(54) **Title:** METHODS FOR REDUCING REMNANT CHOLESTEROL AND OTHER LIPOPROTEIN FRACTIONS BY ADMINISTERING AN INHIBITOR OF PROPROTEIN CONVERTASE SUBTILISIN KEXIN-9 (PCSK9)
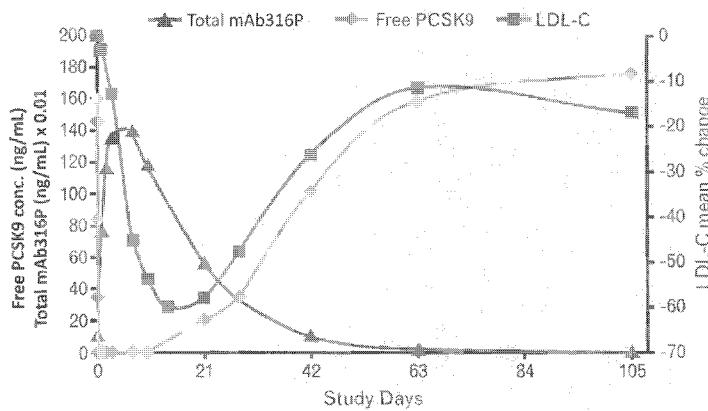
Figure 1

(57) **Abstract:** The present invention provides methods for reducing various lipoprotein fractions in the serum of patients. The methods of the invention include reducing serum remnant cholesterol, and/or the serum concentration of one or more LDL-C subfractions in a patient. The methods of the present invention comprise selecting a patient who exhibits elevated serum lipoproteins, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor. In certain embodiments, the PCSK9 inhibitor is an anti-PCSK9 antibody such as the exemplary antibody referred to herein as mAb316P.

METHODS FOR REDUCING REMNANT CHOLESTEROL AND OTHER LIPOPROTEIN FRACTIONS 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 remnant cholesterol and other lipoprotein subfractions in a patient.

BACKGROUND

[0002]   Remnant cholesterol (also referred to as remnant lipoprotein) is a category of cholesterol that comprises non-HDL and non-LDL cholesterol. Remnant lipoproteins are products of VLDL lipolysis, and include $VLDL_3$ and intermediate-density lipoproteins (IDL, the direct precursor to LDL formation). Serum remnant cholesterol level has been identified as a predictive risk factor for coronary artery disease. In addition to remnant cholesterol, there are several subfractions of low density lipoprotein cholesterol (LDL-C) that may have relevance with regard to cardiovascular health. In particular, LDL-C is composed of a continuum of LDL particles of different densities and states of lipidation. Therapeutic reduction of serum remnant cholesterol and other lipoprotein subfraction levels may be a means for treating or reducing the risk of cardiovascular disorders.

[0003]   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 Nos. 8,062,640, 8,357,371, and US Patent Application Publication No. 2013/0064834.

BRIEF SUMMARY OF THE INVENTION

[0004]   The present invention provides methods for reducing various lipoproteins and lipoprotein fractions in the serum of a patient. The patient can be a patient with hypercholesterolemia or at risk of developing hypercholesterolemia, or who has or is at risk of developing a cardiovascular disease.

[0005]   The methods of the present invention comprise selecting a patient with an elevated serum level of a particular lipoprotein or lipoprotein fraction, and administering to the patient a pharmaceutical composition comprising an inhibitor of proprotein convertase subtilisin kexin-9 (PCSK9). According to certain aspects of the invention, the patient is selected on the basis of having an elevated serum remnant cholesterol level. According to certain other aspects of the invention, the patient is selected on the basis of having an elevated serum very low-density lipoprotein cholesterol level (e.g., elevated serum VLDL-C, $VLDL_1$-C, $VLDL_2$-C, $VLDL_{1+2}$-C, $VLDL_3$-C, etc.). According to yet other aspects of the invention, the patient is selected on the basis of having an elevated serum intermediate-density lipoprotein cholesterol (IDL-C) level.

According to certain other aspects of the invention, the patient is selected on the basis of having an elevated serum level of one or more low-density lipoprotein cholesterol (LDL-C) subfraction (e.g., elevated serum LDL_1-C, LDL_2-C, LDL_3-C, LDL_4-C, LDL_{3+4}-C, etc.). According to certain aspects of the present invention, the patient is additionally or alternatively selected on the basis of having an elevated serum level of Lp(a).

[0006] The patient may also be selected on the basis of exhibiting additional risk factors for such diseases and disorders in which a reduction in lipoprotein 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.

[0007] 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 Nos. 8,062,640, and/or disclosed herein.

[0008] 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.

[0009] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0010] **Figure 1** shows the dynamic relationship between mAb316P, free (unbound) PCSK9, and LDL-C levels following a single dose of mAb316P 150 mg SC in health subjects who were not receiving background statin therapy.

[0011] **Figure 2** shows the mean concentration of free mAb316P in HeFH and non-FH patients receiving mAb316P 50, 100, or 150 mg SC plus atorvastatin or mAb316P 150 mg SC plus diet only. Dotted line indicates quantitation limit for free PCSK9 (0.0312 mg/dL)

[0012] **Figure 3** shows the mean percentage change in LDL-C from baseline versus mean total mAb316P concentration (mg/dL) in patients (HeFH and non-FH combined; n-21 receiving mAb316P 150 mg SC on Study Days 1, 29, and 43. Points indicate sampling times; h, hour; d, day. The hysteresis curve moves in a clockwise direction over time, as indicated by the arrows, demonstrating the temporal relationship between mAb316P concentration and change in LDL-C.

[0013] **Figure 4** shows mean baseline free PCSK9 levels in non-FH patients receiving atorvastatin or diet alone (no statin).

[0014] **Figure 5** shows LDL-C efficacy curves for patients receiving mAb316P 150 mg SC or placebo ± atorvastatin.

[0015] **Figure 6** shows the mean percentage change in LDL-C from baseline over time in hypercholesterolemic patients on concomitant statin therapy following administration of mAb316P 50 mg, 100 mg, or 150 mg Q2W, 200 mg or 300 mg Q4W, or placebo. Delta symbol (Δ) corresponds to LOCF means.

[0016] **Figures 7-9** show the mean (SD) levels of cholesterol in VLDL and serum remnant lipoprotein subfractions and triglycerides before and after treatment for placebo and mAb316P 150 mg Q2W in three different clinical studies (A, B, and C, as summarized in Table 1 herein). **Figure 7** depicts the results for Study A, **Figure 8** depicts the results for Study B, and **Figure 9** depicts the results for Study C.

[0017] **Figures 10-12** show the mean (SD) levels of cholesterol in LDL subfractions before and after treatment for placebo and mAb316P 150 mg Q2W in three different clinical studies (A, B, and C, as summarized in Table 1 herein). **Figure 10** depicts the results for Study A, **Figure 11** depicts the results for Study B, and **Figure 12** depicts the results for Study C.

[0018] **Figure 13, panels A and B,** show the dose response changes in apo CII and apo CIII, respectively, for all doses in Study A (as summarized in Table 1 herein).

DETAILED DESCRIPTION

[0019] 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.

[0020] 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.).

[0021] 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.

Methods for Reducing Remnant Cholesterol and Other Lipoprotein Fractions

[0022] The present invention provides methods for reducing serum remnant cholesterol and other lipoprotein fractions in a patient. The methods of the invention comprise selecting a patient who exhibits an elevated serum level of one or more of remnant cholesterol (also referred to as "remnant lipoprotein cholesterol," "remnant lipoproteins," or RLP-C), very low

density lipoprotein cholesterol (VLDL-C), intermediate density lipoprotein cholesterol (IDL-C), triglycerides (TG), and or Lp(a). In certain embodiments, the methods of the invention comprise selecting a patient who exhibits elevated serum $VLDL_1$, $VLDL_2$, $VLDL_{1+2}$, $VLDL_3$, LDL_1, LDL_2, LDL_3, LDL_4, LDL_{3+4}, and/or other combinations thereof. The methods of the present invention further comprise administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

[0023] As used herein, the expressions "remnant cholesterol," "remnant lipoproteins," "RLP," and the like, means the cholesterol content of triglyceride-rich lipoproteins, composed of VLDLs and IDLs in the fasting state and of these two lipoproteins together with chylomicron remnants in the nonfasting state. Remnant cholesterol can be calculated as: total cholesterol minus HDL-C minus LDL-C (*i.e.*, non-[HDL-C + LDL-C]). Remnant cholesterol includes $VLDL_3$ and IDL.

[0024] Other lipoprotein fractions include, *e.g.*, LDL_1-C ("large buoyant" LDL), LDL_2-C, LDL_3-C and LDL_4-C ("small dense" LDL). The serum levels of these lipoproteins may be ascertained by standard lipoprotein subfractionation techniques such as, *e.g.*, vertical auto profile (VAP) testing, ion mobility, etc.

[0025] In the context of the present invention, "elevated serum lipoprotein" (*e.g.*, elevated serum remnant cholesterol, elevated serum VLDL-C, elevated serum $VLDL_1$-C, elevated serum $VLDL_2$-C, elevated serum $VLDL_{1+2}$-C, elevated serum $VLDL_3$-C, elevated serum IDL-C, elevated serum LDL_1-C, elevated serum LDL_2-C, elevated serum LDL_3-C, elevated serum LDL_4-C, elevated serum LDL_{3+4}-C, etc.) means a serum level of the respective lipoprotein greater than about 8 mg/dL. In certain embodiments, a patient is considered to exhibit an elevated serum lipoprotein if the level of the particular lipoprotein measured in the serum of a patient is greater than about 9 mg/dL, 10 mg/dL, 11 mg/dL, 12 mg/dL, 13 mg/dL, 14 mg/dL, 15 mg/dL, 16 mg/dL, 17 mg/dL, 18 mg/dL, 19 mg/dL, 20 mg/dL, 25 mg/dL, 30 mg/dL, 35 mg/dL, 40 mg/dL, 45 mg/dL, 50 mg/dL. The serum lipoprotein level can be measured in a patient post-prandial. In some embodiments, the lipoprotein 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). Any clinically acceptable diagnostic method can be used in the context of the present invention to measure serum lipoprotein in a patient.

[0026] According to the present invention, a reduction in serum remnant cholesterol or other lipoprotein subfraction (*e.g.*, VLDL-C, $VLDL_1$-C, $VLDL_2$-C, $VLDL_{1+2}$-C, $VLDL_3$-C, IDL-C, IDL_1-C, IDL_2-C, IDL_{1+2}-C, LDL_1-C, LDL_2-C, LDL_{2a}-C, LDL_{2b}-C, LDL_{1+2a}-C, LDL_3-C, LDL_{3a}-C, LDL_{3b}-C, LDL_4-C, LDL_{4a}-C, LDL_{4b}-C, LDL_{4c}-C, $LDL_{4a+4b+4c+3b}$-C, LDL_{3+4}-C, etc.) means that the serum lipoprotein/lipoprotein subfraction level in the patient after receiving a pharmaceutical composition of the invention is reduced by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or more, from baseline. As used herein, "baseline level," when referring to a particular lipoprotein or lipoprotein subfraction, means the level of lipoprotein/lipoprotein subfraction measured in the serum of a subject prior to receiving a pharmaceutical composition of the present invention. The reduction in serum

lipoprotein/lipoprotein subfraction resulting from administration of a pharmaceutical composition of the invention may be achieved and/or observed at 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks , 14 weeks, 16 weeks, 18 weeks, 20 weeks, 22 weeks, or longer, following the initiation of a therapeutic regimen comprising administration of one or more doses of a pharmaceutical composition comprising a PCSK9 inhibitor, examples of which are disclosed elsewhere herein.

[0027]   For example, the present invention includes methods for reducing serum remnant cholesterol in a patient by selecting a patient with elevated serum remnant cholesterol, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum remnant cholesterol is reduced in the patient by at least about 35% to about 45% from baseline following administration of the pharmaceutical composition.

[0028]   The present invention also includes methods for reducing serum VLDL-C in a patient by selecting a patient with elevated serum VLDL-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum VLDL-C is reduced in the patient by at least about 20% to about 28% from baseline following administration of the pharmaceutical composition.

[0029]   The present invention also includes methods for reducing serum $VLDL_{1+2}$-C in a patient by selecting a patient with elevated serum $VLDL_{1+2}$-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum $VLDL_{1+2}$-C is reduced in the patient by at least about 20% to about 32% from baseline following administration of the pharmaceutical composition.

[0030]   The present invention also includes methods for reducing serum $VLDL_3$-C in a patient by selecting a patient with elevated serum $VLDL_3$-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum $VLDL_3$-C is reduced in the patient by at least about 20% to about 27% from baseline following administration of the pharmaceutical composition.

[0031]   The present invention also includes methods for reducing serum IDL-C in a patient by selecting a patient with elevated serum IDL-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum IDL-C is reduced in the patient by at least about 50% to about 56% from baseline following administration of the pharmaceutical composition.

[0032]   The present invention also includes methods for reducing serum LDL_1-C in a patient by selecting a patient with elevated serum LDL_1-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum LDL_1-C is reduced in the patient by at least about 65% to about 78% from baseline following administration of the pharmaceutical composition.

[0033]   The present invention also includes methods for reducing serum LDL_2-C in a patient by selecting a patient with elevated serum LDL_2-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum LDL_2-C is reduced in the patient by at least about 75% to about 85% from baseline following administration of the pharmaceutical composition.

[0034]   The present invention also includes methods for reducing serum LDL_{3+4}-C in a patient by selecting a patient with elevated serum LDL_{3+4}-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum LDL_{3+4}-C is reduced in the patient by at least about 45% to about 70% from baseline following administration of the pharmaceutical composition.

[0035]   The present invention also includes methods for reducing apolipoprotein (apo) CII and/or CIII in a patient by selecting a patient with hypercholesterolemia, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor. According to certain embodiments, the patient has non-familial hypercholesterolemia (non-FH); in certain other embodiments, the patient has heterozygous familial hypercholesterolemia (he-FH). According to certain embodiments of this aspect of the invention, apo CII is reduced in the patient by at least about 9% to about 30% from baseline, and apo CIII is reduced in the patient by at least about 20% to about 25%, following administration of the pharmaceutical composition.

[0036]   The present invention also includes methods for reducing serum Lp(a) in a patient by selecting a patient with elevated serum Lp(a), and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor, wherein serum Lp(a) is reduced in the patient by at least about 10% to about 40% (*e.g.*, about 25%, about 30% or about 35%) from baseline following administration of the pharmaceutical composition.

[0037]   The present invention also includes methods for reducing lipoprotein particle concentration in a patient by selecting a patient with hypercholesterolemia and/or elevated serum lipoprotein particle concentration, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor. The methods according to this aspect of the invention are useful for, *inter alia*, reducing the concentration of low-density lipoprotein particles (LDL-P), intermediate density lipoprotein particles (IDL-P), and very low-density lipoprotein particles (VLDL-P), including methods for reducing the serum concentrations of, *e.g.*, small VLDL-P (diameter 29 to 42 mm); medium VLDL-P (diameter 42 to 60 nm); large VLDL-P (diameter > 60 nM); small LDL-P (diameter 18 to 20.5 nm); large LDL-P (diameter 20.5 to 23 nm); and IDL-P (diameter 23 to 29 nm).

Patient Population

[0038]   The methods of the present invention are useful for reducing serum remnant cholesterol and other lipoprotein fractions (*e.g.*, VLDL-C, $VLDL_1$-C, $VLDL_2$-C, $VLDL_{1+2}$-C, $VLDL_3$-C, IDL-C, LDL_1-C, LDL_2-C, LDL_3-C, LDL_4-C, LDL_{3+4}-C, etc.) in a patient. In some

instances the patient is otherwise healthy except for exhibiting elevated levels of one or more of the aforementioned serum lipoproteins. 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 remnant cholesterol or with elevated serum levels of other lipoproteins or lipoprotein fractions. 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), Autosomal Dominant Hypercholesterolemia (ADH, *e.g.*, ADH associated with one or more gain-of-function mutations in the PCSK9 gene), as well as incidences of hypercholesterolemia that are distinct from Familial Hypercholesterolemia (nonFH).

[0039] 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 an arterial disorder such as cerebrovascular occlusive disease, peripheral vascular disease, peripheral arterial disorder, 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.

[0040] 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 remnant cholesterol and/or other lipoprotein fraction 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.

PCSK9 Inhibitors

[0041]   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.

[0042]   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.

[0043]   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.

[0044]   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.

[0045] 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.

[0046] 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.

[0047] 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)).

[0048] 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.

[0049]   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.

[0050]   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.

[0051]   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*.

[0052]   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.

[0053]   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.

[0054]   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.

[0055]   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.

[0056]   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.

[0057] 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.

[0058] 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).

[0059] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0060] 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.

[0061] According to certain embodiments, the anti-PCSK9 antibody used in the methods of the present invention is an antibody with pH-dependent binding characteristics. As used herein, the expression "pH-dependent binding" means that the antibody or antigen-binding fragment thereof exhibits "reduced binding to PCSK9 at acidic pH as compared to neutral pH" (for purposes of the present disclosure, both expressions may be used interchangeably). For the example, antibodies "with pH-dependent binding characteristics" includes antibodies and antigen-binding fragments thereof that bind PCSK9 with higher affinity at neutral pH than at acidic pH. In certain embodiments, the antibodies and antigen-binding fragments of the present invention bind PCSK9 with at least 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more times higher affinity at neutral pH than at acidic pH.

[0062] According to this aspect of the invention, the anti-PCSK9 antibodies with pH-dependent binding characteristics may possess one or more amino acid variations relative to the parental anti-PCSK9 antibody. For example, an anti-PCSK9 antibody with pH-dependent binding characteristics may contain one or more histidine substitutions or insertions, e.g., in one or more CDRs of a parental anti-PCSK9 antibody. Thus, according to certain embodiments of the present invention, methods are provided comprising administering an anti-PCSK9 antibody which comprises CDR amino acid sequences (e.g., heavy and light chain CDRs) which are identical to the CDR amino acid sequences of a parental anti-PCSK9 antibody, except for the substitution of one or more amino acids of one or more CDRs of the parental antibody with a histidine residue. The anti-PCSK9 antibodies with pH-dependent binding may possess, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or more histidine substitutions, either within a single CDR of a parental antibody or distributed throughout multiple (e.g., 2, 3, 4, 5, or 6) CDRs of a parental anti-PCSK9 antibody. For example, the present invention includes the use of anti-PCSK9 antibodies with pH-dependent binding comprising one or more histidine substitutions in HCDR1, one or more histidine substitutions in HCDR2, one or more histidine substitutions in HCDR3, one or more histidine substitutions in LCDR1, one or more histidine substitutions in LCDR2, and/or one or more histidine substitutions in LCDR3, of a parental anti-PCSK9 antibody.

[0063] As used herein, the expression "acidic pH" means a pH of 6.0 or less (e.g., less than about 6.0, less than about 5.5, less than about 5.0, etc.). The expression "acidic pH" includes pH values of about 6.0, 5.95, 5.90, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3,

5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

Preparation of Human Antibodies

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069]   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.

[0070]   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.

[0071]   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).

[0072]   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

[0073]   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.

[0074]   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.

[0075]   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.

[0076]   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.

[0077]   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.

[0078]   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.

[0079]   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

[0080]   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 (at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or more from baseline) in one or more parameters selected from the group consisting of remnant cholesterol (RLP-C), VLDL-C, $VLDL_1$-C, $VLDL_2$-C, $VLDL_{1+2}$-C, $VLDL_3$-C, IDL-C, LDL_1-C, LDL_2-C, LDL_3-C, LDL_4-C, LDL_{3+4}-C. Alternatively, animal models can be used to establish whether a particular amount of a candidate PCSK9 inhibitor is a therapeutically effective amount.

[0081]   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 75 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.

[0082]   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

[0083]   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

[0084]   According to certain embodiments of the present invention, multiple doses of a PCSK9 inhibitor (*i.e.*, a pharmaceutical composition comprising 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.

[0085]   The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the individual doses of a pharmaceutical composition comprising a 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 the PCSK9 inhibitor, but generally may 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 varies from one another (e.g., adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (e.g., "maintenance doses").

[0086]   According to exemplary embodiments of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (e.g., 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antigen-binding molecule which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0087]   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.

[0088]   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 2, 4, 6, 8 or more weeks 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 2, 4, 6, 8 or more weeks 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.

[0089]   The present invention includes administration regimens comprising an up-titration option (also referred to herein as "dose modification"). As used herein, an "up-titration option" means that, after receiving a particular number of doses of a PCSK9 inhibitor, if a patient has

not achieved a specified reduction in one or more defined therapeutic parameters, the dose of the PCSK9 inhibitor is thereafter increased. For example, in the case of a therapeutic regimen comprising administration of 75 mg doses of an anti-PCSK9 antibody to a patient at a frequency of once every two weeks, if after 8 weeks (i.e., 5 doses administered at Week 0, Week 2 and Week 4, Week 6 and Week 8), the patient has not achieved a serum LDL-C concentration of less than 70 mg/dL, then the dose of anti-PCSK9 antibody is increased to e.g., 150 mg administered once every two weeks thereafter (e.g., starting at Week 10 or Week 12, or later).

EXAMPLES

[0090] 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

[0091] Human anti-PCSK9 antibodies were generated as described in US Patent No. 8,062,640. The exemplary PCSK9 inhibitor used in the following Example is the human anti-PCSK9 antibody designated "mAb316P" (also referred to in the scientific literature as "alirocumab"). 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: Dynamics Between Anti-PCSK9 Antibody Levels and Low-Density Lipoprotein Cholesterol (LDL-C) Levels

Introduction

[0092] Descriptive analyses of pharmacokinetic and pharmacodynamic data from Phase I and II studies of mAb316P were conducted to characterize relationships between mAb316P, PCSK9 and LDL-C levels. Three different clinical trials were included in this analysis:

[0093] (1) Single-dose Study: Phase I, single-dose study in healthy subjects not receiving background statin therapy in which subjects received single subcutaneous (SC) doses of mAb316P 50, 100 ,150, and 250 mg, or placebo.

[0094]   (2) <u>Multiple-dose Study</u>: A Phase I study in cohorts of heterozygous familial hypercholesterolemia (HeFH) and non-familial hypercholesterolemia (FH) patients receiving atorvastatin therapy and non-FH patients receiving diet alone. Patients receiving atorvastatin were randomized to mAb316P (50, 100, or 150 mg) or placebo administered SC on Days 1, 29, and 43. Non-FH patients receiving diet alone were randomized to mAb316P 150 mg or placebo administered SC on Days 1 and 29.

[0095]   (3) <u>Phase II Study</u>: A 12-week study in hypercholesterolemic patients receiving atorvastatin therapy: patients randomized to mAb316P 50, 100, or 150 mg SC every 2 weeks (Q2W), 200 or 300 mg SC every 4 weeks (Q4W), or placebo.

Results

[0096]   Single administration of mAb316P 150 mg SC rapidly bound to and rapidly reduced circulating free PCSK9 levels; this was followed by a drop in LDL-C. The return of free PCSK9 towards starting levels was noted to precede the return of LDL-C levels (Figure 1). Free PCSK9 levels reached nadir by Day 3 following administration of mAb316P 50, 100, or 150 mg SC in HeFH or non-FH patients (Figure 2). The return of free PCSK9 to starting levels was slower in the patients who received mAb316P plus diet alone versus patients who received mAb316P plus atorvastatin (Figure 2). The greatest mean LDL-C reductions were recorded on Day 8 for the 50 mg and 100 mg doses and Day 15 for the 150 mg dose; this delay relative to free PCSK9 concentrations may possibly be due to the time required for the production of new LDL-C receptors. A representative hysteresis curve showing the relationship between total mAb316P and LDL-C following administration of the 150 mg dose is shown in Figure 3.

[0097]   Higher baseline free PCSK9 levels were observed in patients taking statins as compared with diet alone (Figure 4). There was increased target-mediated clearance of mAb316P with concurrent statin therapy (due to higher free PCSK9 levels, likely reflective of an increase in production rate) compared with mAb316P and diet therapy alone. This appeared to affect the duration of LDL-C lowering over a 4-week dosing interval (Days 1 to 29), which was reduced with concurrent statin therapy versus diet only; however, this effect was not seen over the 2-week dosing interval (Days 29 to 43) (Figure 5).

[0098]   In hypercholesterolemic (non-FH) patients receiving concomitant statin therapy, mAb316P Q2W resulted in uniformly strong LDL-C lowering at 12 weeks. Patients on Q2W dosing regimens exhibited lower variability in the LDL-C response at Week 12 versus Q4W dosing (Figure 6); coefficient of variation 16% versus 49% for 150 mg Q2W and 300 mg Q4W, respectively.

Conclusions

[0099]   Dynamics were apparent between mAb316P, free PCSK9 and LDL-C levels. mAb316P treatment resulted in reductions in free PCSK9 levels within 3 days of dosing and peak reductions in LDL-C 8–15 days after dosing.

[0100] The clearance of mAb316P was accelerated by its binding to free PCSK9 (target-mediated clearance). Lower levels of free PCSK9 resulted in a longer duration of efficacy through lower target-mediated clearance. Previous studies have shown that administration of statins increases the production of free PCSK9. In the studies analyzed herein, patients who received mAb316P plus diet alone had lower baseline free PCSK9 levels and higher mAb316P blood concentrations compared with patients who received mAb316P plus atorvastatin therapy.

[0101] In patients receiving concomitant statin therapy, mAb316P administered Q2W achieved lower variation in LDL-C lowering at the end of the dosing interval as compared with Q4W dosing regimens. In Q4W dosing, patient-to-patient differences in target-mediated clearance (among other factors) appeared to contribute to differences in the duration of maximum LDL-C between Weeks 2 and 4 post dose and therefore wider differences in achieved efficacy 4 weeks after dosing. These differences were not observed when doses were administered Q2W.

Example 3A: An Anti-PCSK9 Antibody Reduces Cholesterol Concentrations of Serum Remnant Lipoprotein Fractions, Very Low Density Lipoproteins, Triglycerides, and Lipoprotein(a) [Lp(a)]

Introduction

[0102] Increased very low-density lipoproteins (VLDL) levels form part of a pattern of atherogenic dyslipidemia, which predisposes to premature atherosclerosis. Remnant lipoproteins are products of VLDL lipolysis, and include $VLDL_3$ and intermediate-density lipoproteins (IDL, the direct precursor to LDL formation).

[0103] Lipoprotein(a) [Lp(a)] consists of apolipoprotein(a) [apo(a)] covalently bound to the apo B component of a cholesterol-rich lipoprotein which is approximately the same size as a low-density lipoprotein (LDL) particle. Lp(a) metabolism is under mostly genetic regulation, in that more than 90% of its plasma concentration is influenced by quantitative and qualitative polymorphisms at the apo(a) gene (*LPA*). Elevated Lp(a) levels are considered to be an independent risk factor for cardiovascular disease, with risk continuing to increase as levels rise. Although elevated Lp(a) may promote atherosclerosis through intimal deposition of cholesterol, it may also promote thrombosis due to a high homology with plasminogen, although it lacks protease activity. In patients undergoing coronary angiography, Lp(a) concentrations >30 mg/dL have been shown to be associated with an increased risk of angiographic stenosis and major coronary events. Furthermore, Lp(a) levels and genotype have been shown to be associated with aortic-valve calcification, suggesting it may play a role in causation.

[0104] The objective of this Example was to test the hypothesis that mAb316P, a fully human monoclonal antibody to proprotein convertase subtilisin/kexin type 9 (PCSK9), reduces serum levels of VLDL and lipoprotein remnants, contributing to its ability to reduce serum LDL-C and non-HDL-C, and to confirm the Lp(a) lowering activity of mAb316P in patients.

[0105]   Three multicenter, double-blind, parallel-group, placebo-controlled trials were conducted in patients with primary hypercholesterolemia (Study A, n=183; Study B, n=92) or heterozygous familial hypercholesterolemia (Study C, n=77). A summary of the designs and dosing for the three trials is shown in Table 1.

Table 1. Summary of Designs and Dosing for mAb316P Clinical Trials

Study	A	B	C
Duration	12 weeks	8 weeks	12 weeks
Patients	hypercholesterolemia (n = 183)	hypercholesterolemia (n = 92)	heterozygous familial hypercholesterolemeia (n = 77)
mAb316P Doses	200-300 mg Q4W 50-100 mg Q2W 150 mg Q2W (n=31)	150 mg Q2W + ATV 10 to 80 mg 150 mg Q2W + ATV 10 to 80 mg (total n=61)	150, 200, 300 mg Q4W 150 mg Q2W (n=16)
Placebo	n = 31	Placebo + ATV 10 to 80 mg (n = 31)	n = 15
Pooled Analysis Population	mAb316P 150 mg Q2W (n = 108)		Placebo (n = 77)

[0106]   In Study B, all patients received atorvastatin (ATV) 10 mg prior to randomization, which was uptitrated to ATV 80 mg at the start of randomized treatment in the placebo and one mAb316P arm.

[0107]   Patients on background atorvastatin or statins+/-ezetimibe received mAb316P 50-300 mg administered subcutaneously (SC) either every 2 or 4 weeks (Q2W, Q4W), depending on the study. The mAb316P 150 mg Q2W dose was common to all three trials.

[0108]   In *post hoc* analyses, lipoproteins were subfractionated by vertical auto profile (VAP) testing. The VAP method is a single, direct ultracentrifugation test that separates the lipoprotein fractions according to their densities in a vertical rotor, which allows high resolution of each lipoprotein class and subclass. The bottom of the tube is punctured and the cholesterol in each layer is measured with a spectrophotometer after the addition of an enzymatic cholesterol reagent. Percent changes in VLDL-C, $VLDL_{1+2}$-C (a measure of cholesterol in "large, buoyant" VLDL particles), triglycerides (TG), $VLDL_3$-C, IDL-C and total remnant lipoprotein cholesterol levels (RLP-C; $VLDL_3$-C + IDL-C) in patients treated with mAb316P 150 mg Q2W vs. placebo were analyzed at week 12 (Study A), week 8 (Study B) and week 6 (Study C) using ANCOVA.

[0109]   In addition, baseline and on-treatment Lp(a) levels were assessed in patients from the three different Phase 2 studies receiving mAb316P 150 mg Q2W or placebo. In all Phase 2 studies Lp(a) levels were measured at the same laboratory and using the same method. Data on Lp(a) levels at baseline and end of treatment (Week 8/12 on-treatment value or the last

available on-treatment value carried forward) from the modified intention-to-treat populations of the three studies were pooled, and percentage changes from baseline for mAb316P 150 mg Q2W and placebo were compared using analysis of covariance with treatment group and study as fixed effects and baseline Lp(a) as covariate. *P*-values associated with these exploratory analyses are provided for descriptive purposes only and were not adjusted for multiplicity. The relationship between the percentage changes from baseline in Lp(a) and LDL-C was assessed using linear regression, and the Spearman's correlation coefficient was calculated.

Results/Conclusions

[0110]   In the 3 studies, reductions in TG, VLDL-C, and in the cholesterol content of remnant lipoprotein were observed with mAb316P vs. placebo. Results are summarized in Table 2 and Figures 7-9.

Table 2

	Baseline (mg/dL)	Endpoint (mg/dL)	Percent Change	p-value(s)
VLDL	23.24 to 26.31	16.62 to 18.12	-22.33 to -27.95	0.0023 to <0.0001
VLDL$_{1+2}$-C	9.79 to 10.59	6.59 to 7.31	-21.87 to -31.45	0.0178 to <0.0001
VLDL$_3$	13.55 to 15.62	10.03 to 10.88	-21.9 to -26.66	0.0011 to <0.0001
TG	135.72 to 157.19	99.9 to 124.44	-13.07 to -21.19	0.6945 to 0.0003
IDL	15.38 to 22.06	6.79 to 8.62	-50.28 to -55.75	<0.0001
RLPC	29.34 to 37.69	16.86 to 19.5	-37.05 to -44.04	<0.0001

[0111]   The entire spectrum of atherogenic lipoproteins (LDL-C, IDL-C, VLDL-C and RLP subfractions) was reduced by mAb316P, against a background of lipid lowering therapy (statins ± ezetimibe), in heFH/non-FH patients. This Example therefore illustrates that mAb316P significantly reduced serum TG and VLDL-C, in addition to significant reductions in cholesterol concentrations of remnant lipoprotein fractions separable by VAP, including VLDL$_3$-C and IDL-C.

[0112]   Baseline and on-treatment Lp(a) data were available for 102 of the 108 patients who received mAb316P 150 mg Q2W and 74 of the 77 patients who received placebo in the Pooled Analysis Population. Baseline values are shown in Table 3.

Table 3. Baseline Lp(a) Levels in Patients Included in the Pooled Analysis

Lp(a) values in mg/dL		
	Placebo (n=74)	**mAb316P 150 mg Q2W (n=102)**
Overall Population, median (IQR)	19.0 (6.0-77.0)	29.5 (8.0-70.0)
Range	1.5-299.0	1.5-181.0
Number of Patients Subdivided by Baseline Lp(a)		
	Placebo (n=74)	**mAb316P 150 mg Q2W (n=102)**
≤ 50 mg/dL, n (%)	49 (66.2)	68 (66.7)
> 50 mg/dL, n (%)	25 (33.8)	36 (35.3)

*Patients from the mITT population with Lp(a) data available at baseline and end of treatment (Week 8/12 on-treatment value or the last available on-treatment value carried forward).

[0113]   As shown in Table 3, 36 (35%) patients treated with mAb316P and 25 (33%) patients treated with placebo had baseline Lp(a) >50 mg/dL, considered as the high-risk cut-point by the EAS guidelines.

[0114]   Absolute and percentage median reductions from baseline in Lp(a) are summarized in Table 4.

Table 4. Lp(a) Change From Baseline

All Patients		Patients Subdivided by Baseline Lp(a)			
		≤ 50 mg/dL		> 50 mg/dL	
pooled placebo treated (n=74)	pooled mAb316P treated (n=102)	pooled placebo treated (n=49)	pooled mAb316P treated (n=68)	pooled placebo treated (n=25)	pooled mAb316P treated (n=34)
Median Lp(a) Change From Baseline, mg/dL (IQR; LOCF)					
-0.5 (-5.0– 2.0)	-9.0 (-19.0– -2.0)*	0.0 (-3.0– 1.5)	-3.5 (-11.5– -1.5)*	-5.0 (-11.0– 6.0)	-26.5 (-39.0– -16.0)*
Median Lp(a) Percent Change From Baseline, (IQR; LOCF)					
-0.3% (-16.7– 11.5)	-30.3% (-50.0– -19.4)*	0.0% (-16.7– 16.7)	-36.1% (-51.1– -17.4)*	-4.4% (-9.4– 7.1)	-27.0% (-32.6– -19.6)*

* *P*<0.0001 vs placebo.

[0115]   As shown in Table 4, the median percentage reduction from baseline in Lp(a) was – 30.3% with mAb316P 150 mg Q2W versus –0.3% with placebo (*P*<0.0001). The absolute median reductions in Lp(a) from baseline were substantially greater in patients with the higher baseline Lp(a). In summary, an analysis of data pooled from three Phase 2 trials conducted with mAb316P 150 mg Q2W demonstrated significant reductions in Lp(a) versus placebo, including in patients with baseline Lp(a) >50 mg/dL. In those patients considered at higher cardiovascular disease risk due to elevated Lp(a), the percentage reductions in Lp(a) appeared to be of similar magnitude resulting in greater absolute reductions in Lp(a).

Example 3B: An Anti-PCSK9 Antibody Reduces Apolipoprotein CII and CIII Levels in Serum

Introduction

[0116]   Apoprotein (apo) CIII inhibits lipoprotein lipase (LPL)-mediated catabolism of VLDL triglycerides. Apo CII appears to have a more complex relationship with VLDL and LPL activity that may depend on baseline triglyceride levels. Apo CII is, in general, an important activator of LPL

[0117]   LPL hydrolyzes the triglyceride mass of VLDL and its remnants. In this Example, the ability of an anti-PCSK9 antibody, mAb316P, to reduce VLDL-C and remnants by impacting serum levels of apoproteins CII and CIII was investigated.

[0118]   Three multicenter, double-blind, parallel-group, placebo-controlled trials were conducted in patients with primary (non-familial) hypercholesterolemia (non-FH) (Study A, n=183; Study B, n=92) or heterozygous familial hypercholesterolemia (heFH) (Study C, n=77). Patients (who were receiving atorvastatin or statins+/-ezetimibe) were treated with mAb316P 50–150 mg every 2 wks (Q2W) or 150–300mg every 4 wks (Q4W) depending on the study. The mAb316P 150 mg Q2W dose was common to all three trials. (*See* Example 3A, Table 1).

[0119]   In *post hoc* analyses, apo CII and CIII concentrations were measured via immunoassay. (using reagent kits from Randox Laboratories Limited, UK [apo CII, Cat. No. LP3866; apo CIII, Cat. No. LP3865] and an Architect Ci8200 analyzer [Abbott Laboratories, IL]). The immunoassay methods were based on the reaction of a sample containing human apo CII (or apo CIII) and specific antiserum to apo CII (or CIII) to form an insoluble complex whose concentration can be measured turbidimetrically at 340 nm. Both assays were validated for analytical performance.

[0120]   Percent change from baseline in apo CII and apo CIII in the placebo group was compared with patients treated with mAb316P, analyzed at week 12 (Study A), week 8 (Study B) and week 6 (Study C) using analysis of covariance. Study C had a Week 12 endpoint; however, 6-week data were used due to reduced number of patients with available stored samples at 12 weeks (n=17) compared with 6 weeks (n=75).

Results

[0121]   Percent change in apo CII and CIII from baseline by treatment group for mAb316P 150 mg Q2W in Studies A, B and C are shown in Table 5. Values shown are mean (SD), except for apo CIII, where values are median (IQR). Data are from Week 12 (study A), Week 8 (study B) and Week 6 (study C). Figure 13 shows the dose response changes in apo CII (panel A) and CIII (panel B) for study A.

Table 5

Study A	Apo CII % change	P-value versus placebo	Apo CIII % change	P-value versus placebo
Placebo	12.0 (33.0)		3.76 (-5.5 to 27.6)	
mAb316P 150mg Q2W	-21.4 (26.5)	<0.00001	-24.7 (-28.4 to -2.9)	<0.00001
Study B				
Placebo	-10.6 (30.2)		-7.0 (29.8 to 7.3)	
mAb316P 150mg Q2W +	-10.0 (22.7)	0.98	-4.9	0.4426

atorvastatin 10 mg			(-24.4 to 1.0)	
mAb316P 150mg Q2W + atorvastatin 80 mg	-29.3 (19.29)	0.0067	-22.9 (-28 to -14.0)	0.0165
Study C				
Placebo	1.6 (15.5)		-3.3 (-15.3 to 5.6)	
mAb316P 150mg Q2W	-9.4 (28.1)	0.0794	-20.2 (-29.0 to 2.5)	0.019

[0122]   mAb316P reduced apo CII and CIII at all doses, but a clear dose response was observed (Table 5, Figure 13). In all three studies, mAb316P reduced apo CII in patients from baseline (Table 5). The mean reductions from baseline at Week 12 (study A), Week 8 (study B) and Week 6 (study C) were 21.4% (P<0.00001), 29.3% (P=0.0067) and 9.4% (P=0.08), respectively (P-values compared with placebo). Apo CIII was reduced by 24.7% (P<0.00001), 22.9% (P=0.017) and 20.2% (P=0.019), for studies A, B and C, respectively (P-values compared with placebo).

Conclusions

[0123]   Therapy with anti-PCSK9 antibody mAb316P was associated with significant reductions in serum apo CII and CIII. There was a nearly 1:1 reduction in both apo CII and CIII in patients with non-FH. In patients with heFH, apo CIII decreased approximately two-fold more than apo CII. The reductions in apo CII and CIII may be a manifestation of either increased clearance or reduced production/secretion of VLDL particles, known carriers of these apoproteins.

Example 4: An Anti-PCSK9 Antibody Reduces Cholesterol Concentrations of All Serum Low-Density Lipoprotein Fractions

Introduction

[0124]   Low-density lipoprotein cholesterol (LDL-C) is composed of a continuum of LDL particles of different densities and states of lipidation. Analysis of effects of lipid-modifying therapies on LDL particle composition may aid understanding of treatment mode-of-action. The purpose of this Example was to test the hypothesis that mAb316P, a fully human monoclonal antibody to proprotein convertase subtilisin/kexin 9 (PCSK9), reduces LDL-C by decreasing multiple LDL fractions.

[0125]   Three multicenter, double-blind, parallel-group, placebo-controlled trials were conducted in patients with primary hypercholesterolemia (Study A, n=183; Study B, n=92) or heterozygous familial hypercholesterolemia (Study C, n=77). Patients (who were receiving atorvastatin or statins+/-ezetimibe) were treated with mAb316P 50–150 mg every 2 wks (Q2W) or 150–300mg every 4 wks (Q4W) depending on the study. The mAb316P 150 mg Q2W dose was common to all three trials. (See Example 3A, Table 1).

[0126]   In *post hoc* analyses, lipoproteins were subfractionated by vertical auto profile (VAP) testing. Percent change in LDL_1-C ("large buoyant" LDL), LDL_2-C, LDL_3-C, and LDL_4-C ("small, dense" LDL) levels in patients receiving mAb316P 150 mg Q2W vs. placebo from baseline to week 12 for Study A, week 8 for Study B, and week 6 for Study C were assessed using ANCOVA. The LDL_3-C and LDL_4-C fractions were analyzed individually and in a pooled analysis. This was done because LDL_4-C typically exists at substantially lower concentrations than LDL_{3-4}-C. There was much more substantial variation in percent reductions in LDL_4-C. The sum of LDL_{3+4}-C represents a sum of the two smallest and densest LDL-C fractions.

Results/Conclusions

[0127]   Significant reductions from baseline in cholesterol content of all LDL subfractions separable by VAP were observed in patients receiving mAb316P 150mg Q2W in the 3 studies, vs. placebo. Results are summarized in Table 6 and Figures 10-12.

Table 6

	Baseline(mg/dL)	Endpoint (mg/dL)	Percent Change	p-value
LDL_1	16.18 to 25.92	3.56 to 6.81	-68.87 to -77.84	<0.0001
LDL_2	20.29 to 31.46	3.04 to 7.84	-77.85 to -84.17	<0.0001
LDL_{34}	46.33 to 49.16	14.9 to 22.29	-48.77 to -68.49	<0.0001

[0128]   This Example therefore illustrates that therapy with a monoclonal antibody directed against PCSK9 (mAb316P) significantly reduced cholesterol concentrations in LDL fractions separable by VAP. The previously demonstrated LDL-C reductions with mAb316P were confirmed across the spectrum of LDL-C sub-fractions.

Example 5: Effects of an Anti-PCSK9 Antibody on Lipoprotein Particle Concentrations

Background

[0129]   The cholesterol content of low-density lipoprotein particles (LDL-P) and high-density lipoprotein particles (HDL-P) can vary greatly among individuals. For example, one individual may have larger, cholesterol-rich LDL-P, and another will have smaller, cholesterol-poor LDL-P. Therefore measurements of serum levels of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) often do not correlate with circulating LDL-P and HDL-P number.

[0130]   Epidemiological studies have demonstrated that atherosclerotic cardiovascular disease risk tracks with both LDL-C and LDL-P. However, in patient populations where these measures are discordant (e.g., patients with diabetes or metabolic syndrome), risk has been shown to track more consistently with LDL-P than LDL-C. Consequently, multiple expert panels and consensus statements have advocated the adjunctive use of LDL-P as a target of therapy in the management of high-risk patients.

[0131]   Nuclear magnetic resonance (NMR) technology can accurately measure the number of circulating lipoprotein particles to identify patients with discordantly high LDL-P levels that require intensification of therapy.

[0132]   The anti-PCSK9 antibody mAb316P, has been shown to reduce LDL-C by up to 72.4% in patients on stable doses of atorvastatin over 12 weeks with similar rates of adverse events between placebo and mAb316P-treatment groups. mAb316P reduced apolipoprotein B by up to 56% proportionally with the changes in LDL-C. The effect of mAb316P on lipoprotein particle numbers in hypercholesterolemic patients has heretofore not been reported.

[0133]   The present Example sets forth an evaluation of the effects of mAb316P on the concentration and size of lipoprotein particles.

Methods

[0134]   A sub-study of a randomized double-blind Phase II trial was carried out with respect to patients with LDL-C ≥100mg/dL who received placebo (n=31) or mAb316P 150 mg administered subcutaneously (SC) every 2 weeks (Q2W) (n=28) on top of stable daily atorvastatin (10, 20 or 40 mg daily). Lipid and lipoprotein tests were performed using samples collected after a 12-hour overnight fast. Lipoprotein particle profiles were measured by NMR spectroscopy using the LipoProfile-3 algorithm at LipoScience, Inc. (Raleigh, NC). LDL-P and HDL-P subclasses were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals, and weighted-average LDL and HDL sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal.

[0135]   Diameter range estimates for the subclasses were as follows:

[0136]   VLDL-P: small VLDL-P = 29 to 42 nm; medium VLDL-P = 42 to 60 nm; large VLDL-P >60 nm;

[0137]   LDL-P: small LDL-P = 18 to 20.5 nm; large LDL-P = 20.5 to 23 nm; intermediate-density lipoprotein particles (IDL-P) = 23 to 29 nm;

[0138]   HDL-P: small HDL-P = 7.3 to 8.2 nm; medium HDL-P = 8.2 to 9.4 nm; and large HDL-P = 9.4 to 14 nm.

[0139]   Total VLDL-P, LDL-P, and HDL-P equal the sum of the particle number concentrations of the VLDL, LDL, and HDL subclasses, respectively. Endpoints for the analysis were percentage change in the concentration of LDL-P, VLDL-P and HDL-P from baseline to Week 12.

[0140]   Any variables that were not normally distributed were transformed for all statistical tests, but the untransformed variables are presented in the tables herein. Mean (standard deviation [SD]) are reported for continuous normally distributed variables, while median (interquartile range [IQR]) are reported for any non-normally distributed variables. Count and percentage are reported for categorical variables. To determine if the treatment group was

significantly different than placebo, Fisher's exact tests were performed and the associated *P*-values are reported. To determine if each of the treatment groups were significantly different than placebo, *t*-tests were performed and the associated *P*-values are reported. To determine if there was a significant difference in percent change between baseline and Week 12 for the placebo compared to the treatment groups analysis of covariances were performed with the baseline value as a covariate.

Results

[0141]   Table 7 shows lipoprotein particle concentrations before and after mAb316P 150mg SC Q2W compared to placebo.

Table 7. Lipoprotein particle concentrations (nmol/L) for placebo (n=31) and mAb316P 150mg Q2W (n=28). *P<0.05; †P<0.0001 vs. placebo

Mean (sd) or median (Q1:Q3) [proportion of total]		**PLACEBO**		
		Baseline	**Week 12**	**% change**
LDL	Total LDL	1422.5 (321.3)	1383.8 (327.9)	-1.0%
	Intermediate density lipoprotein	110 (51:166.5)	57 (24.5:144.5)	-15.0%
	Large LDL	546.6 (205.3)	431.8 (217.4)	-21.8%
	Small LDL	755.3 (304.9)	847.6 (375.1)	17.8%
VLDL +chylomicron	Total VLDL +chylomicron	61.9 (47.8:95.6)	83.9 (45:102.2)	33.4%
	Large VLDL +chylomicron	3.5 (2.1:8.5)	4.3 (1.8:9.4)	14.3%
	Medium VLDL	19.3 (13.1:33.9)	33.1 (13.1:51.9)	24.0%
	Small VLDL	35.3 (28.2:47.8)	37.3 (23.4:56.3)	21.4%
HDL	Total HDL	32.9 (6.4)	33.2 (7.4)	1.4%
	Large HDL	3.8 (2.0)	3.9 (2.2)	6.99%
	Medium HDL	9.2 (5.9:14.4)	8 (5.4:10.2)	-13.9%
	Small HDL	18.8 (5.3)	21.3 (5.8)	18.4%
		mAb316P 150 mg Q2W		
		Baseline	**Week 12**	**% change**
LDL	Total LDL	1320 (304.0)	475.4 (167.3)†	-63.3%†
	Intermediate density lipoprotein	84.5 (33:115)	37 (12:66)*	-52.8%

	Large LDL	532.2 (212.9)	152.4 (107.6)†	-71.3%
	Small LDL	666.5 (333.8)	279.9 (191.0)†	-54.0%
VLDL +chylomicron	Total VLDL +chylomicron	71.5 (36.9:94.1)	42.0 (30.5:54.1)†	-36.4%†
	Large VLDL +chylomicron	3.6 (1.8:8.5)	3.1 (1.7:6.9)	5.37%
	Medium VLDL	18.3 (10.7:50)	14.4 (8.2:26.9)†	-38.92%
	Small VLDL	35.7 (25:49.5)	21.4 (19.9:26.7)*	-33.4%
HDL	Total HDL	32.6 (6.3)	36.1 (6.5)*	11.2%*
	Large HDL	4.8 (3.1)	6.1 (3.5)*	44.6%
	Medium HDL	7.8 (5.6:10.7)	9.8 (6.6:11.3)	17.65%
	Small HDL	19.4 (4.1)	20.0 (5.7)*	2.8%

[0142]   mAb316P reduced mean LDL-P by 63% vs 1% for placebo (P<0.0001) and median VLDL-P by 36% vs an increase of 33% for placebo (P<0.0001). HDL-P levels increased 11% for mAb316P vs 1% for placebo (P<0.05). Changes in all particle subclasses were directionally similar.

Conclusion

[0143]   mAb316P significantly reduced LDL-P and other lipoprotein particles in a manner similar to its previously reported effect on LDL-C and apolipoprotein B.

Example 6: Effects of an Anti-PCSK9 Antibody on Lipoprotein Subfractions As Determined by Ion Mobility

Background

[0144]   mAb316P (also known as Alirocumab) has demonstrated significant reductions in low-density lipoprotein cholesterol (LDL-C) in Phase 2 clinical trials. LDL-C tracks closely with cardiovascular disease (CVD) risk and subfractions of LDL, defined by differences in particle size and density, have also been associated with varying degrees of CVD risk. Analysis of lipoprotein subfractions may provide further insight into CVD risk evaluation and the effects of lipid-lowering treatments seen in individual patients.

Methods

[0145]   A Phase 2 randomized, double-blind, clinical trial was conducted in patients with hypercholesterolemia and LDL-C levels ≥100mg/dL, in which 31 patients received placebo and 27 patients received alirocumab 150 mg every 2 weeks (Q2W) via a 1-mL subcutaneous auto-

injection in addition to stable atorvastatin (10-40 mg daily). In the present sub-study, lipoprotein subfractions were determined at baseline and at Week 12 using ion mobility.

Results

[0146] Changes in lipoprotein subfractions and lipid parameters from baseline to Week 12 are set forth in Tables 8A (placebo-treated) and 8B (mAb316P-treated).

Table 8A: Placebo

Lipoprotein subfractions			
	Baseline	**Week 12**	**Percent Change**
VLDL + IDL + Total LDL	1648.44 (453.5)	1728.88 (434.58)	8.25
VLDL (large + intermediate + small)	134.97 (109.01 to 152.85)	140.48 (106.18 to 193.49)	6.64
IDL (IDL 1+2)	314.28 (282.74 to 407.95)	303.35 (277.99 to 376.17)	-3.3
Total LDL	1172.99 (362.85)	1233.76 (331.13)	8.8
Large LDL (LDL 1+2a)	448.23 (173.52)	419.36 (160.98)	-0.26
Medium LDL (LDL 2b)	233.2 (105.34)	228.92 (88.12)	5.07
Small LDL (LDL 3a)	195.17 (85.53)	225.98 (105.1)	24.39
Very Small LDL (LDL 4a+4b+4c+ 3b)	296.4 (91.75)	359.49 (136.92)	24.61
HDL (HDL 2b; HDL3+2a)	23532.48 (5392.57)	24400.97 (5798.21)	0.06
Lipid parameters			
	Baseline	**Week 12**	**Percent Change**
LDL-C, mg/dL	130.2 (27.3)	120.5 (27.0)	-5.1
HDL-C, mg/dL	49.0 (10.3)	48.9 (13.2)	-1.0
TG, mg/dL	124.0 (92.0 to 187.5)	127.0 (98.0 to 197.0)	9.7
Apo-B, mg/dL	108.3 (19.3)	109.2 (27.0)	2.2
Apo-A1, g/L	1.4 (1.3 to 1.6)	1.4 (1.3 to 1.7)	0.0
Lp(a), g/L	0.2 (0.1 to 0.9)	0.2 (0.07 to 0.85)	0.0

Table 8B: mAb316P 150 mg Q2W

Lipoprotein subfractions			
	Baseline	**Week 12**	**Percent Change (p-value)**
VLDL + IDL + Total LDL	1542.82 (435.5)	767.78 (212.39)	-48.68 (<0.0001)

VLDL (large + intermediate + small)	138.17 (96.41 to 167.8)	77.2 (51.81 to 95.8)	-51.37 (<0.0001)
IDL (IDL 1+2)	309.64 (268.32 to 363.95)	155.19 (134.05 to 194.5)	-52.29 (<0.0001)
Total LDL	1078.28 (347.61)	529.35 (171)	-49.84 (<0.0001)
Large LDL (LDL 1+2a)	421.08 (130.03)	152.66 (53.4)	-62.66 (<0.0001)
Medium LDL (LDL 2b)	200.86 (91.4)	78.67 (40.76)	-58.69 (<0.0001)
Small LDL (LDL 3a)	172.02 (104.89)	79.24 (38.36)	-48.56 (<0.0001)
Very Small LDL (LDL 4a+4b+4c+ 3b)	284.31 (101.87)	218.77 (55.66)	-17.91 (<0.0001)
HDL (HDL 2b; HDL3+2a)	23480.25 (5281.63)	21888.87 (4515.15)	-0.05 (0.0339)

Lipid parameters

	Baseline	Week 12	Percent Change (p-value)
LDL-C, mg/dL	123.9 (26.7)	34.2 (15.6)	-72.4 (<0.0001)*
HDL-C, mg/dL	53.3 (16.1)	55.1 (14.8)	5.5 (0.570)‡
TG, mg/dL	140.5 (92.5 to 177.5)	99.0 (79.0 to 139.0)	-18.9 (0.0006)‡
Apo-B, mg/dL	101.6 (26.6)	44.1 (14.1)	-56.1 (<0.0001)‡
Apo-A1, g/L	1.5 (1.3 to 1.7)	1.6 (1.4 to 1.7)	1.4 (0.1524)‡
Lp(a), g/L	0.3 (0.1 to 0.6)	0.1 (0.05 to 0.41)	-28.6 (<0.0001)‡

*Statistically significant p-value according to the hierarchical procedure. ‡P-values are not adjusted for multiplicity and are for descriptive purposes only.

[0147]   Tables 8A and 8B illustrate changes in lipoprotein subfractions and lipid parameters in placebo and mAb316P 150 mg Q2W groups, respectively, from baseline to Week 12. Compared with placebo, mAb316P reduced very low-density lipoprotein (VLDL) + intermediate-density lipoprotein (IDL) + total LDL by 48.7% (p<0.0001), VLDL by 51.4% (p<0.0001), IDL by 52.3% (p<0.0001) and total LDL by 49.8% (p<0.0001); changes in other subfractions were directionally similar. No significant change was noted for HDL. There was a highly significant difference in percent change reduction in very small LDL versus the other LDL fractions (p<0.0001).

Conclusions

[0148] As determined by ion mobility, mAb316P significantly reduced LDL subfractions when added to stable atorvastatin therapy, similar to its previously reported effect on LDL-C.

[0149] 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 serum remnant cholesterol in a patient, the method comprising selecting a patient with elevated serum remnant cholesterol, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

2.   The method of claim 1, wherein serum remnant cholesterol is reduced in the patient by at least about 35% to about 45% from baseline following administration of the pharmaceutical composition.

3.   A method for reducing serum VLDL in a patient, the method comprising selecting a patient with elevated serum VLDL, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

4.   The method of claim 3, wherein serum VLDL is reduced in the patient by at least about 20% to about 28% from baseline following administration of the pharmaceutical composition.

5.   A method for reducing serum $VLDL_{1+2}$-C in a patient, the method comprising selecting a patient with elevated serum $VLDL_{1+2}$-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

6.   The method of claim 5, wherein serum $VLDL_{1+2}$-C is reduced in the patient by at least about 20% to about 32% from baseline following administration of the pharmaceutical composition.

7.   A method for reducing serum $VLDL_3$-C in a patient, the method comprising selecting a patient with elevated serum $VLDL_3$-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

8.   The method of claim 7, wherein serum $VLDL_3$-C is reduced in the patient by at least about 20% to about 27% from baseline following administration of the pharmaceutical composition.

9.   A method for reducing serum IDL-C in a patient, the method comprising selecting a patient with elevated serum IDL-C, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

10.   The method of claim 9, wherein serum IDL-C is reduced in the patient by at least about 50% to about 56% from baseline following administration of the pharmaceutical composition.

11.   A method for reducing the serum concentration of at least one low-density lipoprotein cholesterol (LDL-C) subfraction in a patient, the method comprising selecting a patient with an elevated serum level of at least one LDL-C subfraction, and administering to the patient a pharmaceutical composition comprising a PCSK9 inhibitor.

12.   The method of claim 11, wherein the LDL-C subfraction is LDL_1-C.

13.   The method of claim 12, wherein serum LDL_1-C is reduced in the patient by at least about 65% to about 78% from baseline following administration of the pharmaceutical composition.

14.   The method of claim 11, wherein the LDL-C subfraction is LDL_2-C.

15.   The method of claim 14, wherein serum LDL_2-C is reduced in the patient by at least about 75% to about 85% from baseline following administration of the pharmaceutical composition.

16.   The method of claim 11, wherein the LDL-C subfraction is LDL_3-C.

17.   The method of claim 11, wherein the LDL-C subfraction is LDL_4-C.

18.   The method of claim 11, wherein the LDL-C subfraction is LDL_{3+4}-C.

19.   The method of claim 18 wherein serum LDL_{3+4}-C is reduced in the patient by at least about 45% to about 70% from baseline following administration of the pharmaceutical composition.

20.   The method of any one of claims 1 to 19, wherein the PCSK9 inhibitor is an antibody or antigen-binding fragment thereof that specifically binds PCSK9.

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

22.   The method of claim 21, wherein the pharmaceutical composition comprises 50 mg to 150 mg of the PCSK9 inhibitor.

23.   The method of claim 22, wherein the pharmaceutical composition comprises 50 mg of the PCSK9 inhibitor.

24.   The method of claim 22, wherein the pharmaceutical composition comprises 75 mg of the PCSK9 inhibitor.

25.   The method of claim 22, wherein the pharmaceutical composition comprises 100 mg of the PCSK9 inhibitor.

26.   The method of claim 22, wherein the pharmaceutical composition comprises 150 mg of the PCSK9 inhibitor.

27.   The method of any one of claims 20 to 26, 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.

28.   The method of claim 27, 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.

29.   The method of claim 28, 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.

30.   The method of claim 27, 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.

31.   The method of claim 30, 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.

32.   The method of claim 20, 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.

33.   The method of claim 20, 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.

34.   The method of claim 20, wherein the antibody or antigen-binding fragment thereof exhibits pH-dependent binding characteristics to PCSK9.

39

35.   The method of claim 34, wherein the antibody or antigen-binding fragment thereof binds PCSK9 at neutral pH with a higher affinity than at acidic pH.

36.   The method of any one of claims 1 to 35, wherein the patient is on a therapeutic statin regimen at the time of or just prior to administration of the pharmaceutical composition.

37.   The method of claim 36 wherein the therapeutic statin regimen comprises a statin selected from the group consisting of cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin and pravastatin.

38.   The method of claim 36, wherein the statin is atorvastatin.

39.   The method of any one of claims 1 to 35, wherein the patient is not on a therapeutic statin regimen at the time of administration of the pharmaceutical composition.

RECTIFIED SHEET (RULE 91) ISA/EP
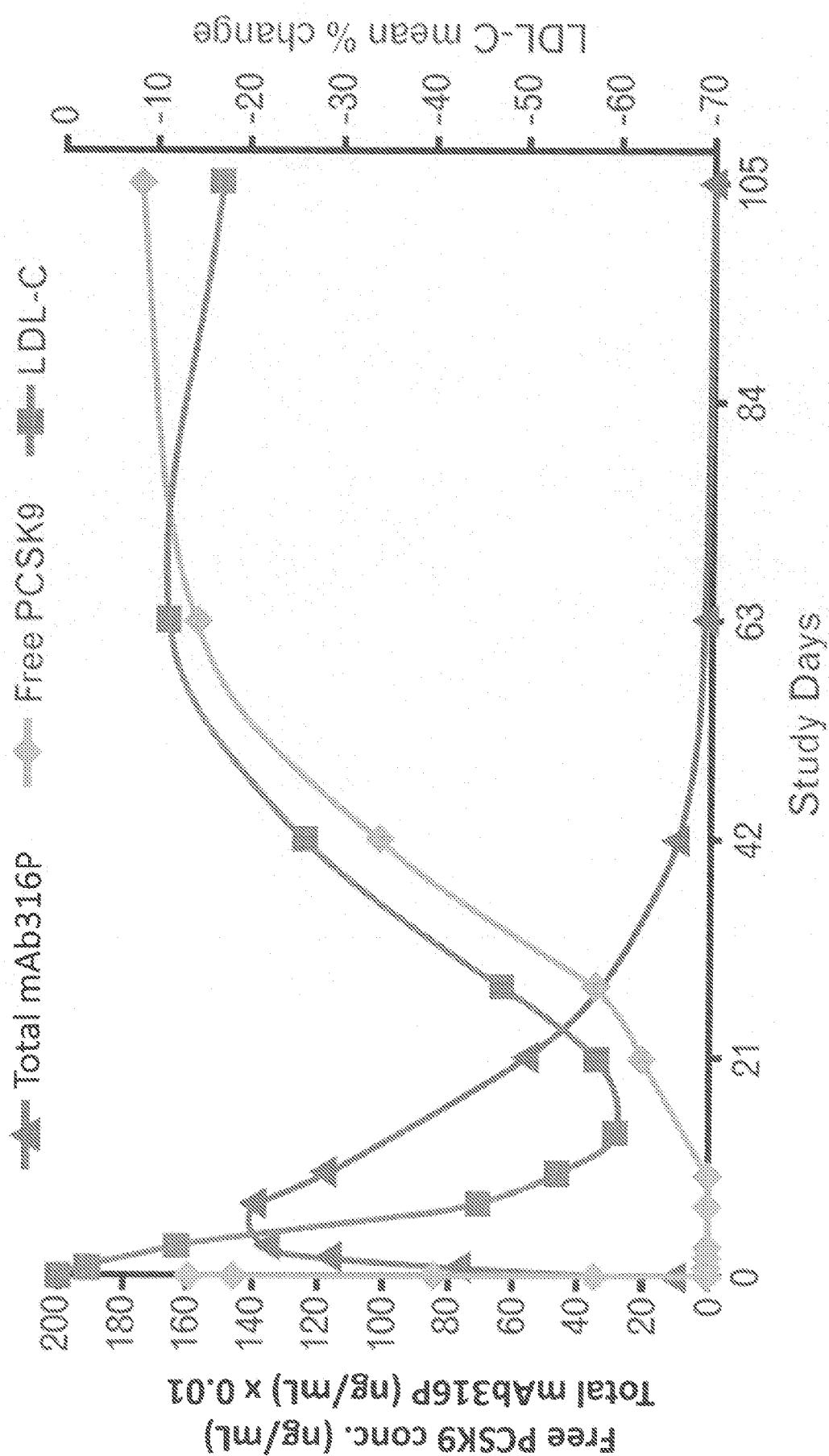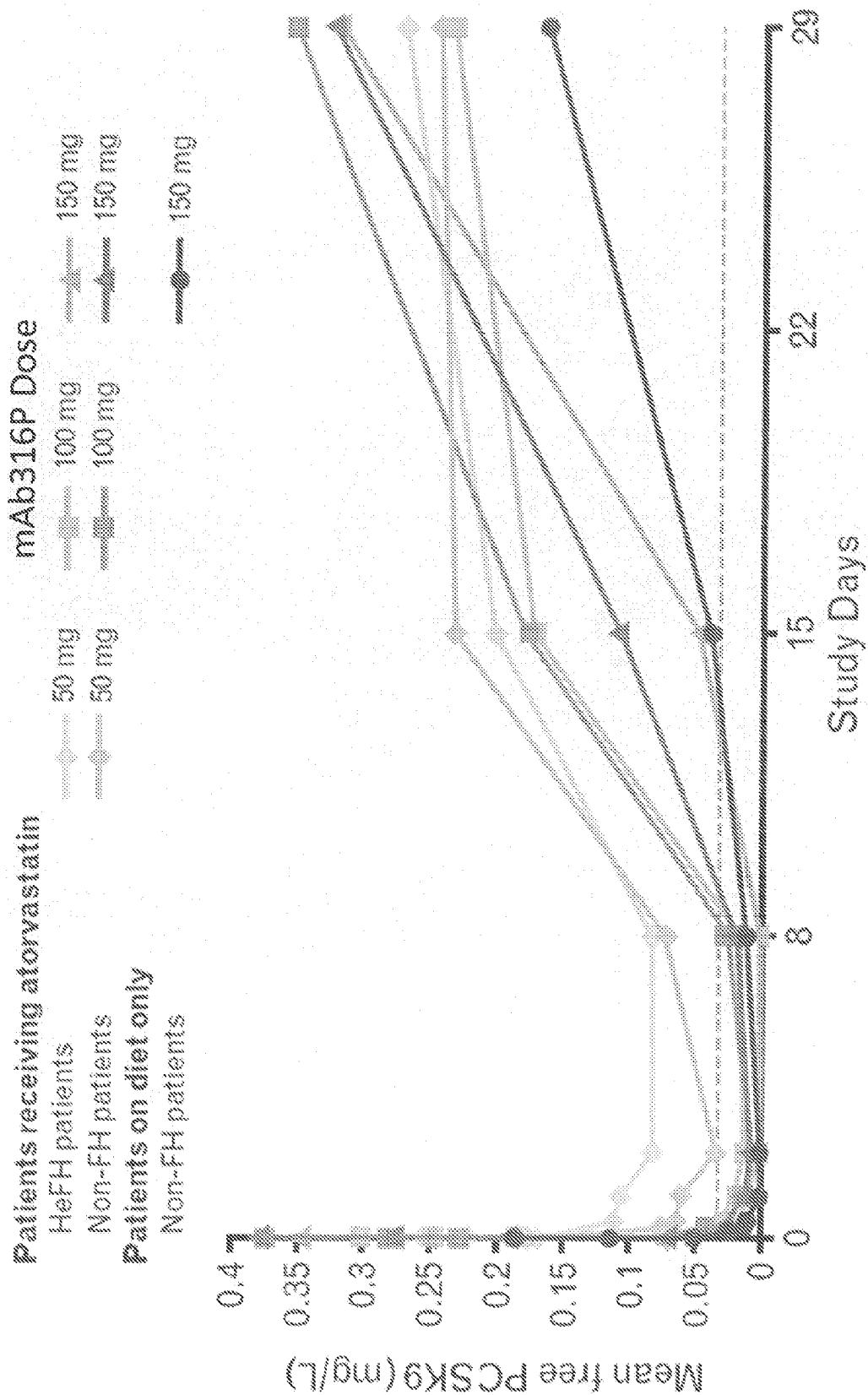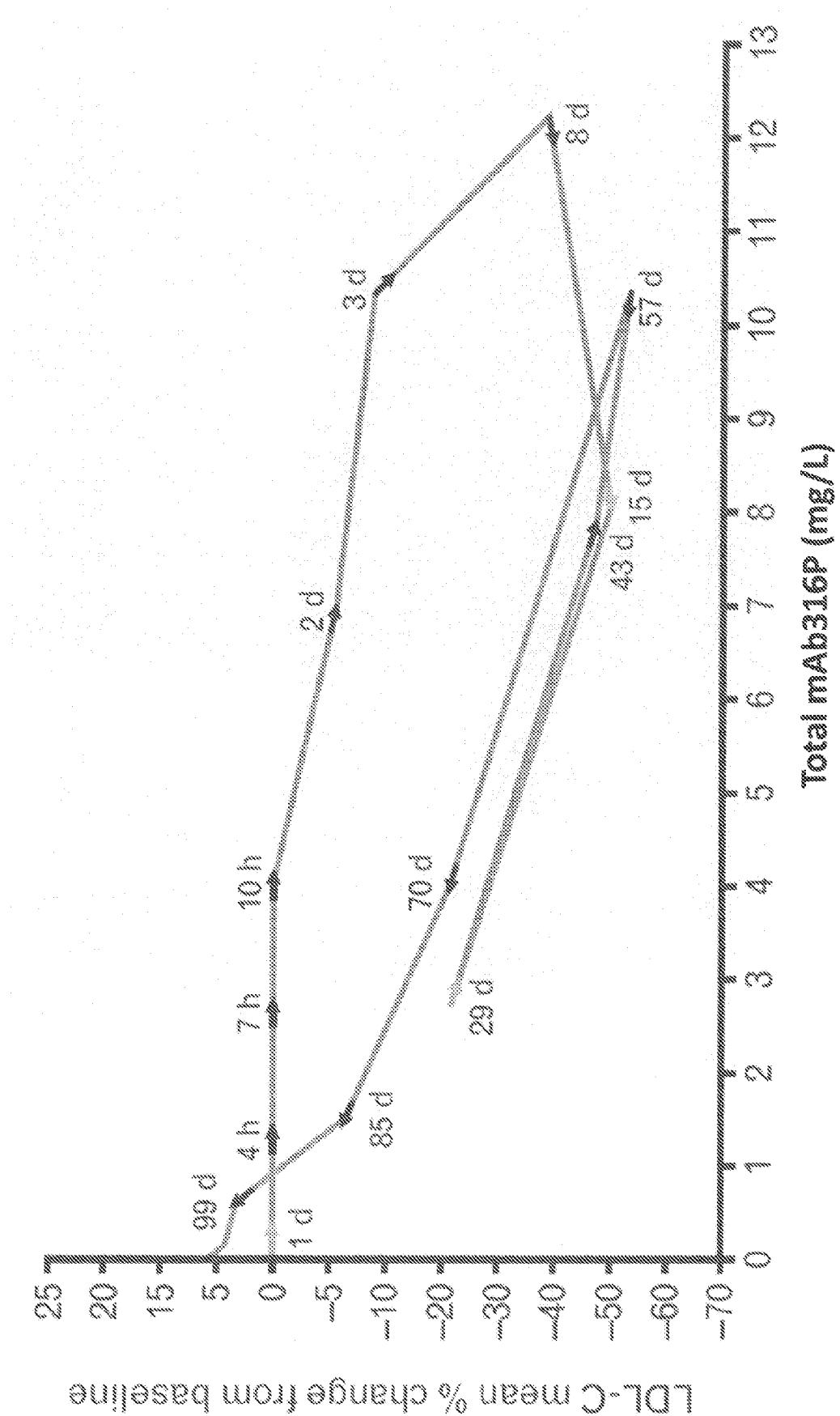
Figure 1

Figure 2

Figure 3
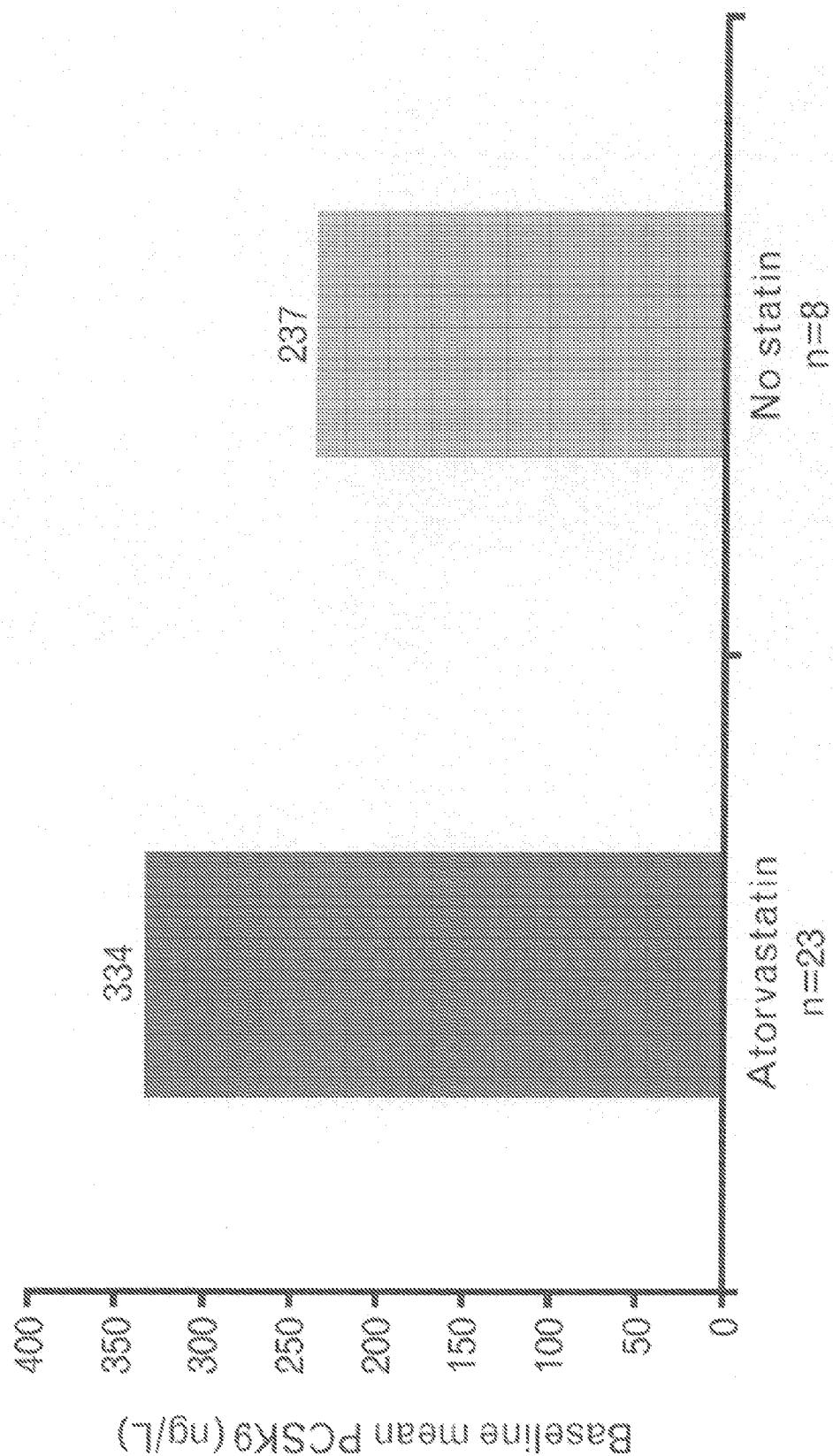
Figure 4
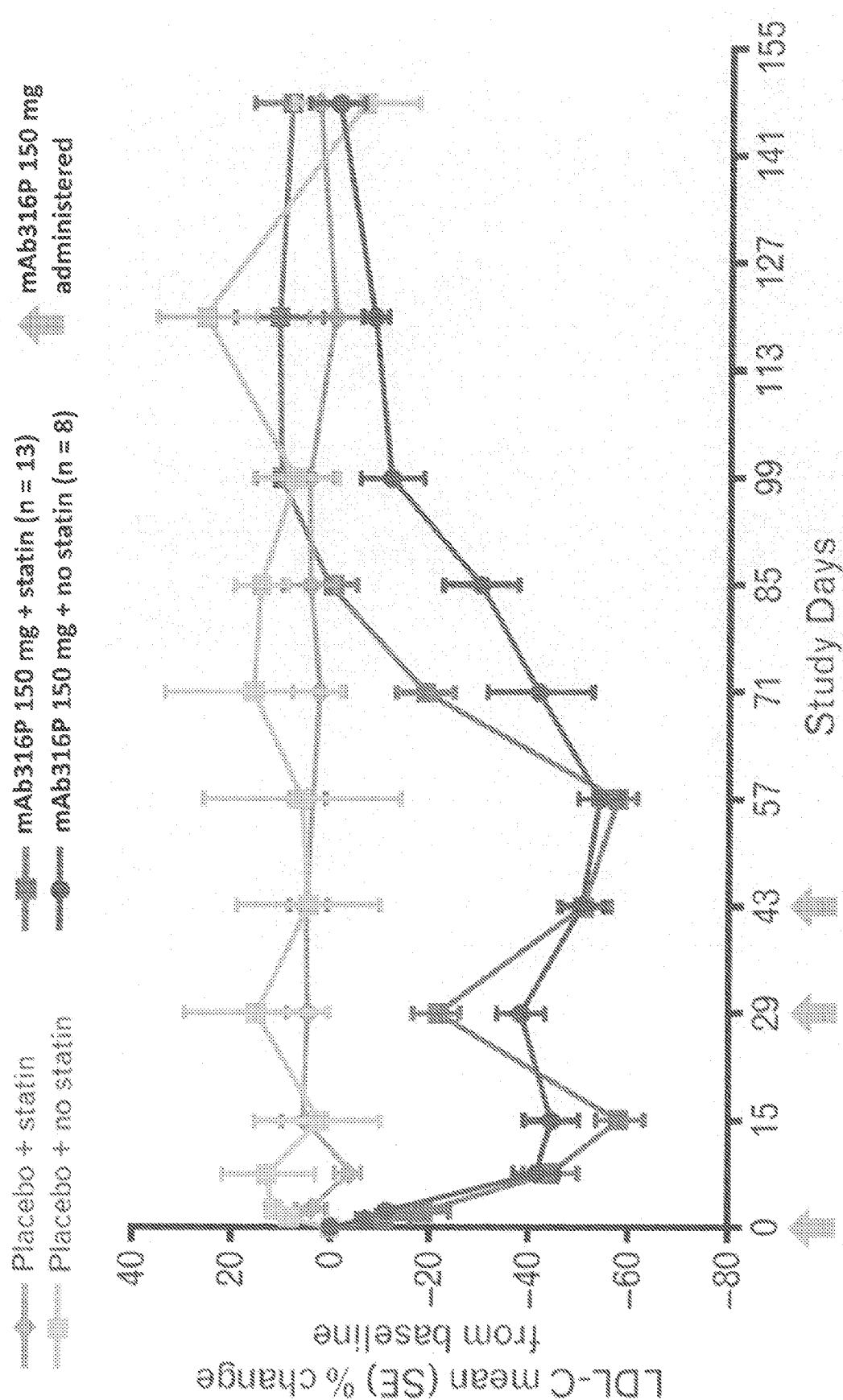
Figure 5
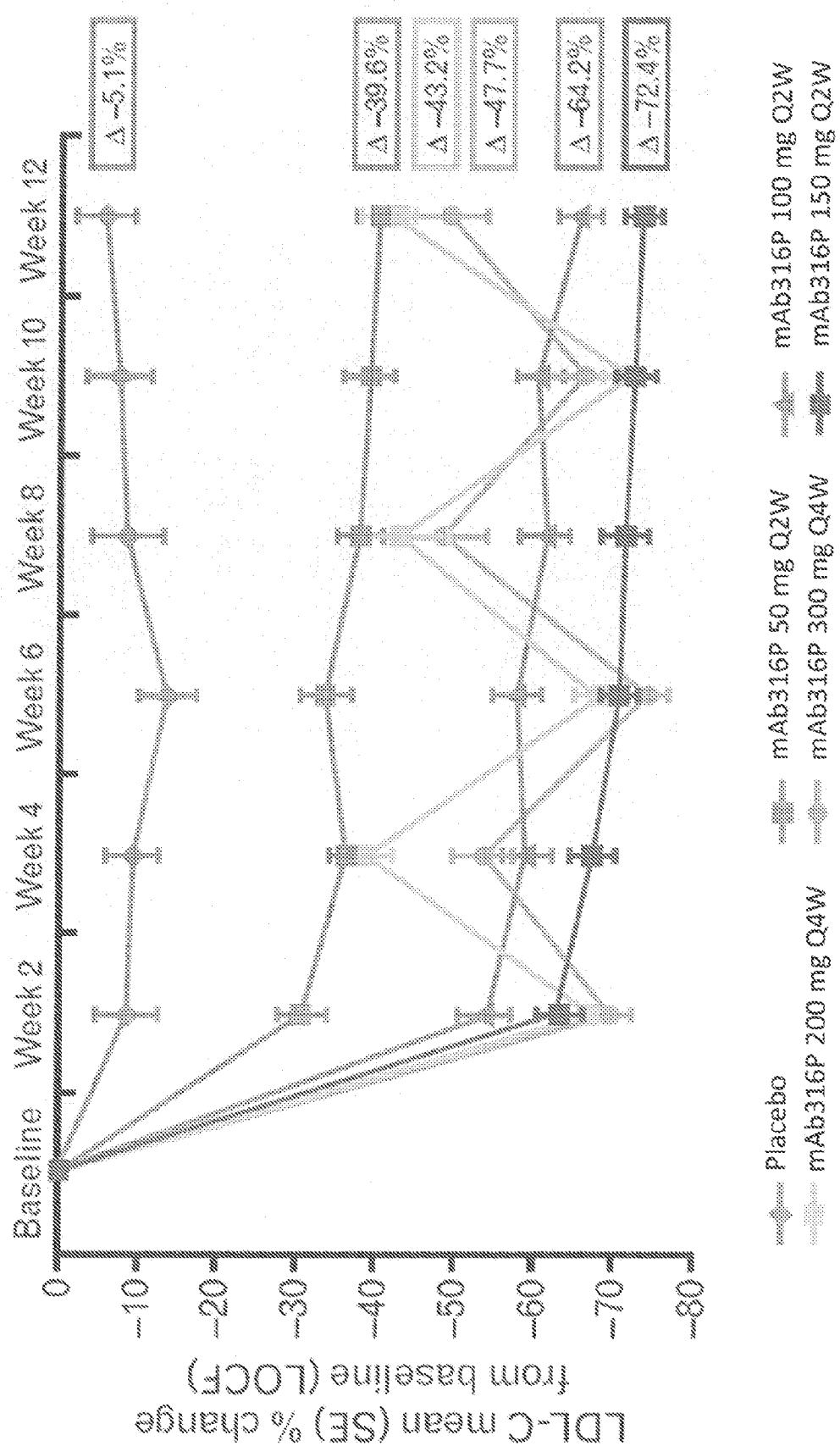
Figure 6
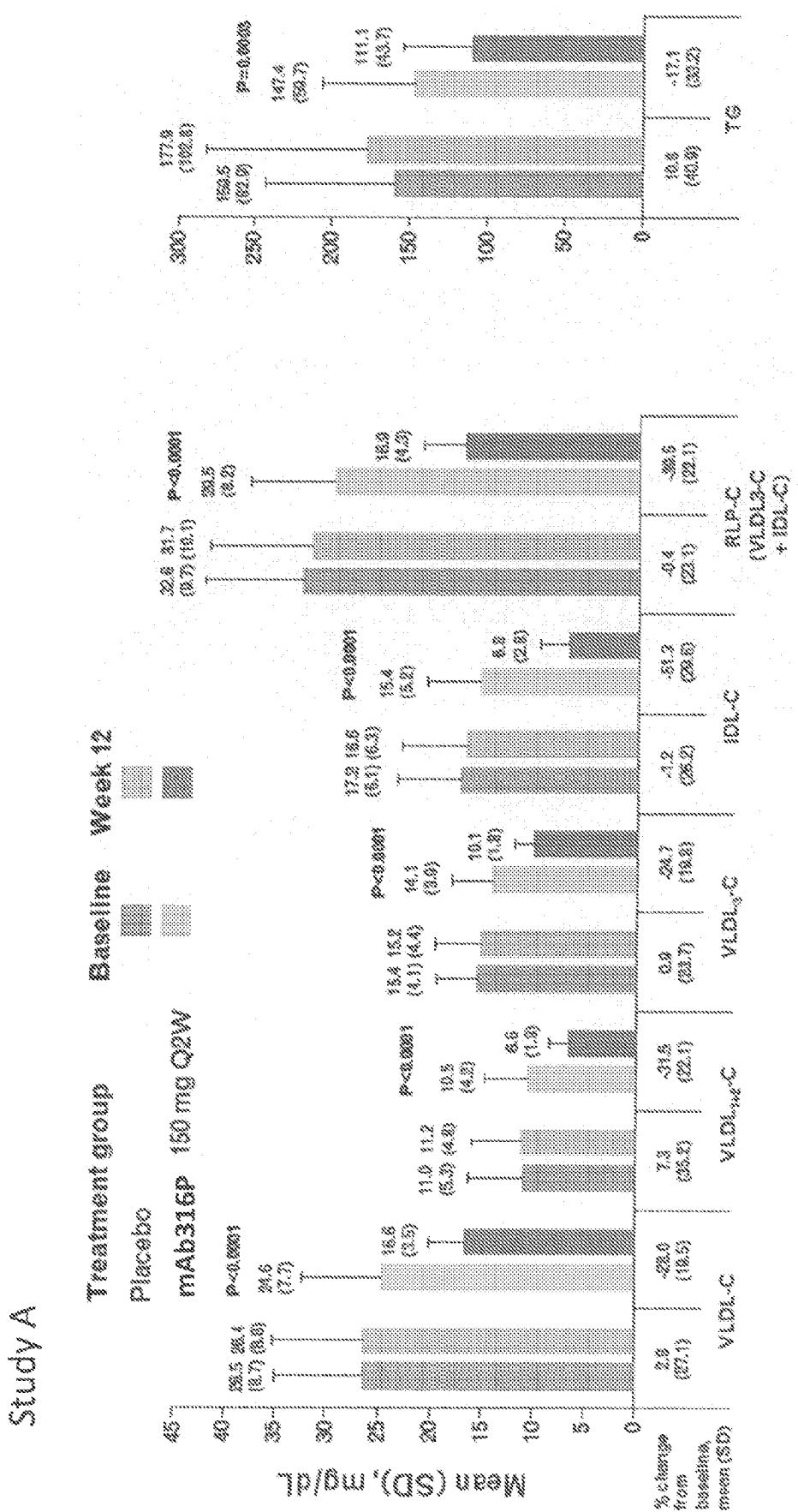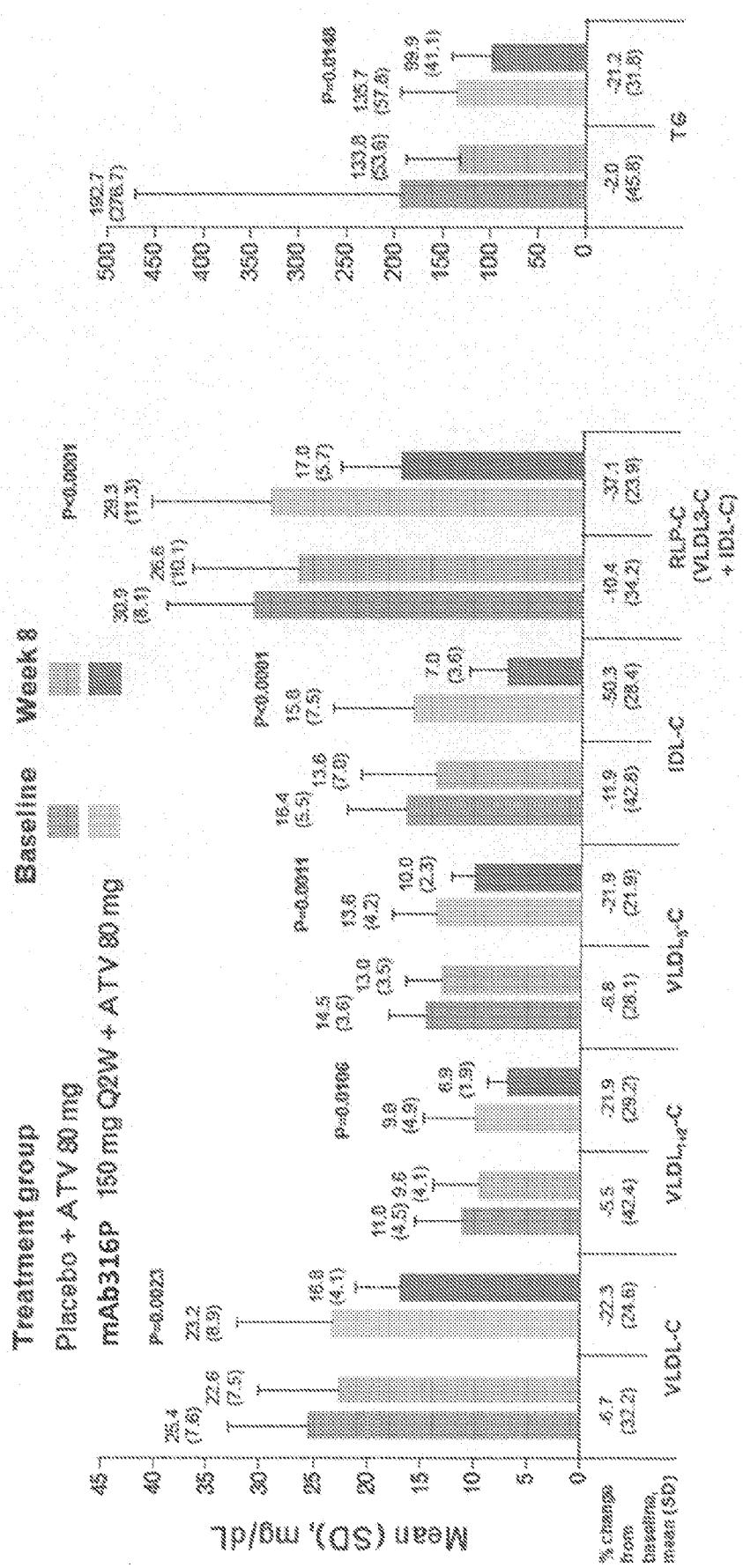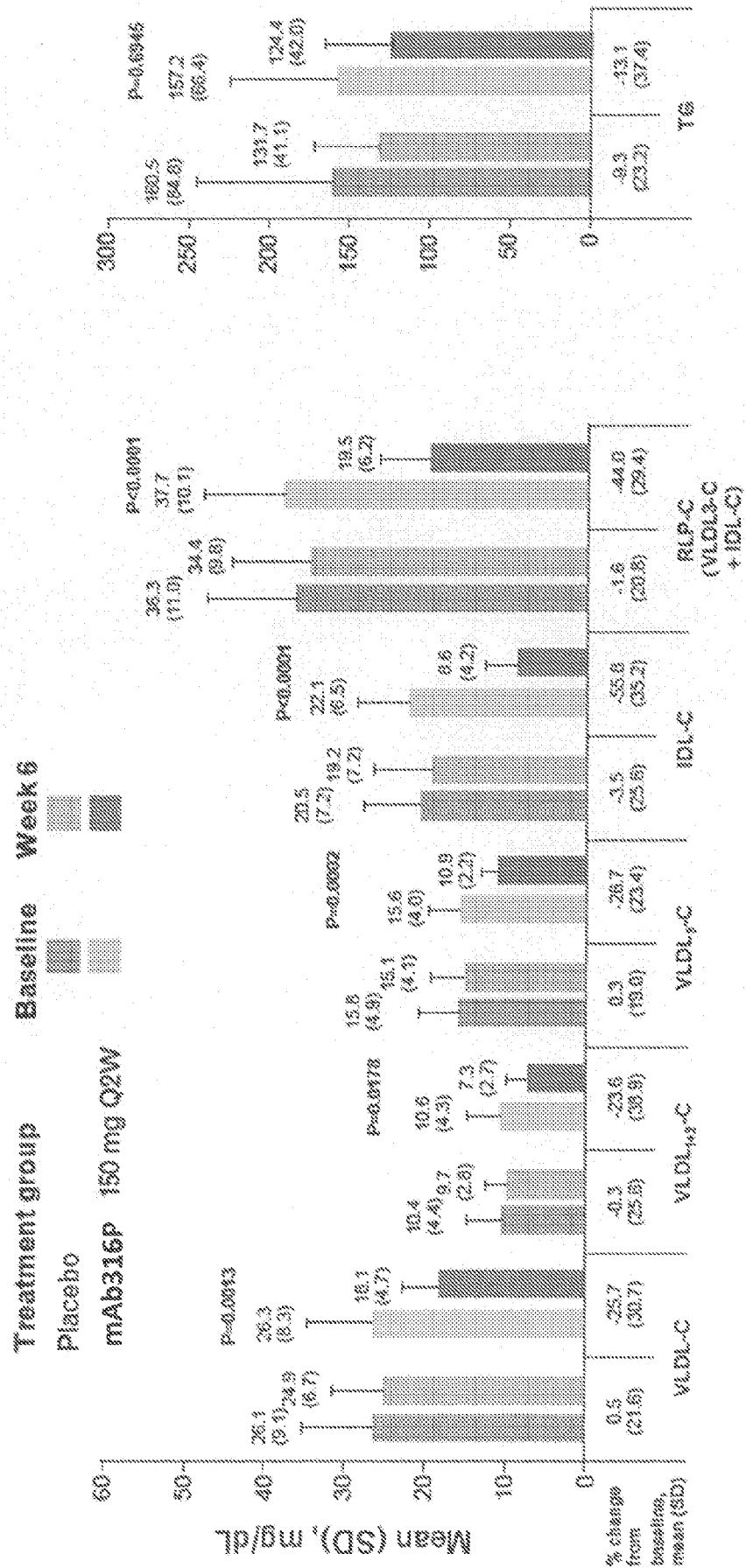
Figure 7
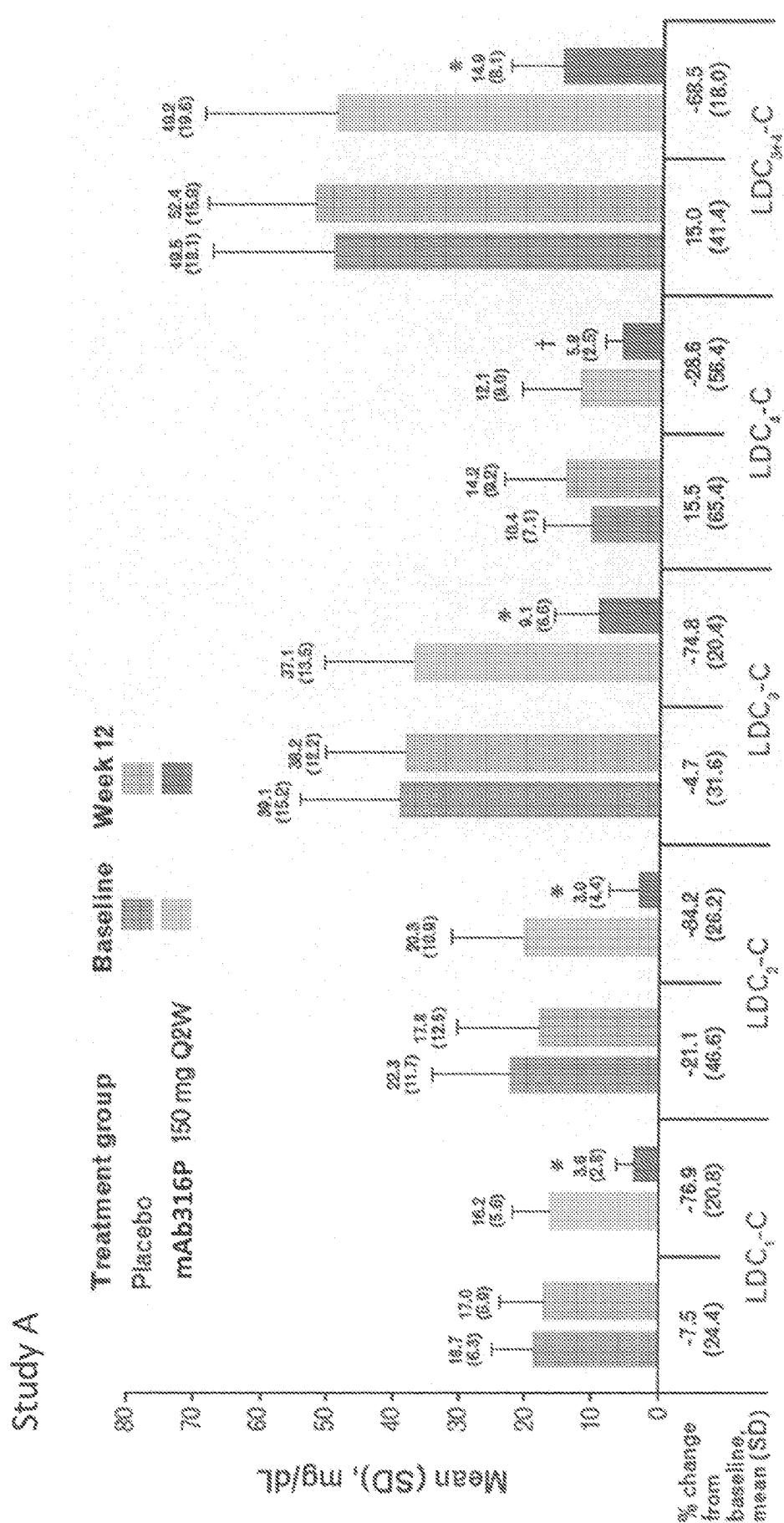
Figure 8
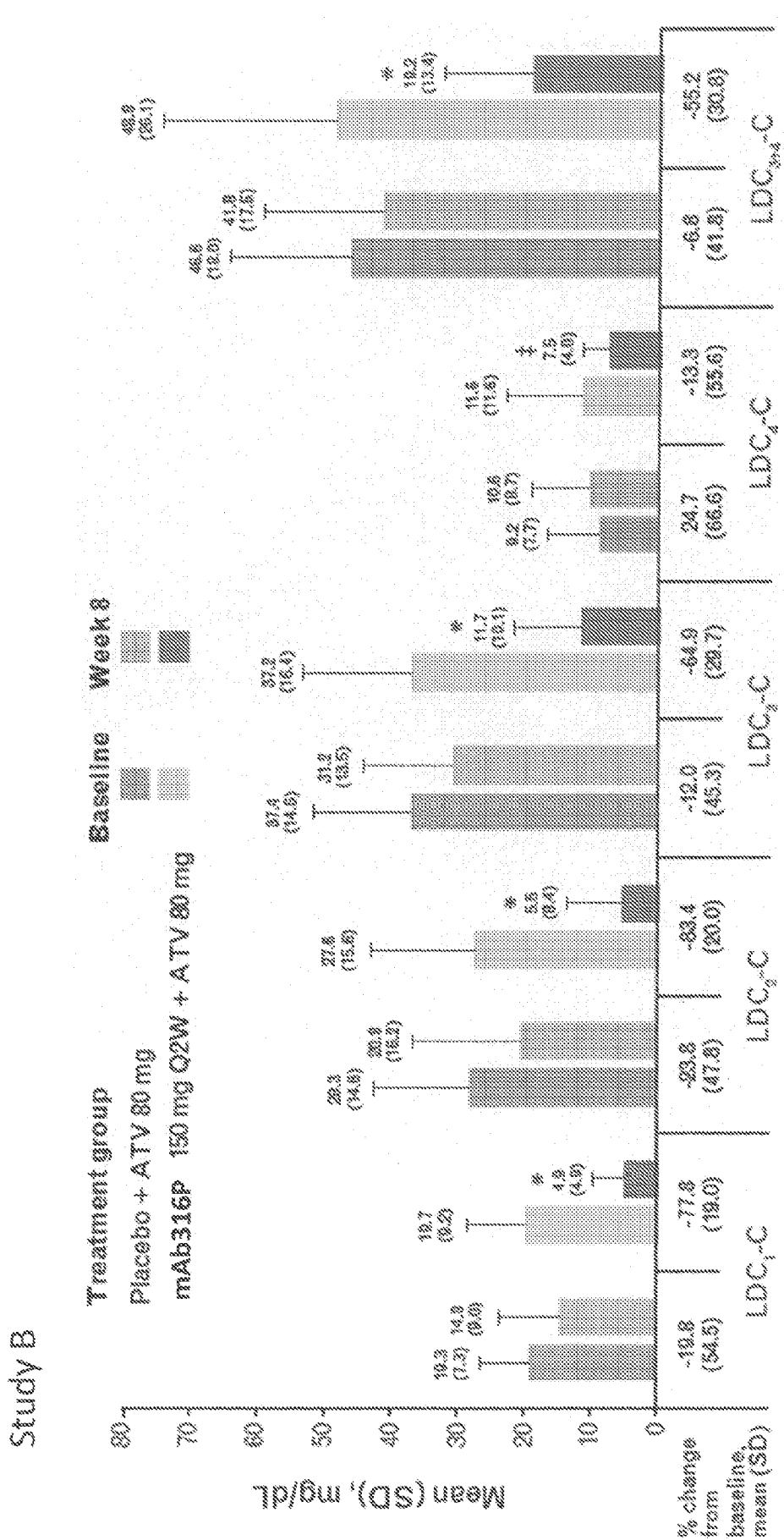
Figure 9

Figure 10

Figure 11

Figure 12

Figure 13