriosclerosis, coronary heart disease, vascular inflammation or xanthoma, in an subject, comprising administering, to said subject, a therapeutically effective amount of an EGF-A polypeptide or an antibody or antigen-binding fragment thereof that binds specifically to PCSK9, which antibody or fragment or EGF-A polypeptide inhibits binding between PCSK9 and LDL receptor; optionally in association with a further chemotherapeutic agent.

43. The method of claim 42 wherein the antibody binds specifically to a PCSK9 catalytic domain or to a domain of PCSK9 which interacts with an LDL receptor EGF-A domain.

44. A method for reducing:
total cholesterol level;
low density lipoprotein cholesterol level;
apolipoprotein B level;
total cholesterol/high density lipoprotein ratio; or
low density lipoprotein/high density lipoprotein ratio; or
for treating:
hypercholesterolemia;
hyperlipidemia;
hypertriglyceridaemia;
sitosterolemia;
atherosclerosis;

arteriosclerosis;
coronary heart disease;
vascular inflammation; or
xanthoma,
in a subject, comprising administering, to said subject, a therapeutically effective amount of the antibody or antigen-binding fragment thereof of claim 1.

45. The method of claim 44 wherein the antibody or antigen-binding fragment thereof of claim 1 in administered in association with a further chemotherapeutic agent.

46. The method of claim 45 wherein the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor.

47. The method of claim 46 wherein the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate, bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride, tolamolol fenspiride hydrochloride, proroxan, alfuzosin hydrochloride, labetalol hydrochloride, bretylium tosylate, dihydroergtamine mesylate, carvedilol, labetalol, bretylium tosylate, phentolamine mesylate, solypertine tartrate, zolertine hydrochloride, candesartan cilexetil, telmisartan, candesartan; losartan potassium, benazepril hydrochloride, captopril, fosinopril, moexipril hydrochloride, perindopril erbumine, quinapril, ramipril, enalapril maleate, lisinopril, delapril, spirapril, delapril hydrochloride, libenzapril, pentopril, perindopril 1H-Indole-2-carboxylic acid, quinaprilat, spirapril hydrochloride, spiraprilat, teprotide, zofenopril, amlodipine besylate, clentiazem maleate, isradipine, nimodipine, felodipine, nilvadipine; diltiazem hydrochloride, verapamil hydrochloride, teludipine hydrochloride, belfosdil, fostedil,

ranolazine, butoprozine hydrochloride, tosifen, molsidomine, ranolazine hydrochloride, tosifen, diltiazem hydrochloride, isosorbide dinitrate, sosorbide mononitrate, nitroglycerin, verapamil hydrochloride, chromonar, clonitate, droprenilamine, lidoflazine, prenylamine, propatyl nitrate, mioflazine hydrochloride, mixidine, molsidomine, isosorbide mononitrate, erythrityl tetranitrate, clonitrate, dipyridamole; nicorandil, pyridinecarboxamide, nifedipine; perhexiline maleate; oxprenolol, pentrinitrol, verapamil, althiazide, benzthiazide, buthiazide, chlorothiazide, spironolactone, triamterene, guanfacine hydrochloride, methyldopa-hydrochlorothiazide combined with Hydrochlorothiazide, methyldopa-chlorothiazide, clonidine hydrochloride, chlorthalidone, clonidine hydrochloride, clonidine, althiazide, benzthiazide, captopril, carvedilol, chlorothiazide, clonidine hydrochloride, cyclothiazide, delapril hydrochloride, dilevalol hydrochloride, delapril hydrochloride, doxazosin mesylate, fosinopril sodium, moexipril hydrochloride, monatepil maleate, metoprolol succinate, guanfacine hydrochloride, methyldopa, quinaprilat, quinapril hydrochloride, primidolol, prazosin hydrochloride, pelanserin hydrochloride, phenoxybenzamine hydrochloride, candesartan cilexetil, telmisartan, candesartan, amlodipine maleate, terazosin hydrochloride, bevantolol hydrochloride or ramipril.

48. The method of claim 47 wherein the further chemotherapeutic agent is ezetimibe and, optionally, simvastatin.

49. The method of claim 44 wherein the antibody or antigen-binding fragment thereof is a humanized antibody.

50. A method for reducing:
total cholesterol level;
low density lipoprotein cholesterol level;
apolipoprotein B level;
total cholesterol/high density lipoprotein ratio; or
low density lipoprotein/high density lipoprotein ratio; or
for treating:
hypercholesterolemia;
hyperlipidemia;
hypertriglyceridaemia;
sitosterolemia;

atherosclerosis;
arteriosclerosis;
coronary heart disease;
vascular inflammation; or
xanthoma,
in a subject, comprising administering, to said subject, a therapeutically effective amount of the antibody or antigen-binding fragment thereof of claim 24.

51. The method of claim 50 wherein the antibody or antigen-binding fragment thereof of claim 1 in administered in association with a further chemotherapeutic agent.

52. The method of claim 51 wherein the further chemotherapeutic agent is a cardiovascular agent, an adrenergic blocker, an antihypertensive agent, an angiotensin system inhibitor, an angiotensin-converting enzyme inhibitor, a coronary vasodilator, a diuretic, an adrenergic stimulant or an HMG-CoA reductase inhibitor.

53. The method of claim 51 wherein the further chemotherapeutic agent is ezetimibe, lovastatin, atorvastatin, pravastatin, rosuvastatin, fluvastatin, rivastatin, simvastatin, an azetidinone, bunolol hydrochloride, acebutolol, alprenolol hydrochloride, atenolol, carteolol hydrochloride, celiprolol hydrochloride; cetamolol hydrochloride, labetalol hydrochloride, esmolol hydrochloride, levobetaxolol hydrochloride, levobunolol hydrochloride, nadolol, practolol, propranolol hydrochloride, sotalol hydrochloride, timolol, timolol maleate, bisoprolol; bisoprolol fumarate, nebivalol, cicloprolol hydrochloride, dexpropranolol hydrochloride, diacetolol hydrochloride, dilevalol hydrochloride, exaprolol hydrochloride, flestolol sulfate, metalol hydrochloride, metoprolol 2-Propanol, metoprolol tartrate, pamatolol sulfate, penbutolol sulfate, practolol, tiprenolol hydrochloride, tolamolol fenspiride hydrochloride, proroxan, alfuzosin hydrochloride, labetalol hydrochloride, bretylium tosylate, dihydroergtamine mesylate, carvedilol, labetalol, bretylium tosylate, phentolamine mesylate, solypertine tartrate, zolertine hydrochloride, candesartan cilexetil, telmisartan, candesartan; losartan potassium, benazepril hydrochloride, captopril, fosinopril, moexipril hydrochloride, perindopril erbumine, quinapril, ramipril, enalapril maleate, lisinopril, delapril, spirapril, delapril hydrochloride, libenzapril, pentopril, perindopril 1H-Indole-2-carboxylic acid, quinaprilat, spirapril hydrochloride, spiraprilat, teprotide, zofenopril, amlodipine besylate, clentiazem maleate, isradipine, nimodipine, felodipine, nilvadipine; diltiazem

hydrochloride, verapamil hydrochloride, teludipine hydrochloride, belfosdil, fostedil, ranolazine, butoprozine hydrochloride, tosifen, molsidomine, ranolazine hydrochloride, tosifen, diltiazem hydrochloride, isosorbide dinitrate, sosorbide mononitrate, nitroglycerin, verapamil hydrochloride, chromonar, clonitate, droprenilamine, lidoflazine, prenylamine, propatyl nitrate, mioflazine hydrochloride, mixidine, molsidomine, isosorbide mononitrate, erythrityl tetranitrate, clonitrate, dipyridamole; nicorandil, pyridinecarboxamide, nifedipine; perhexiline maleate; oxprenolol, pentrinitrol, verapamil, althiazide, benzthiazide, buthiazide, chlorothiazide, spironolactone, triamterene, guanfacine hydrochloride, methyldopa-hydrochlorothiazide combined with Hydrochlorothiazide, methyldopa-chlorothiazide, clonidine hydrochloride, chlorthalidone, clonidine hydrochloride, clonidine, althiazide, benzthiazide, captopril, carvedilol, chlorothiazide, clonidine hydrochloride, cyclothiazide, delapril hydrochloride, dilevalol hydrochloride, delapril hydrochloride, doxazosin mesylate, fosinopril sodium, moexipril hydrochloride, monatepil maleate, metoprolol succinate, guanfacine hydrochloride, methyldopa, quinaprilat, quinapril hydrochloride, primidolol, prazosin hydrochloride, pelanserin hydrochloride, phenoxybenzamine hydrochloride, candesartan cilexetil, telmisartan, candesartan, amlodipine maleate, terazosin hydrochloride, bevantolol hydrochloride or ramipril.

54. The method of claim 53 wherein the further chemotherapeutic agent is ezetimibe and, optionally, simvastatin.

55. The method of claim 50 wherein the antibody or antigen-binding fragment thereof is a humanized antibody.

56. A method for producing an antibody or antigen-binding fragment therof of claim 1 comprising introducing one or more polynucleotides into one or more host cells; which polynucleotides encodes and direct expression of said heavy and/or light chain immunoglobulins; and growing said host cells under conditions whereby said heavy and light chain immunoglobulins are expressed.

57. The method of claim 56 wherein said polynucleotide encoding and directing expression of said light chain and said polynucleotide encoding and directing expression of said heavy chain are in separate host cells.

Residue numbering according to Kabat

HEAVY CHAINS

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                                                                -----CDR-H1--------
11B5   E V Q L Q Q S G A E L V K P G A S V T L S C T A S G F N I K D T Y M H
75B9   E V Q L Q Q S G A D L V K P G A S V K L S C T A S G F N I K D T Y I H
77D10  E V Q L Q Q S G A E L V R S G A S V K L S C T T S G F N I K D Y Y I H
29C10  E V L L Q Q S V A E L V R P G A S V R L S C T A S G F N I K D T Y I H
22D11  E V Q L V D S G G G L V Q P G R S L K L S C A A S G F T F S N H D M A
1F11   E V Q L Q Q S G P E L V K P G A S V K I S C K V S G Y T F T D Y Y M N
1G11   E V Q L Q Q S G P E L V K P G A S V K I S C K V S G Y T F T D Y Y M N
       1               10                  20          26      30        35
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                                        -------------CDR-H2--------------
11B5   W V N Q R P E Q G L V W I G R I D P A N G H T E Y D P K F Q D
75B9   W V K Q R P E Q G L E W I G R I D P A N G H T E Y D P K F Q G
77D10  W V K Q R P E Q G L E W I G W I D P E N G D T E Y A P K F Q G
29C10  W V R Q R P E Q G L E W F G W I D P A N G Y T K Y A P N F Q G
22D11  W V R Q A P T K G L E W V A S I T P S G G T T Y Y R D S V E G
1F11   W V K Q S H G K S L E W I G D I N P N N G G A I Y N Q K F K G
1G11   W V K Q S H G K S L E W I G D I N P N N G G A I Y N Q K F K G
            40                  50     a            60        65
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11B5    K A T I T T D T S S N T A Y L H L S S L T S G D T A V Y Y C A R
75B9    R A T L T T D T S S N T A Y L Q L F S L T S E D S A V Y F C A R
77D10   K A T M T A D T S S N T A Y L Q L S S L T S A D T A V Y Y C N A
29C10   K A T L T T D T S S N T A Y L H L S S L T S E D S A I Y Y C A R
22D11   R F T V S R D N V K S S L H L Q M D S L T S E D T A T Y Y C A R
1F11    K A T L T V D K S S S I A Y M E L R S L T S E D S A V Y Y C T S
1G11    K A T L T V D K S S S I A Y M E L R S L T S E D S A V Y Y C T S
                    70                  80    a b c                90

        ----------CDR-H3----------
11B5    S Y F G S I         F A Y W G Q G T L V T V S A
75B9    S Y Y G S I         F A Y W G Q G T L V T V S A
77D10   Y Y R Y D D GTW     F P Y W G Q G T L V T V S A
29C10   G Y Y R Y Y S       L D Y W G Q G T S V T V S S
22D11   Q N Y Y D G SYYYGLYY F D Y W G Q G V M V T V S S
1F11    G I I T E I A       E D F W G Q G T T L T V S S
1G11    G I I T E I A       E D F W G Q G T T L T V S S
       95                     102              110
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LIGHT CHAINS

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                                                     --------L1-------
11B5    Q I V L T Q S P A I M S A S P G E K V T I S C S A S         S S
75B9    Q I V L T Q S P A I M S A S P G E K V T I S C S A S         S S
77D10   D I Q L T Q S P A S L S A S V G E T V T I T C R A S G       N I
29C10   D I Q M T Q T T S S L S A S L G D R V T I S C R A S Q       D I
22D11   D V L M T Q T P V S L P V S L G G Q V S I S C R S S Q SLVYS D G
1F11    D I V M T Q S Q K F M S T S V G D R V S V T C K A S Q       N V
1G11    D I V M T Q S Q K F M S T S V G D R V S V T C K A S Q       N V
        1                 10                   20      24

        -L1------                                -----L2------
11B5    V S Y L Y W Y Q Q K P G S S P K P W I F R S S H R A S G V P
75B9    V S Y L F W Y Q Q K P G S S P K P W I F R T S Y L A S G V P
77D10   H S Y L A W Y Q Q K Q G K S P Q F L V D N A K T L P D G V P
29C10   S N Y L N W Y Q Q K P D G T V K L L I Y Y S S R L H S G V P
22D11   N T Y L H W Y L Q K P G Q S P Q L L I Y R V S N R F S G V P
1F11    G T N V V W Y Q Q K P G Q S P K A L I H S A S Y R Y S G V P
1G11    G T N V V W Y Q Q K P G Q S P K A L I H S A S Y R Y S G V P
       30       34          40                   50          56
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11B5    A R F S G S G S G T S Y S L T I S S M E A E D A A T Y Y C
75B9    A R F S G S G S G T S F S L T I S S M E A E D A A T Y Y C
77D10   S R F S V S G S G T Q Y S L K I N S L Q P E D F G T Y Y C
29C10   S R F S G R G S G T D Y S L T I S T L E Q E D I A T Y F C
22D11   D R F S G S G S G T D F T L K I S R V E P E D L G L Y Y C
1F11    D R F K G S G S G T D F T L T I T N V Q S E D L A G F F C
1G11    D R F K G S G S G T D F T L T I T N V Q S E D L A G F F C
       60                  70                  80

        -------L3--------
11B5    H Q Y Q S Y P P T F G G G T K L E I K R A
75B9    H Q Y H T Y P P T F G G G T K L E I K R A
77D10   Q H F W N T P W T F G G G T K L E I K R A
29C10   Q Q G K T L P L T F G A G T K L E L K R A
22D11   L Q S T H F P P T F G S G T K L E I K R A
1F11    Q Q Y K T Y P Y T F G G G T Q L E I K R A
1G11    Q Q Y K T Y P Y T F G G G T Q L E I K R A
       89              97   100
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