METHODS FOR REDUCING LIPOPROTEN(a) LEVELS BY ADMINISTERING AN INHIBITOR OF PROPROTEIN CONVERTASE SUBTILISIN KEXIN-9 (PCSK9)

FIELD OF THE INVENTION

[0001] The present invention relates to the field of therapeutic treatments of diseases and disorders which are associated with elevated levels of lipoproteins. More specifically, the invention relates to the administration of PCSK9 inhibitors to reduce the levels of serum Lp(a) in a patient.

BACKGROUND

[0002] Lipoprotein(a) (Lp(a)) is a low-density lipoprotein-like particle formed by the association of apolipoprotein(a) (Apo(a)) with apoplipoprotein B100 (ApoB100). The Apo(a) component is covalently linked to the ApoB100 component in the assembled Lp(a) particle via a disulfide bond. Elevated serum Lp(a) has been shown in several studies to correlate with a variety of atherosclerotic and thrombotic disorders. (*See, e.g.*, Marcovina and Koschinsky (1998), *Am. J. Cardiol. 82*:57U-66U; Ignatescu *et al.* (1998), *Thromb. Haemost. 80*:231-232; Lippi and Guidi (2000), *Q. J. Med. 93*:75-84; Bennet *et al.* (2008), *Arch. Intern. Med. 168*:598-608; The Emerging Risk Factors Collaboration (2009), *J. Am. Med. Assoc. 302*:412-423; and Lamon-Fava *et al.* (2011), *J. Lipid Res. 52*:1181-1187). Thus, therapeutic reduction of serum Lp(a) levels has been suggested as a means for treating or reducing the risk of cardiovascular disorders. There are few available therapeutic options for lowering serum Lp(a) levels. Examples of treatments that have been tested and/or proposed for lowering serum Lp(a) include administration of acetylsalicylic acid, L-carnitine, niacin or anacetrapib, or LDL apheresis. (*See, e.g.*, Parhofer (2011), *Curr. Pharm Des 17*:871-876). No currently available treatments, however, provide adequate and practical therapy for elevated Lp(a).

[0003] Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a proprotein convertase belonging to the proteinase K subfamily of the secretory subtilase family. The use of PCSK9 inhibitors (anti-PCSK9 antibodies) to reduce serum total cholesterol, LDL cholesterol and serum triglycerides is described in US Patent Appl. Publ. No. 2010/0166768. Nonetheless, heretofore, PCSK9 inhibitors have not been shown to lower Lp(a) levels in patients. Thus, there remains a need in the art for therapeutic methods of lowering serum Lp(a) levels.

BRIEF SUMMARY OF THE INVENTION

[0004] The present invention addresses the aforementioned need in the art by providing methods for lowering serum Lp(a) levels in an individual. The methods of the invention comprise selecting a patient who exhibits elevated serum Lp(a), and administering a pharmaceutical composition comprising a PCSK9 inhibitor to the patient. The patient is selected on the basis of having an elevated serum Lp(a) level that is indicative of enhanced risk

for cardiovascular and/or thrombotic occlusive diseases and disorders. The patient may also be selected on the basis of exhibiting additional risk factors for such diseases and disorders in which a reduction in Lp(a) levels would be beneficial or risk-lowering. For example, patients with hypercholesterolemia (*e.g.*, heFH, nonFH, etc.) may be good candidates for treatment with the therapeutic methods of the present invention.

[0005] PCSK9 inhibitors which may be administered in accordance with the methods of the present invention include, *e.g.*, anti-PCSK9 antibodies or antigen-binding fragments thereof. Specific exemplary anti-PCSK9 antibodies which may be used in the practice of the methods of the present invention include any antibodies or antigen-binding fragments as set forth in US Patent Appl. Publ. No. 2010/0166768, and/or disclosed herein.

[0006] The PCSK9 inhibitor may be administered to a subject subcutaneously or intravenously. Furthermore, the PCSK9 inhibitor may be administered to a patient who is on a therapeutic statin regimen at the time of therapeutic intervention.

[0007] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0008] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0009] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0010] Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe in their entirety.

Elevated Serum Lp(a) Levels

[0011] The present invention provides methods for reducing serum Lp(a) levels in a patient. The methods of the invention comprise selecting a patient who exhibits elevated serum Lp(a), and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor. As used herein, the expressions "Lp(a)" or "lipoprotein(a)" refer to a low-density lipoprotein-like

particle formed by the association of apolipoprotein(a) (apo(a)) with apolipoprotein B100 (apo B100).

[0012] In the context of the present invention, "elevated serum Lp(a)" means a serum Lp(a) level greater than about 14 mg/dL. In certain embodiments, a patient is considered to exhibit elevated serum Lp(a) if the level of serum Lp(a) measured in the patient is greater than about 15 mg/dL, 20 mg/dL, 25 mg/dL, 30 mg/dL, 35 mg/dL, 40 mg/dL, 45 mg/dL, 50 mg/dL, 60 mg/dL, 70 mg/dL, 80 mg/dL, 90 mg/dL, 100 mg/dL, 120 mg/dL, 140 mg/dL, 160 mg/dL, 180 mg/dL, or 200 mg/dL. The serum Lp(a) level can be measured in a patient post-prandial. In some embodiments, the Lp(a) level is measured after a period of time of fasting (*e.g.*, after fasting for 6 hrs, 8 hrs, 10 hrs, 12 hrs or more). An exemplary method for measuring serum Lp(a) in a patient is by rate immune-nephelometry, although any clinically acceptable diagnostic method can be used in the context of the present invention.

Patient Population

[0013] The methods of the present invention are useful for reducing serum Lp(a) levels in human subjects that exhibit an elevated level of serum Lp(a). In some instances the patient is otherwise healthy except for exhibiting elevated serum Lp(a). For example, the patient may not exhibit any other risk factor of cardiovascular, thrombotic or other diseases or disorders at the time of treatment. In other instances, however, the patient is selected on the basis of being diagnosed with, or at risk of developing, a disease or disorder that is caused by or correlated with elevated serum Lp(a). For example, at the time of, or prior to administration of the pharmaceutical composition of the present invention, the patient may be diagnosed with or identified as being at risk of developing a cardiovascular disease or disorder, such as, *e.g.*, coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, peripheral artery occlusive disease, etc. The cardiovascular disease or disorder, in some instances, is hypercholesterolemia. For example, a patient may be selected for treatment with the methods of the present invention if the patient is diagnosed with or identified as being at risk of developing a hypercholesterolemia condition such as, *e.g.*, heterozygous Familial Hypercholesterolemia (heFH), homozygous Familial Hypercholesterolemia (hoFH), as well as incidences of hypercholesterolemia that are distinct from Familial Hypercholesterolemia (nonFH).

[0014] In other instances, at the time of, or prior to administration of the pharmaceutical composition of the present invention, the patient may be diagnosed with or identified as being at risk of developing a thrombotic occlusive disease or disorder, such as, *e.g.*, pulmonary embolism, central retinal vein occlusion, etc. In certain embodiments, the patient is selected on the basis of being diagnosed with or at risk of developing a combination of two or more of the abovementioned diseases or disorders. For example, at the time of, or prior to administration of the pharmaceutical composition of the present invention, the patient may be diagnosed with or

identified as being at risk of developing coronary artery disease and pulmonary embolism. Other diagnostic combinations (*e.g.*, atherosclerosis and central retinal vein occlusion, heFH and stroke, etc.) are also included in the definition of the patient populations that are treatable by the methods of the present invention.

[0015] In yet other instances, the patient who is to be treated with the methods of the present invention is selected on the basis of one or more factors selected from the group consisting of age (*e.g.*, older than 40, 45, 50, 55, 60, 65, 70, 75, or 80 years), race, gender (male or female), exercise habits (*e.g.*, regular exerciser, non-exerciser), other preexisting medical conditions (*e.g.*, type-II diabetes, high blood pressure, etc.), and current medication status (*e.g.*, currently taking statins [*e.g.*,cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, etc.], beta blockers, niacin, etc.). The present invention also includes methods for reducing serum Lp(a) levels in patients who are intolerant of, non-responsive to, or inadequately responsive to conventional statin therapy. Potential patients can be selected/screened on the basis of one or more of these factors (*e.g.*, by questionnaire, diagnostic evaluation, etc.) before being treated with the methods of the present invention.

[0016] The present invention also includes methods for increasing transintestinal cholesterol excretion (TICE) in a subject by administering a PCSK9 inhibitor to the subject. For example, the present invention provides methods for increasing TICE in a subject by administering to the subject an anti-PCSK9 antibody, *e.g.*, the antibody referred to herein as "mAb316P". According to certain embodiments, the present invention includes methods comprising identifying a subject for which enhanced TICE would be beneficial, or identifying a subject that exhibits impaired TICE, and administering a PCSK9 inhibitor (*e.g.*, mAb316P) to the subject.

PCSK9 Inhibitors

[0017] The methods of the present invention comprise administering to a patient a therapeutic composition comprising a PCSK9 inhibitor. As used herein, a "PCSK9 inhibitor" is any agent which binds to or interacts with human PCSK9 and inhibits the normal biological function of PCSK9 *in vitro* or *in vivo*. Non-limiting examples of categories of PCSK9 inhibitors include small molecule PCSK9 antagonists, peptide-based PCSK9 antagonists (*e.g.*, "peptibody" molecules), and antibodies or antigen-binding fragments of antibodies that specifically bind human PCSK9.

[0018] The term "human proprotein convertase subtilisin/kexin type 9" or "human PCSK9" or "hPCSK9", as used herein, refers to PCSK9 having the nucleic acid sequence shown in SEQ ID NO:754 and the amino acid sequence of SEQ ID NO:755, or a biologically active fragment thereof.

[0019] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain

comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-PCSK9 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0020] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0021] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0022] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally

comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0023] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0024] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0025] The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0026] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may nonetheless include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to

include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0027] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0028] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0029] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0030] An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also

includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0031] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" PCSK9, as used in the context of the present invention, includes antibodies that bind PCSK9 or portion thereof with a K_D of less than about 1000 nM, less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. An isolated antibody that specifically binds human PCSK9, however, have cross-reactivity to other antigens, such as PCSK9 molecules from other (non-human) species.

[0032] The anti-PCSK9 antibodies useful for the methods of the present invention may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes methods involving the use of antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*,

only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. The use of antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0033] The present invention also includes methods involving the use of anti-PCSK9 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes the use of anti-PCSK9 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0034] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0035] The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0036] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

Preparation of Human Antibodies

[0037] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human PCSK9.

[0038] Using VELOCIMMUNE™ technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to PCSK9 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0039] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0040] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc, using standard procedures known to those skilled in the art. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0041] In general, the antibodies that can be used in the methods of the present invention possess high affinities, as described above, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0042] Specific examples of human antibodies or antigen-binding fragments of antibodies that specifically bind PCSK9 which can be used in the context of the methods of the present invention include any antibody or antigen-binding fragment which comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 22, 26, 42, 46, 50, 66, 70, 74, 90, 94, 98, 114, 118, 122, 138, 142, 146, 162, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258, 262, 266, 282, 286, 290, 306, 310, 314, 330, 334, 338, 354, 358, 362, 378, 382, 386, 402, 406, 410, 426, 430, 434, 450, 454, 458, 474, 478, 482, 498, 502, 506, 522, 526, 530, 546, 550, 554, 570, 574, 578, 594, 598, 602, 618, 622, 626, 642, 646, 650, 666, 670, 674, 690, 694, 698, 714, 718, 722, 738 and 742, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. The antibody or antigen-binding fragment may comprise the three light chain CDRs (LCVR1, LCVR2, LCVR3) contained within a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 20, 24, 34, 44, 48, 58, 68, 72, 82, 92, 96, 106, 116, 120, 130, 140, 144, 154, 164, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260, 264, 274, 284, 288, 298, 308, 312, 322, 332, 336, 346, 356, 360, 370, 380, 384, 394, 404, 408, 418, 428, 432, 442, 452, 456, 466, 476, 480, 490, 500, 504, 514, 524, 528, 538, 548, 552, 562, 572, 576, 586, 596, 600, 610, 620, 624, 634, 644, 648, 658, 668, 672, 682, 692, 696, 706, 716, 720, 730, 740 and 744, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0043] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises the six CDRs (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3) from the heavy and light chain variable region amino acid sequence pairs (HCVR/LCVR) selected from the group consisting of SEQ ID NOs: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, 502/504, 506/514, 522/524, 526/528, 530/538, 546/548, 550/552, 554/562, 570/572, 574/576, 578/586, 594/596, 598/600, 602/610, 618/620, 622/624, 626/634, 642/644, 646/648, 650/658, 666/668, 670/672, 674/682, 690/692, 694/696, 698/706, 714/716, 718/720, 722/730, 738/740 and 742/744.

[0044] In certain embodiments of the present invention, the anti-PCSK9 antibody, or antigen-binding fragment thereof, that can be used in the methods of the present invention has HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 amino acid sequences selected from SEQ ID

NOs: 76/78/80/84/86/88 (mAb316P) and 220/222/224/228/230/232 (mAb300N) (*See* US Patent App. Publ No. 2010/0166768).

[0045] In certain embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises HCVR/LCVR amino acid sequence pairs selected from the group consisting of SEQ ID NOs: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, 502/504, 506/514, 522/524, 526/528, 530/538, 546/548, 550/552, 554/562, 570/572, 574/576, 578/586, 594/596, 598/600, 602/610, 618/620, 622/624, 626/634, 642/644, 646/648, 650/658, 666/668, 670/672, 674/682, 690/692, 694/696, 698/706, 714/716, 718/720, 722/730, 738/740 and 742/744.

Pharmaceutical Compositions and Methods of Administration

[0046] The present invention includes methods which comprise administering a PCSK9 inhibitor to a patient, wherein the PCSK9 inhibitor is contained within a pharmaceutical composition. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0047] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0048] A pharmaceutical composition of the present invention can be delivered

subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0049] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0050] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0051] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be

prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0052] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

Dosage

[0053] The amount of PCSK9 inhibitor (*e.g.*, anti-PCSK9 antibody) administered to a subject according to the methods of the present invention is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of PCSK9 inhibitor that results in a detectable reduction in serum Lp(a). For example, "therapeutically effective amount" of a PCSK9 inhibitor includes, *e.g.*, an amount of PCSK9 inhibitor that causes a reduction of at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50% or more in Lp(a) levels when administered to a human patient, *e.g.*, as illustrated in Example 2, herein. Alternatively, animal models can be used to establish whether a particular amount of a candidate PCSK9 inhibitor is a therapeutically effective amount.

[0054] In the case of an anti-PCSK9 antibody, a therapeutically effective amount can be from about 0.05 mg to about 600 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the anti-PCSK9 antibody.

[0055] The amount of anti-PCSK9 antibody contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the anti-PCSK9 antibody may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

Combination Therapies

[0056] The methods of the present invention, according to certain embodiments, may comprise administering a pharmaceutical composition comprising an anti-PCSK9 antibody to a patient who is on a therapeutic regimen for the treatment of hypercholesterolemia at the time of, or just prior to, administration of the pharmaceutical composition of the invention. For example, a patient who has previously been diagnosed with hypercholesterolemia may have been prescribed and is taking a stable therapeutic regimen of another drug prior to and/or concurrent with administration of a pharmaceutical composition comprising an anti-PCSK9 antibody. The prior or concurrent therapeutic regimen may comprise, *e.g.*, (1) an agent which induces a cellular depletion of cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase, such as a statin (e.g., cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, etc.); (2) an agent which inhibits cholesterol uptake and or bile acid re-absorption; (3) an agent which increase lipoprotein catabolism (such as niacin); and/or (4) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol. In certain embodiments, the patient, prior to or concurrent with administration of an anti-PCSK9 antibody is on a fixed combination of therapeutic agents such as ezetimibe plus simvastatin; a statin with a bile resin (e.g., cholestyramine, colestipol, colesevelam); niacin plus a statin (e.g., niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor).

Administration Regimens

[0057] According to certain embodiments of the present invention, multiple doses of a PCSK9 inhibitor may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of a PCSK9 inhibitor. As used herein, "sequentially administering" means that each dose of PCSK9 inhibitor is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of a PCSK9 inhibitor, followed by one or more secondary doses of the PCSK9 inhibitor, and optionally followed by one or more tertiary doses of the PCSK9 inhibitor.

[0058] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the PCSK9 inhibitor. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose");

the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of PCSK9 inhibitor, but will generally differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of PCSK9 inhibitor contained in the initial, secondary and/or tertiary doses will vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment.

[0059] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 30 (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more) days after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of PCSK9 inhibitor which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0060] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of a PCSK9 inhibitor. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0061] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 29 days after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 1 to 60 days after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

EXAMPLES

[0062] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be

accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to Human PCSK9

[0063] Human anti-PCSK9 antibodies were generated as described in US Patent App. Publ No. 2010/0166768. The exemplary PCSK9 inhibitor used in the following Example is the human anti-PCSK9 antibody designated mAb316P. mAb316P has the following amino acid sequence characteristics: heavy chain variable region (HCVR) comprising SEQ ID NO:90; light chain variable domain (LCVR) comprising SEQ ID NO:92; heavy chain complementarity determining region 1 (HCDR1) comprising SEQ ID NO:76; HCDR2 comprising SEQ ID NO:78; HCDR3 comprising SEQ ID NO:80; light chain complementarity determining region 1 (LCDR1) comprising SEQ ID NO:84; LCDR2 comprising SEQ ID NO:86; and LCDR3 comprising SEQ ID NO:88.

Example 2: Clinical Trials of An Anti-PCSK9 Monoclonal Antibody, As Mono- or Add-On Therapy in Heterozygous Familial and Non-Familial Hypercholesterolemia

Introduction

[0064] This example describes the results from two clinical trials in which single doses of the anti-PCSK9 monoclonal antibody mAb316P were administered intravenously (IV) or subcutaneously (SC) to healthy volunteers, and a multiple-dose clinical trial of mAb316P in subjects with either Familial Hypercholesterolemia (FH) or nonFH, receiving stable doses of atorvastatin or dietary intervention only.

Methods

A. Study Design

[0065] Three clinical trials were conducted using mAb316P administered to human patients. Two of the trials were single dose placebo-controlled studies of mAb316P administered IV or SC, respectively. The third trial was a phase 1b, double-blind, randomized, placebo-controlled, ascending multiple-dose evaluation of mAb316P administered SC to subjects with FH or nonFH. The third trial was conducted in two parts: Part A and Part B. In Part A of the third trial, each subject received a total of 3 administrations of mAb316P (50 mg, 100 mg or 150 mg) or matching placebo. In Part B of the third trial, each subject received 2 administrations of either mAb316P (200 mg) or matching placebo. In both parts of the multiple SC dose trial, the first dose was administered in an inpatient unit where subjects were confined and observed for 48 hours post-dose. Subsequent doses were administered in an outpatient setting with at least 2 hours of post-dose observation.

B. Dose and Dose Escalation

[0066] The single dose IV study included 5 sequential cohorts (0.3, 1.0, 3.0, 6.0, or 12.0 mg/kg of mAb316P), and the single SC dose trial included 4 sequential dose cohorts (50, 100, 150, and 250 mg of mAb316P). Based on initial results from these trials, the third trial evaluated SC mAb316P doses of 50 mg, 100 mg, 150 mg, or placebo administered on days 1, 29 and 43 to 7 groups of subjects with FH or nonFH (Part A), and 200 mg or placebo administered on days 1 and 29 to an eighth group of subjects with FH or nonFH (Part B). The dosing regimens and patient compositions of the three trials are summarized in Table 1.

Table 1

mAb 316 Dose	Patient Group	Total No. Patients (mAb316:Pbo)	Patient Type	Screening LDL-C	Atorvastatin Dose
Single IV Dose					
0.3 mg/kg	1	8 (6:2)	Healthy Volunteers	> 100 mg/dL	None
1.0 mg/kg	2				
3.0 mg/kg	3				
6.0 mg/kg	4				
12.0 mg/kg	5				
Single SC Dose					
50 mg	1	8 (6:2)	Healthy Volunteers	> 100 mg/dL	None
100 mg	2				
150 mg	3				
250 mg	4				
Multiple SC Dose					
Part A					
50 mg	1	7 (5:2)	FH	> 100 mg/dL	10-40 mg OD
	2	10 (8:2)	nonFH		
100 mg	3	7 (5:2)	FH		
	4	10 (8:2)	nonFH		
150 mg	5	7 (5:2)	FH		
	6	10 (8:2)	nonFH		
	7	10 (8:2)	nonFH	> 130 mg/dL	None
Part B					
200 mg	8	10 (8:2)	FH & nonFH	> 100 mg/dL	10-40 mg OD

[0067] In the multiple SC dose trial, each dose level commenced with a sentinel group of 3 (FH, non-FH, or both) subjects, with at least 1, but not more than 2, subjects receiving mAb316P. Enrollment of additional subjects at a given dose level only proceeded after the initial 3 subjects had safely completed their day 3 post-treatment assessments. Enrollment in

the next higher dose level was opened once 10 subjects had completed the day 15 safety assessments. Enrollment, once started at each dose level, continued independent of the dose escalation decision and day 15 safety review.

C. Subjects

[0068] Subjects in the single-dose studies were healthy men and women (18-65 years of age, 50-95 kg in body weight, body mass index [BMI] = 18-30 kg/m^2) with serum LDL-C >100 mg/dL (2.59 mmol/L). The use of all non-study agents to alter lipids was prohibited throughout these studies.

[0069] Subjects in the multiple-dose study had heterozygous FH or nonFH (18-65 years of age, BMI=18.0-35.0 kg/m^2) and were on stable atorvastatin therapy (10 to 40 mg per day) with LDL cholesterol >100 mg/dL (2.59 mmol/L), or nonFH and were on diet only with LDL cholesterol >130 mg/dL (3.36 mmol/L) (Table 1). All subjects reviewed and signed an informed consent previously approved by an institutional review board prior to any study related procedures. Subjects in the FH group met Simon Broome criteria for definite or possible FH (Simon Broome Register Group (1991), BMJ 303:893-896; Neil *et al.*, (2000), BMJ 321:148) while nonFH subjects did not. Those taking atorvastatin were on a stable dose, 10 to 40 mg daily, for at least 28 days prior to baseline, and remained on the same dose throughout the study. NonFH patients on diet only could not have received lipid-lowering therapy for at least 28 days prior to baseline, and remained off such treatment throughout the study. Potentially eligible patients not on atorvastatin could enter a 4 week run-in period during which were switched to a dose of atorvastatin (10 to 40 mg per day) that was likely to maintain LDL cholesterol near their pre-study level and >100 mg/dL (2.59 mmol/L). All subjects had triglycerides ≤300 mg/dL and blood pressure controlled on fewer than 3 antihypertensive medications. Patients with a history of cerebrovascular, cardiovascular, or peripheral vascular atherosclerotic disease, diabetes, or disorders known to produce secondary elevations of LDL cholesterol were excluded as were those with hepatic transaminases (ALT or AST) >1.2 times the upper limit of normal (x ULN), >2+ proteinuria or creatine kinase (CK) >3 x ULN, unless clearly exercise related in which case they were required to have repeat testing with CK <3 x ULN.

D. Laboratory Measurements and Methods

[0070] In the multi-dose study, serum lipid (total-, HDL-, LDL-, non-HDL-, and VLDL cholesterol) and apolipoprotein (Apo) B, A1 and Lp(a), hs-CRP and safety laboratory tests were performed after 12 hour overnight fasts (water only) on screening (day -21 to -3), day -2 (for those on atorvastatin), day -1, days 1, 2, 3, 8, 15, 29, 43, 57, 71, 85, 99, 120, and the end-of-study (day 148). All laboratory measurements were performed by a central laboratory which maintained Part III certification by CDC Lipid Standardization Program and accreditation by the College of American Pathologists. Triglycerides and cholesterol were measured with enzymatic

colorimetric tests (Olympus AU2700 or AU5400 Analyzer, Olympus, Center Valley, Pennsylvania, USA) with calibration directly traceable to CDC reference procedures. Apo B containing lipoproteins were precipitated with dextran sulphate and HDL cholesterol was measured on the supernatant. (Warnick *et al.*, (1978), *J. Lipid Res. 19*:65-76). LDL- and VLDL cholesterol were measured after preparative ultracentrifugation (beta-quantification). Apo A1, B, Lp(a) and hs-CRP were measured with rate immune-nephelometry (Dade Behring BNII nephelometer, Siemens Healthcare Diagnostics, Deerfield, Illinois, USA). All lipid, apolipoprotein, and hs-CRP values were blinded to the investigators, study staff, and patients from after day -2 for the atorvastatin treated groups or day -1 for diet only group.

E. Statistical Plan

[0071] Effects of mAb316P on lipid parameters in the single-dose studies were assessed using analysis of covariance (ANCOVA) models. Least squares means of differences between treatment groups and the pooled placebo group, 95% confidence intervals, and p-values for comparison between treatment groups versus placebo by visit were obtained within the framework of ANCOVA. Significance for all tests was set at 0.05.

[0072] In the multi-dose study, all subjects who received placebo and atorvastatin were pooled into two placebo groups of either FH or nonFH subjects. The 6 subjects in both the FH and nonFH (atorvastatin treated) placebo groups, and 5 and 8 subjects in each of the respective FH and nonFH mAb316P-treated dose groups provided at least 80% power to detect a treatment difference of 30% (SD=15%) versus placebo in mean percentage change from baseline of LDL cholesterol at each study visit when comparing each dose with placebo with a 2-sided test at the 5% significance level. No adjustments were made for multiple comparisons. Group 7 results were summarized separately by treatment and placebo and the two groups were compared individually. ANCOVA with treatment arm as the fixed effect and the relevant baseline value as a covariate was used to analyze the continuous variables. Missing values were imputed by the Last Observation Carried Forward (LOCF) method. All p-values, except for triglycerides and Lp(a), which were derived from the Rank-based analysis of covariates, were drawn from the ANCOVA model.

[0073] To summarize the lipid and lipoprotein effects, results were selected from study day 57 on which the effects of two subsequent 2-week dosing periods could be observed. The study was not powered for a direct statistical comparison of the response of FH subjects to nonFH subjects.

Results

A. Study Population

[0074] A total of 40 subjects were randomized in the single dose IV study and 32 were randomized in the single dose SC study. For Part A of the multiple-dose SC study, a total of 97

subjects were screened and 62 were randomized (21 FH and 41 nonFH). A total of 10 subjects were included in Part B of the multi-dose SC study (4 FH and 6 nonFH). Baseline characteristics of the subjects in the single dose studies and Part A of the multi-dose study are shown in Table 2A. Baseline characteristics of the subjects in Part B of the multi-dose study are shown in Table 2B.

Table 2A

	Single Dose IV	Single Dose SC	Multiple Dose SC – Part A		
			FH	nonFH	nonFH Diet Alone (no atorvastatin)
No. Patients	40	32	21	30	10
Median Age	36	34	40	52	52
Gender					
Male	65%	74%	81%	59%	56%
Female	35%	26%	19%	41%	44%
Median BMI (kg/m^2)	26	25	27	27	27
Race (%)					
White	55%	44%	86%	93%	100%
Black/African American	37.5%	50%	14%	7%	0%
American Indian/ Alaskan Native	7.5%	6%	0%	0%	0%
Atorvastatin Dose					
10 mg	None	None	14%	63%	None
20 mg	None	None	33%	33%	None
40 mg	None	None	52%	4%	None
Baseline Value					
LDL-C (mg/dL)	133	127	134	111	174
ApoB (mg/dL)	1.1	1.0	1.1	1.0	1.3
HDL-C (mg/dL)	54	55	44	51	51
TG (mg/dL)	108	103	113	136	133

Table 2B

	Multiple Dose SC – Part B (200 mg dose)					
	FH and NonFH Combined		FH		NonFH	
	Pbo	mAb316P	Pbo	mAb316P	Pbo	mAb316P
No. Patients	2	8	1	3	1	5
Median Age	35	45.5	27	50	43	41
Gender						
Male	50%	37.5%	100%	66.7%	0%	20%
Female	50%	62.5%	0%	33.3%	100%	80%
Median BMI (kg/m^2)	29.39	26.02	28.26	25.43	30.52	26.61
Race (%)						
White	100%	100%	100%	100%	100%	100%
Atorvastatin Dose						
10 mg	50%	75%	0%	33.3%	100%	100%
20 mg	0%	12.5%	0%	33.3%	0%	0%
40 mg	50%	12.5%	100%	33.3%	0%	0%
Baseline Value						
LDL-C (mmol/L)	3.05	3.32	3.76	3.78	2.33	3.08
ApoB (g/L)	0.85	1.06	0.92	1.05	0.77	1.07
HDL-C (mmol/L)	1.03	1.23	0.88	1.19	1.17	1.30
TG (mmol/L)	1.17	1.23	1.01	0.98	1.33	1.30

B. Lipid and Lipoprotein Response

[0075] Administration of a single IV or SC dose of mAb316P to healthy volunteers produced similar mean maximum percent reductions in LDL cholesterol of 55-60%. The degree and duration of LDL cholesterol lowering was dose dependent and LDL cholesterol reductions were sustained at least until 29 and 22 days after administration of higher dose levels of IV or SC mAb316P, respectively. Similarly, in the multiple-dose study, the mean percent reduction from baseline in LDL cholesterol was dose dependent and exceeded 50% two weeks after dosing with 150 mg in the FH and nonFH on atorvastatin and the nonFH diet alone populations . All but 1 subject in each population that received 150 mg in the multiple-dose study experienced a reduction of at least 40% from baseline.

[0076] Lipid and apolipoprotein baseline and day 57 levels for all treatment groups in Part A of the multiple-dose study are shown in Tables 3A (atorvastatin-treated subjects) and 3B (diet only – no atorvastatin treatment).

Table 3A

		Atorvastatin-Treated Subjects			
		Placebo (Pbo)	mAb316P Dose		
			50 mg	100 mg	150 mg
Parameter		N=12	N=13	N=13	N=13
LDL Cholesterol mean (SD) mg/dL	Baseline	125 (19)	114 (15)	121 (31)	123 (27)
	day 57	195 (17)	148 (29)	130 (16)	127 (26)
	% change vs Pbo		-39.2	-53.7	-61.0
	p value vs Pbo		<0.0001	<0.0001	<0.0001
Total Cholesterol mean (SD) mg/dL	Baseline	193 (20)	191 (24)	192 (27)	197 (32)
	day 57	195 (17)	148 (29)	130 (16)	127 (26)
	% change vs Pbo		-24.6	-33.2	-36.4
	p value vs Pbo		<0.0001	<0.0001	<0.0001
HDL Cholesterol mean (SD) mg/dL	Baseline	43 (10)	52 (16)	48 (10)	48 (11)
	day 57	42 (10)	55 (14)	51 (10)	54 (12)
	% change vs Pbo		13.2	11.3	18.2
	p value vs Pbo		0.0153	0.0336	0.0009
LDL Cholesterol mean (SD) mg/dL	Baseline	125 (20)	115 (16)	119 (30)	123 (29)
	day 57	129 (15)	74 (15)	58 (14)	52 (21)
	% change vs Pbo		-41.7	-55.8	-62.4
	p value vs Pbo		<0.0001	<0.0001	<0.0001
Triglycerides median (min:max) mg/dL	Baseline	113 (60:244)	118 (43:171)	135 (46:210)	117 (65:278)
	day 57	111 (55:186)	97 (40:189)	111 (31:173)	92 (60:181)
	% change vs Pbo		-16.3	-7.5	-11.7
	p value vs Pbo		0.0142	0.2515	0.0609
Apolipo-protein B mean (SD) mg/dL	Baseline	107 (12)	104 (15)	100 (21)	106 (22)
	day 57	108 (14)	74 (14)	60 (12)	58 (14)
	% change vs Pbo		-31.5	-42.0	-46.4
	p value vs Pbo		<0.0001	<0.0001	<0.0001
Apolipo-protein A1 mean (SD) mg/dL	Baseline	132 (19)	155 (36)	144 (23)	145 (23)
	day 57	132 (20)	162 (31)	150 (20)	161 (24)
	% change vs Pbo		9.9	6.9	13.5
	p value vs Pbo		0.019	0.086	0.013
Lipoprotein (a) median (min:max) mg/dL	Baseline	22 (2:121)	46 (5:151)	50 (7:142)	61 (5:154)
	day 57	13 (2:119)	42 (4:145)	26 (3:95)	47 (5:119)
	% change vs Pbo		-15.5	-24.1	-18.3
	p value vs Pbo		0.1109	0.0015	0.1226

Table 3B

		Diet Only – No Atorvastatin Treatment	
		Placebo (Pbo)	150 mg mAb316P
Parameter		N=2	N=8
LDL Cholesterol mean (SD) mg/dL	Baseline	152 (16)	179 (49)
	day 57	242 (45)	154 (29)
	% change vs Pbo		-57.0
	p value vs Pbo	<0.0001	0.0023
Total Cholesterol mean (SD) mg/dL	Baseline	228 (6)	257 (58)
	day 57	242 (45)	154 (29)
	% change vs Pbo		-43.3
	p value vs Pbo		0.0025
HDL Cholesterol mean (SD) mg/dL	Baseline	54 (14	50 (8)
	day 57	64 (8)	51 (10)
	% change vs Pbo		-18.9
	p value vs Pbo		0.17
LDL Cholesterol mean (SD) mg/dL	Baseline	152 (12)	177 (49)
	day 57	159 (30)	79 (24)
	% change vs Pbo		-58.4
	p value vs Pbo		<0.0025
Triglycerides median (min:max) mg/dL	Baseline	116 (86:146)	127 (82:268)
	day 57	93 (68:118)	116 (70:186)
	% change vs Pbo		-16.86
	p value vs Pbo		0.4820
Apolipoprotein B mean (SD) mg/dL	Baseline	118 (23)	138 (37)
	day 57	118 (6)	76 (19)
	% change vs Pbo		-45.0
	p value vs Pbo		0.003
Apolipoprotein A1 mean (SD) mg/dL	Baseline	138 (6)	151 (15)
	day 57	170 (32)	154 (16)
	% change vs Pbo		-19.2
	p value vs Pbo		0.073
Lipoprotein (a) median (min:max) mg/dL	Baseline	47 (30:63)	34 (5:111)
	day 57	58 (41:75)	39 (4:119)
	% change vs Pbo		-43.8
	p value vs Pbo		0.0415

[0077] The LDL cholesterol response at day 57 was similar in all subjects irrespective of FH or nonFH, atorvastatin treated or on diet alone. Corresponding changes were also observed in total- and non-HDL cholesterol and Apo B (Tables 3A and 3B). Importantly, reductions were also observed in Lp(a). Additionally, in patients taking atorvastatin favorable changes were seen in both HDL cholesterol and apoA1.

[0078] Similar improvements were observed in the mAb316P-treated patients in Part B of the multiple-dose study. That is, subcutaneous administration of 200 mg of mAb316P induced rapid, substantial, and sustained reductions in LDL-C, total cholesterol, non-HDL-C, apoB, and in the ratio of apoB/apoA. Patients who received 200 mg of mAb316P SC also appeared to demonstrate a trend toward higher levels of HDL-C and ApoA1.

Discussion

[0079] This Example describes human trials of the anti-PCSK9 antibody referred to herein as mAb316P, starting with two single ascending dose studies in healthy volunteers and extending into a large multiple-dose proof of concept trial in patients with both FH and nonFH treated with either a statin or on diet alone. These trials confirm the potential to bring about rapid and significant reductions in LDL cholesterol with PCSK9 inhibition in both familial and nonfamilial hypercholesterolemia, whether on a statin or on diet alone. The populations tested encompass the large majority of patients with hypercholesterolemia.

[0080] The robust and reproducible effect on LDL cholesterol of mAb316P from single IV dosing, through single SC dosing, to multiple SC doses is seen within an unexpectedly short time after administration, reaching a maximum at approximately 2 weeks. This rapidity exceeds that for all other therapeutic modalities for hypercholesterolemia other than removal of LDL cholesterol via apheresis, and is more rapid than for statins. That large reductions of LDL cholesterol can be achieved with mAb316P in patients already on relatively high doses of atorvastatin known to produce 40% to 50% LDL cholesterol reductions clearly distinguishes the potential of mAb316P from other investigational therapies that have been added to statins, such as ezetimibe, bile acid sequestrants, and squalene synthase inhibitors. The LDL cholesterol lowering efficacy of mAb316P, combined with apparent tolerability and safety, highlights the surprising therapeutic advantages of mAb316P over several other Apo B/LDL-C lowering agents in development such as Apo B antisense, MTP inhibitors, and thyroid hormone analogues. In fact, the achievement in many of the subjects in these studies of low LDL cholesterol levels of 50 mg per deciliter (1.29 mmol/L) or less without any increase in hepatic transaminases is in marked contrast to results obtained with agents that inhibit hepatic VLDL and LDL formation. Epidemiology studies demonstrating that genetic under-expression or even the absence of PCSK9 and life-long low levels of LDL cholesterol appear not to be associated with unexpected morbidity or mortality, provide a level of comfort with regard to the therapeutic potential of drug inhibition of PCSK9.

[0081] The anti-PCSK9 antibodies of the present invention such as mAb316P also provide patients unable to tolerate statins (now estimated to be approximately 5% to 10% of all statin treated patients) a significant opportunity to achieve large (at least 50%) reductions in LDL cholesterol. For many of the estimated 10 million patients worldwide with FH, the anti-PCSK9 antibodies of the invention, including mAb316P, offer the real possibility that their use along with statins may finally achieve optimal LDL cholesterol control and potentially further reduce their substantial risk of the early and recurrent cardiovascular disease. The currently disclosed therapeutic methodologies may also offer many patients the potential to discontinue LDL apheresis, an invasive, time consuming, expensive every 2-week procedure that provides only short-term LDL cholesterol reduction.

[0082] Additional unexpected beneficial effects on other lipids were seen in HDL cholesterol, Apo A1 and, most surprisingly, Lp(a) which trended to, or reached, statistical significance, including in patients treated with atorvastatin. Importantly, before now, no therapeutic biologic agents (*e.g.*, anti-PCSK9 antibodies) have been shown clinically to reduce Lp(a), which has been thought not to be cleared via the LDL receptor. Thus, the current experiments are the first demonstration of the ability of a PCSK9 inhibitor to reduce serum Lp(a) levels in patients.

[0083] Regarding safety, mAb316P injection site reactions were minimal. mAb316P was also generally safe and well tolerated with no trend in drug-related adverse events and no evidence of hepato- or myo-toxicity. The few CK elevations that were seen were exercise related.

[0084] In summary, this Example shows that mAb316P inhibition of PCSK9, besides effectively lowering LDL cholesterol in human patients, surprisingly reduced Lp(a) levels as well.

Example 3: A Randomized, Double-Blind, Placebo Controlled, 12 Week Study of the Safety and Efficacy of An Anti-PCSK9 Monoclonal Antibody in Patients with Heterozygous Familial Hypercholesterolemia

[0085] A clinical trial was conducted to assess the efficacy of varying subcutaneous (SC) doses and dosing regimens of mAb316P on serum low-density lipoprotein cholesterol (LDL-C) and other lipids/apolipoproteins (*e.g.*, total cholesterol, high-density lipoprotein cholesterol, triglycerides, apo B, Apo A1, and Lp[a]) in patients with heterozygous familial hypercholesterolemia (heFH).

Patient Population

[0086] The patient population for this study included men and women, ages 18-75 years old, who were diagnosed with heFH, exhibited LDL-C levels of 100 mg/dL or greater, and who were on a stable daily statin dose (either with or without ezetimibe) at the start of the study. Patients were excluded if they were taking any additional lipid lowering compounds such as fibrates, niacin, omega-3 fatty acids, bile acid resins, plant stanols (*e.g.*, Benecol, flaxseed oil, psyllium), or red yeast rice. A total of 77 patients completed the study.

Drug Formulation

[0087] The drug formulation comprised 150 mg/mL of mAb316P, 10 mM histidine, 0.2% polysorbate 20, 10% sucrose, and had a pH of 6.0.

Method of Administration

[0088] The drug formulation (or placebo) was administered to the patients by subcutaneous injection into the abdomen. Each treatment included two subcutaneous injections of 1 mL each.

Administration Regimens

[0089] The patients were divided into five groups. Group 1 included 15 patients who received placebo once every two weeks; Group 2 included 16 patients who received 150 mg of mAb316P once every two weeks; Group 3 included 15 patients who received 150 mg of mAb316P once every four weeks; Group 4 included 16 patients who received 200 mg of mAb316P once every four weeks; and Group 5 included 15 patients who received 300 mg of mAb316P once every four weeks. The study duration was 12 weeks.

Efficacy Assessment

[0090] Blood samples were collected from the patients throughout the study, and during an 8 week follow-up period. The samples were collected after at least a 12 hour fast (in the morning before any drug intake). Measurements of the following parameters were determined from the blood samples: (1) Total Cholesterol; (2) LDL-C; (3) HDL-C; (4) Triglycerides (TG); (5) Apo B; (6) Apo A1; and (7) Lp(a). The median percent changes in these parameters compared to placebo at the end of the study (week 12) are summarized in Table 4.

Table 4

Group	1	2	3	4	5
No. of Patients	15	16	15	16	15
Treatment	Placebo	mAb316P	mAb316P	mAb316P	mAb316P
Dose	N/A	150 mg	150 mg	200 mg	300 mg
Dosing Frequency	Q2W	Q2W	Q4W	Q4W	Q4W
Total Cholesterol	-6.52	-38.27	-18.89	-15.31	-28.84
LDL-C	-4.90	-66.71	-24.30	-24.07	-49.35
HDL-C	+2.46	+14.63	+6.33	+6.53	+4.48
Triglycerides	-10.55	-16.23	-16.73	-9.87	-4.92
Apo B	-7.32	-48.97	-19.09	-14.69	-36.27
Apo A1	-7.24	+9.98	+3.13	+0.33	+5.39
Lp(a)	-11.07	-22.50	-11.30	-8.00	-14.29

Example 4: A Randomized, Double-Blind, Parallel-Group, Placebo Controlled, 12 Week Study of the Safety and Efficacy of An Anti-PCSK9 Monoclonal Antibody in Patients with Primary Hypercholesterolemia on Stable Atorvastatin Therapy

[0091] A clinical trial was conducted to assess the efficacy of five doses and two dose regimens of mAb316P over 12 weeks in patients with primary hypercholesterolemia and LDL-C levels greater than 100 mg/dL on ongoing stable atorvastatin therapy. Efficacy was assessed based on changes in serum low-density lipoprotein cholesterol (LDL-C) and other lipids/apolipoproteins (*e.g.*, total cholesterol, high-density lipoprotein cholesterol, triglycerides, apo B, Apo A1, and Lp[a]) over the course of the study.

Patient Population

[0092] The patient population for this study included men and women, ages 18-75 years old, who were diagnosed with primary hypercholesterolemia, exhibited LDL-C levels of 100 mg/dL or greater, and who were on stable atorvastatin therapy of 10, 20 or 40 mg for at least six weeks prior to the start of the study. Patients were excluded if they were taking any additional lipid lowering compounds such as fibrates, niacin, omega-3 fatty acids, bile acid resins, plant stanols (*e.g.*, Benecol, flaxseed oil, psyllium), or red yeast rice. A total of 118 patients completed the study.

Drug Formulation

[0093] The drug formulation comprised 150 mg/mL of mAb316P, 10 mM histidine, 0.2% polysorbate 20, 10% sucrose, and had a pH of 6.0.

Method of Administration

[0094] The drug formulation (or placebo) was administered to the patients by subcutaneous injection into the abdomen. Each treatment included two subcutaneous injections of 1 mL each.

Administration Regimens

[0095] The patients were divided into six groups. Group 1 included 20 patients who received placebo once every two weeks; Group 2 included 19 patients who received 50 mg of mAb316P once every two weeks; Group 3 included 20 patients who received 100 mg of mAb316P once every two weeks; Group 4 included 18 patients who received 150 mg of mAb316P once every two weeks; Group 5 included 20 patients who received 200 mg of mAb316P once every four weeks; and Group 6 included 21 patients who received 300 mg of mAb316P once every four weeks. The study duration was 12 weeks.

Efficacy Assessment

[0096] Blood samples were collected from the patients throughout the study, and during an 8 week follow-up period. The samples were collected after at least a 12 hour fast (in the morning

before any drug intake). Measurements of the following parameters were determined from the blood samples: (1) Total Cholesterol; (2) LDL-C; (3) HDL-C; (4) Triglycerides (TG); (5) Apo B; (6) Apo A1; and (7) Lp(a). The median percent changes in these parameters compared to placebo at the end of the study (week 12) are summarized in Table 5.

Table 5

Group	1	2	3	4	5	6
No. of Patients	20	19	20	18	20	21
Treatment	Placebo	mAb316P	mAb316P	mAb316P	mAb316P	mAb316P
Dose	N/A	50 mg	100 mg	150 mg	200 mg	300 mg
Dosing Frequency	Q2W	Q2W	Q2W	Q2W	Q4W	Q4W
Total Cholesterol	-3.73	-23.34	-40.21	-45.03	-29.51	-33.48
LDL-C	-6.92	-37.04	-64.28	-74.83	-49.46	-51.98
HDL-C	+1.81	+5.08	+7.93	+8.96	+5.59	+12.09
Triglycerides	+22.42	-7.85	-11.41	-22.93	+1.27	-9.17
Apo B	+0.76	-28.67	-48.67	-54.16	-30.67	-32.95
Apo A1	+0.58	+1.40	-0.88	+1.11	+0.66	+3.42
Lp(a)	0.00	-13.33	-27.27	-28.57	-18.92	-11.11

Example 5: A Randomized, Double-Blind, Parallel-Group, Placebo Controlled, Fixed Dose Study of the Safety and Efficacy of An Anti-PCSK9 Monoclonal Antibody Co-Administered with 80 mg Atorvastatin in Patients with Primary Hypercholesterolemia

[0097] A clinical trial was conducted to assess the efficacy of mAb316P when co-administered with 80 mg of atorvastatin over 8 weeks in patients with primary hypercholesterolemia and LDL-C levels greater than 100 mg/dL. Efficacy was assessed based on changes in serum low-density lipoprotein cholesterol (LDL-C) and other lipids/apolipoproteins (*e.g.*, total cholesterol, high-density lipoprotein cholesterol, triglycerides, apo B, Apo A1, and Lp[a]) over the course of the study.

Patient Population

[0098] The patient population for this study included men and women, ages 18-75 years old, who were diagnosed with primary hypercholesterolemia, exhibited LDL-C levels of 100 mg/dL or greater, and who were on stable atorvastatin therapy of 10 mg for at least six weeks prior to the start of the study. Patients were excluded if they were taking any additional lipid lowering compounds such as fibrates, niacin, omega-3 fatty acids, bile acid resins, plant stanols (*e.g.*, Benecol, flaxseed oil, psyllium), or red yeast rice. A total of 88 patients completed the study.

Drug Formulation

[0099] The drug formulation comprised 150 mg/mL of mAb316P, 10 mM histidine, 0.2% polysorbate 20, 10% sucrose, and had a pH of 6.0.

Method of Administration

[0100] The drug formulation (or placebo) was administered to the patients by subcutaneous injection into the abdomen. Each treatment included one subcutaneous injection of 1 mL.

Administration Regimens

[0101] The patients were divided into three groups. Group 1 included 29 patients who received placebo once every two weeks plus 80 mg atorvastatin daily; Group 2 included 30 patients who received 150 mg of mAb316P once every two weeks plus 80 mg atorvastatin daily; and Group 3 included 29 patients who received 150 mg of mAb316P once every two weeks plus 10 mg atorvastatin daily. The study duration was 8 weeks.

Efficacy Assessment

[0102] Blood samples were collected from the patients throughout the study, and during an 8 week follow-up period. The samples were collected after at least a 12 hour fast (in the morning before any drug intake). Measurements of the following parameters were determined from the blood samples: (1) Total Cholesterol; (2) LDL-C; (3) HDL-C; (4) Triglycerides (TG); (5) Apo B; (6) Apo A1; (7) Apo B/Apo A1 ratio; and (8) Lp(a). The percent changes in these parameters compared to placebo at the end of the study are summarized in Table 6.

Table 6

Group	1	2	3
No. of Patients	29	30	29
Treatment	Placebo	mAb316P	mAb316P
Dose	N/A	150 mg	150 mg
Dosing Frequency	Q2W	Q2W	Q2W
Daily Atorvastatin	80 mg	80 mg	10 mg
Total Cholesterol	-16.59	-47.21	-40.45
LDL-C	-26.87	-70.62	-70.37
HDL-C	-5.71	+5.17	+1.39
Triglycerides	-11.89	-24.67	-3.98
Apo B	-12.00	-58.00	-54.39
Apo A1	-5.56	-4.55	-0.69
Lp(a)	-2.70	-31.01	-34.65

[0103] Taken together, the results from these studies (Examples 3-5) confirm the ability of mAB316P to effectively lower LDL cholesterol and beneficially affect other lipid/apolipoprotein parameters in patients when administered at various doses and dosing regimens. These clinical Examples also confirm the unexpected finding that mAB316P is able to significantly reduce Lp(a) levels in patients under various circumstances.

[0104] 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 and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A method for reducing lipoprotein(a) (Lp(a)) levels, the method comprising selecting a patient who exhibits elevated serum Lp(a), and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

2. The method of claim 1, wherein the patient is selected on the basis of a serum Lp(a) level greater than about 20 mg/dL.

3. The method of claim 2, wherein the patient is selected on the basis of a serum Lp(a) level greater than about 100 mg/dL.

4. The method of any one of claims 1 to 3, wherein prior to or at the time of administration of the pharmaceutical composition, the patient is diagnosed with or identified as being at risk of developing a cardiovascular disease or disorder.

5. The method of claim 4, wherein the cardiovascular disease or disorder is selected from the group consisting of coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, and peripheral artery occlusive disease.

6. The method of claim 4, wherein the cardiovascular disease or disorder is hypercholesterolemia.

7. The method of claim 6, wherein the hypercholesterolemia is heterozygous Familial Hypercholesterolemia (heFH).

8. The method of claim 6, wherein the hypercholesterolemia is not Familial Hypercholesterolemia (nonFH).

9. The method of any one of claims 1 to 3, wherein prior to or at the time of administration of the pharmaceutical composition, the patient is diagnosed with or identified as being at risk of developing a thrombotic occlusive disease or disorder.

10. The method of claim 9, wherein the thrombotic occlusive disease or disorder is selected from the group consisting of pulmonary embolism and central retinal vein occlusion.

11. The method of any one of claims 1 to 10, wherein the PCSK9 inhibitor is an antibody or antigen-binding fragment thereof that specifically binds PCSK9.

12. The method of claim 11, wherein the pharmaceutical composition comprises 20 mg to 200 mg of the PCSK9 inhibitor.

13. The method of claim 12, wherein the pharmaceutical composition comprises 50 mg to 150 mg of the PCSK9 inhibitor.

14. The method of claim 13, wherein the pharmaceutical composition comprises 50 mg of the PCSK9 inhibitor.

15. The method of claim 13, wherein the pharmaceutical composition comprises 100 mg of the PCSK9 inhibitor.

16. The method of claim 13, wherein the pharmaceutical composition comprises 150 mg of the PCSK9 inhibitor.

17. The method of any one of claims 11 to 16, wherein the antibody or antigen binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 90/92 and 218/226.

18. The method of claim 17, wherein the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232.

19. The method of claim 18, wherein the antibody or antigen-binding fragment thereof comprises an HCVR having the amino acid sequence of SEQ ID NO:218 and an LCVR having the amino acid sequence of SEQ ID NO:226.

20. The method of claim 17, wherein the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR amino acid sequences having SEQ ID NOs:76, 78, 80, 84, 86 and 88.

21. The method of claim 20, wherein the antibody or antigen-binding fragment thereof comprises an HCVR having the amino acid sequence of SEQ ID NO:90 and an LCVR having the amino acid sequence of SEQ ID NO:92.

22. The method of any one of claims 11 to 16, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on PCSK9 as an antibody comprising heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232; or SEQ ID NOs: 76, 78, 80, 84, 86 and 88.

23. The method of any one of claims 11 to 16, wherein the antibody or antigen-binding fragment thereof competes for binding to PCSK9 with an antibody comprising heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232; or SEQ ID NOs: 76, 78, 80, 84, 86 and 88.

24. The method of any one of claims 1 to 23, wherein the patient is on a therapeutic statin regimen at the time of or just prior to administration of the pharmaceutical composition.

25. The method of claim 24, wherein the therapeutic statin regimen comprises a statin selected from the group consisting of cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin and pravastatin.

26. The method of claim 25, wherein the statin is atorvastatin.

27. The method of any one of claims 1 to 23, wherein the patient is not on a therapeutic statin regimen at the time of administration of the pharmaceutical composition.

28. A pharmaceutical composition comprising a PCSK9 inhibitor for use in reducing lipoprotein(a) (Lp(a)) levels in a patient who exhibits an elevated serum Lp(a) level.

29. The pharmaceutical composition of claim 28, wherein the patient exhibits a serum Lp(a) level greater than about 20 mg/dL.

30. The pharmaceutical composition of claim 29, wherein the patient exhibits a serum Lp(a) level greater than about 100 mg/dL.

31. The pharmaceutical composition of any one of claims 28 to 30, wherein the patient is diagnosed with or identified as being at risk of developing a cardiovascular disease or disorder.

32. The pharmaceutical composition of claim 31, wherein the cardiovascular disease or disorder is selected from the group consisting of coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, and peripheral artery occlusive disease.

33. The pharmaceutical composition of claim 31, wherein the cardiovascular disease or disorder is hypercholesterolemia.

34. The pharmaceutical composition of claim 33, wherein the hypercholesterolemia is heterozygous Familial Hypercholesterolemia (heFH).

35. The pharmaceutical composition of claim 33, wherein the hypercholesterolemia is not Familial Hypercholesterolemia (nonFH).

36. The pharmaceutical composition of any one of claims 28 to 30, wherein the patient is diagnosed with or identified as being at risk of developing a thrombotic occlusive disease or disorder.

37. The pharmaceutical composition of claim 36, wherein the thrombotic occlusive disease or disorder is selected from the group consisting of pulmonary embolism and central retinal vein occlusion.

38. The pharmaceutical composition of any one of claims 28 to 37, wherein the PCSK9 inhibitor is an antibody or antigen-binding fragment thereof that specifically binds PCSK9.

39. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition comprises 20 mg to 200 mg of the PCSK9 inhibitor.

40. The pharmaceutical composition of claim 39, wherein the pharmaceutical composition comprises 50 mg to 150 mg of the PCSK9 inhibitor.

41. The pharmaceutical composition of claim 40, wherein the pharmaceutical composition comprises 50 mg of the PCSK9 inhibitor.

42. The pharmaceutical composition of claim 40, wherein the pharmaceutical composition comprises 100 mg of the PCSK9 inhibitor.

43. The pharmaceutical composition of claim 40, wherein the pharmaceutical composition comprises 150 mg of the PCSK9 inhibitor.

44. The pharmaceutical composition of any one of claims 38 to 43, wherein the antibody or antigen binding fragment thereof comprises the heavy and light chain CDRs of a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 90/92 and 218/226.

45. The pharmaceutical composition of claim 44, wherein the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232.

46. The pharmaceutical composition of claim 45, wherein the antibody or antigen-binding fragment thereof comprises an HCVR having the amino acid sequence of SEQ ID NO:218 and an LCVR having the amino acid sequence of SEQ ID NO:226.

47. The pharmaceutical composition of claim 44, wherein the antibody or antigen-binding fragment thereof comprises heavy and light chain CDR amino acid sequences having SEQ ID NOs:76, 78, 80, 84, 86 and 88.

48. The pharmaceutical composition of claim 47, wherein the antibody or antigen-binding fragment thereof comprises an HCVR having the amino acid sequence of SEQ ID NO:90 and an LCVR having the amino acid sequence of SEQ ID NO:92.

49. The pharmaceutical composition of any one of claims 38 to 43, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on PCSK9 as an antibody comprising heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232; or SEQ ID NOs: 76, 78, 80, 84, 86 and 88.

50. The pharmaceutical composition of any one of claims 38 to 43, wherein the antibody or antigen-binding fragment thereof competes for binding to PCSK9 with an antibody comprising heavy and light chain CDR amino acid sequences having SEQ ID NOs:220, 222, 224, 228, 230 and 232; or SEQ ID NOs: 76, 78, 80, 84, 86 and 88.

51. The pharmaceutical composition of any one of claims 28 to 50, wherein the patient is on a therapeutic statin regimen.

52. The pharmaceutical composition of claim 51, wherein the therapeutic statin regimen comprises a statin selected from the group consisting of cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin and pravastatin.

53. The pharmaceutical composition of claim 52, wherein the statin is atorvastatin.

54. The pharmaceutical composition of any one of claims 28 to 50, wherein the patient is not on a therapeutic statin regimen.