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[Continued on next page]

(54) Title: TREATMENT WITH ANTI-PCSK9 ANTIBODIES

(57) Abstract: The present invention concerns dosages for the treatment of human patients susceptible to or diagnosed with a disorder characterized by marked elevations of low density lipoprotein particles in the plasma with a PCSK9 antagonist antibody alone or in combination with a statin.

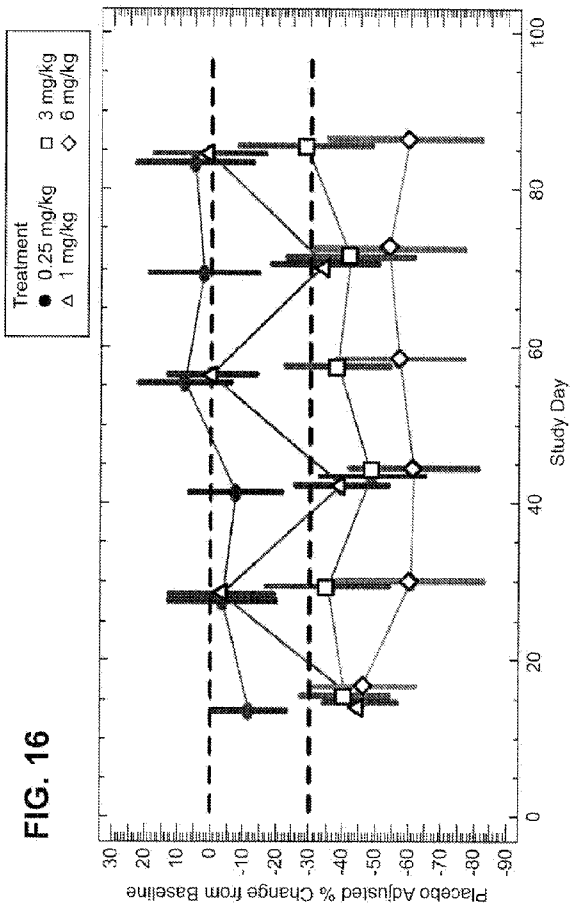

FIG. 16

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TREATMENT WITH ANTI-PCSK9 ANTIBODIES

RELATED APPLICATIONS

This application claims the benefits of U.S. Provisional Application No. 61/507,865 filed July 14, 2011, U.S. Provisional Application No. 61/614,312 filed March 22, 2012, and U.S. Provisional Application No. 61/643,063 filed May 4, 2012, all of which are hereby incorporated by reference in their entireties.

FIELD

The present invention concerns therapeutic regimens for treatment of disorders characterized by marked elevations of low density lipoprotein ("LDL") particles in the plasma. The subject therapeutic regimens involve administration of an anti-proprotein convertase subtilisin kexin type 9 (PCSK9) antibody, alone or in combination with a statin. The subject therapeutic regimens provide for enhanced reduction of LDL-cholesterol levels in blood, and can be used in the prevention and/or treatment of cholesterol and lipoprotein metabolism disorders, including familial hypercholesterolemia, atherogenic dyslipidemia, atherosclerosis, acute coronary syndrome and, more generally, cardiovascular disease.

BACKGROUND

Millions of people in the U.S. are at risk for heart disease and resulting cardiac events. Cardiovascular disease and underlying atherosclerosis is the leading cause of death among all demographic groups, despite the availability of therapies directed at its multiple risk factors. Atherosclerosis is a disease of the arteries and is responsible for coronary heart disease associated with many deaths in industrialized countries. Several risk factors for coronary heart disease have now been identified: dyslipidemias, hypertension, diabetes, smoking, poor diet, inactivity and stress. The most clinically relevant and common dyslipidemias are characterized by an increase in beta-lipoproteins (very low density lipoprotein (VLDL) and LDL) with hypercholesterolemia in the absence or presence of hypertriglyceridemia. Fredrickson et al., 1967, N Engl J Med. 276:34-42, 94-103, 148-156, 215-225, and 273-281. There is a long-felt significant unmet need with respect to cardiovascular disease with 60-70% of cardiovascular events, heart attacks and strokes occurring despite the treatment with statins (the

current standard of care in atherosclerosis). Moreover, new guidelines suggest that even lower LDL levels should be achieved in order to protect high risk patients from premature cardiovascular disease (National Cholesterol Education Program (NCEP) 2004).

PCSK9 has been implicated as a major regulator of plasma low density lipoprotein cholesterol (LDL-C) and has emerged as a promising target for prevention and treatment of coronary heart disease (CHD). Hooper et al., 2011, Expert Opin Ther Targets 15(2):157-68. Human genetic studies identified gain-of-function mutations, which were associated with elevated serum levels of LDL-C and premature and incidences of CHD, whereas loss-of-function mutations were associated with low LDL-C and reduced risk of CHD. Abifadel, 2003, Nat Genet. 43(2):154-6; Cohen, 2005, Nat Genet. 37(2):161-5; Cohen, 2006, N Engl J Med. 354(12):1264-72; Kotowski, 2006, Am J Hum Genet. 78(3):410-22. In humans, the complete loss of PCSK9 results in low serum LDL-C of <20 mg/dL, in otherwise healthy subjects. Hooper, 2007, 193(2):445-8; Zhao, 2006, Am J Hum Genet. 79(3):514-523.

PCSK9 belongs to the subtilisin family of serine proteases and is formed by an N-terminal prodomain, a subtilisin-like catalytic domain and a C-terminal cysteine/histidine-rich domain (CHRD). Highly expressed in the liver, PCSK9 is secreted after the autocatalytic cleavage of the prodomain, which remains non-covalently associated with the catalytic domain. The catalytic domain of PCSK9 binds to the epidermal growth factor-like repeat A (EGF-A) domain of low density lipoprotein receptor (LDLR) at serum pH of 7.4 and higher affinity at endosomes pH of approximately 5.5-6.0. Bottomley, 2009, J Biol Chem. 284(2):1313-23. The C-terminal domain is involved in the internalization of the LDLR-PCSK9 complex, while not binding to catalytic domain. Nassoury, 2007, Traffic 8(7):950; Ni, 2010, J Biol Chem. 285(17):12882-91; Zhang, 2008, Proc Natl Acad Sci USA, 2008, 105(35):13045-50. Both functionalities of PCSK9 are required for targeting the LDLR-PCSK9 complex for lysosomal degradation and lowering LDL-C, which is in agreement with mutations in both domains linked to loss-of-function and gain-of-function. Lambert, 2009, Atherosclerosis 203(1):1-7.

Various therapeutic approaches for inhibiting PCSK9 are currently in development, including gene silencing by siRNA or anti-sense oligonucleotides and disruption of the PCSK9-LDLR interaction by antibodies. Brautbar et al., 2011, Nature Reviews Cardiology 8, 253-265. For example, Chan, 2009, and Ni, 2011, each report an

anti-PCSK9 monoclonal antibody having LDL-C lowering activity in mice and non-human primates; the half-life of each antibody was reported as approximately 61 h and 77 h, respectively, in non-human primates when administered at 3 mg/kg of the PCSK9 antagonist antibody. Chan, 2009, Proc Natl Acad Sci USA 106(24):9820-5; Ni, 2011, J Lipid Res. 52(1):78-86. The PSCK9 antagonist antibody 7D4 has been reported to effectively reduce serum cholesterol levels in cynomoglus monkey; the half-life of 7D4 in cynomolgus monkeys was less than 2 days at a single dose of 10 mg/kg of the PCSK9 antagonist antibody. PCT Patent Application Publication No. WO 2010/029513.

From the information available in the art, and prior to the present invention, it remained unclear whether low, infrequent doses of PCSK9 antagonist antibody would be effective to reduce hypercholesterolemia and the associated incidence of CVD in human patients and, if so, what dosage regimens are needed for such in vivo effectiveness.

SUMMARY

This invention relates to therapeutic regimens for prolonged reduction of LDL-C levels in blood by inhibiting PCSK9 activity and the corresponding effects of PCSK9 on LDL-C plasma levels.

In some embodiments, the invention provides a method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, the method comprising administering to the patient an initial dose of at least about 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 8 mg/kg, 12 mg/kg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, or 400 mg of a proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about one, two, three, or four weeks. The invention can be practiced using, for example, the PCSK9 antagonist antibody L1L3. In some embodiments, the invention can be practiced using an antibody comprising three CDRS from a heavy chain variable region having the amino acid sequence shown in SEQ ID NO: 11 and three CDRS from a light chain variable region having the amino acid sequence shown in SEQ ID NO: 12.

In some embodiments, the initial dose can be about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg or about 1.5 mg/kg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by about one week.

In other embodiments, the initial dose can be about 2 mg/kg, about 4 mg/kg, about 8 mg/kg, or about 12 mg/kg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by at least about two weeks.

In other embodiments, the initial dose can be about 50 mg, about 100 mg, about 150 mg, or about 175 mg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by at least about two weeks.

In other embodiments, the initial dose can be about 3 mg/kg or about 6 mg/kg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by at least about four weeks. In other embodiments, the initial dose can be about 200 mg or about 300 mg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by at least about four weeks. In some embodiments, the PCSK9 antagonist antibody is administered subcutaneously. In some embodiments, the PCSK9 antagonist antibody is administered intravenously.

In some embodiments, the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by about four weeks. In some embodiments, the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by about eight weeks. Each of the plurality of subsequent doses can be about the same amount or less than the initial dose.

In some embodiments, the disorder can be hypercholesterolemia, dyslipidemia, atherosclerosis, cardiovascular disease, coronary heart disease, or acute coronary syndrome (ACS). The human patient may have a fasting total cholesterol level of, for example, about 600 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody. The human patient may have a fasting LDL cholesterol level of, for example, about 130 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody. In some embodiments, the human patient may have a fasting LDL

cholesterol level of about 145 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody.

In some embodiments, the patient is being treated with a statin. In some embodiments, the patient is being treated with a daily dose of a statin. In some embodiments, the human patient may have been administered an effective amount of a statin prior to administration of the initial dose of PCSK9 antagonist antibody. In some embodiments, the patient is on stable doses of a statin prior to administration of an initial dose of PCSK9 antibody. The stable doses can be, for example, a daily dose or an every-other-day dose. In some embodiments, the human patient is on a daily stable dose of a statin for at least about two, three, four, five, or six weeks prior to administration of the initial dose of PCSK9 antagonist antibody. In some embodiments, the human patient on stable doses of a statin has a fasting LDL cholesterol level of, for example, about 70 or 80 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody.

In some embodiments, the method further comprises administering an effective amount of a statin.

In some embodiments, the initial dose of PCSK9 antagonist antibody can be about 3 mg/kg or about 6 mg/kg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by about four weeks or about one month. In some embodiments, the initial dose of PCSK9 antagonist antibody can be about 200 mg or about 300 mg, and the initial dose and the first subsequent dose and additional subsequent doses can be separated from each other in time by about four weeks or about one month.

The statin can be, for example, atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or a combination therapy selected from the group consisting of simvastatin plus ezetimibe, lovastatin plus niacin, atorvastin plus amlodipine, and simvastatin plus niacin. In some embodiments, the statin dose can be, for example, 40 mg atorvastatin, 80 mg atorvastatin, 20 mg rosuvastatin, 40 mg rosuvastatin, 40 mg simvastatin, or 80 mg simvastatin.

In some embodiments, the method comprises administering to the patient an initial dose of at least about 3 mg/kg or about 6 mg/kg of PCSK9 antagonist antibody L1L3; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same or less than the initial dose, wherein the initial dose

and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks, wherein the patient is being treated with a stable daily dose of a statin. In some embodiments, the stable daily dose of a statin can be 40 mg atorvastatin, 80 mg atorvastatin, 20 mg rosuvastatin, 40 mg rosuvastatin, 40 mg simvastatin, or 80 mg simvastatin.

In some embodiments, the method comprises administering to the patient an initial dose of at least about 200 mg or about 300 mg of PCSK9 antagonist antibody L1L3; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks, wherein the patient is being treated with a stable daily dose of a statin. In some embodiments, the method comprises administering to the patient an initial dose of at least about 50 mg, about 100 mg, about 150 mg, or about 175 mg of PCSK9 antagonist antibody L1L3; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about two weeks, wherein the patient is being treated with a stable daily dose of a statin. In some embodiments, the stable daily dose of a statin can be 40 mg atorvastatin, 80 mg atorvastatin, 20 mg rosuvastatin, 40 mg rosuvastatin, 40 mg simvastatin, or 80 mg simvastatin.

In some embodiments, the PCSK9 antagonist antibody is administered subcutaneously or intravenously.

The invention also provides article of manufacture, comprising a container, a composition within the container comprising a PCSK9 antagonist antibody, and a package insert containing instructions to administer an initial dose of PCSK9 antagonist antibody of at least about 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 12 mg/kg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg or 400 mg, and at least one subsequent dose that is the same amount or less than the initial dose. In some embodiments, the invention can be practiced using an antibody comprising three CDRS from a heavy chain variable region having the amino acid sequence shown in SEQ ID NO: 11 and three CDRS from a light chain variable region having the amino acid sequence shown in SEQ ID NO: 12. In some

embodiments, the invention can be practiced using the PCSK9 antagonist antibody L1L3.

The administration of the initial dose and subsequent doses can be separated in time by, for example, at least about one, at least about two, three, four, five, six, seven or eight weeks. In some embodiments, instructions can be, for example, for administration of an initial dose by intravenous injection and at least one subsequent dose by intravenous or subcutaneous injection. In other embodiments, instructions can be, for example, for administration of an initial dose by subcutaneous injection and at least one subsequent dose by intravenous or subcutaneous injection.

In some embodiments, a plurality of subsequent doses can be administered. The plurality of subsequent doses can be separated in time from each other by, for example, at least two, three, four, five, six, seven or eight weeks.

In some embodiments, the package insert can further include instructions for administration of the PCSK9 antagonist antibody to a patient being treated with a statin. The statin can be, for example, atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or a combination therapy selected from the group consisting of simvastatin plus ezetimibe, lovastatin plus niacin, atorvastin plus amlodipine, and simvastatin plus niacin.

In some embodiments, the article of manufacture can further comprise a label on or associated with the container that indicates that the composition can be used for treating a condition characterized by an elevated low-density lipoprotein cholesterol level in the blood. The label can indicate that the composition can be used for the treatment of, for example, hypercholesterolemia, atherogenic dyslipidemia, atherosclerosis, cardiovascular disease, and/or acute coronary syndrome (ACS).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a graph showing absolute fasting LDL-C levels in mg/dL after L1L3 antibody administration.

FIG. 2 depicts a graph showing the percentage change from baseline of fasting LDL-C levels after L1L3 antibody administration.

FIG. 3 depicts a graph showing the percentage change from baseline of fasting total cholesterol levels after L1L3 antibody administration.

FIG. 4 depicts a graph showing the percentage change from baseline of fasting apolipoprotein B levels after L1L3 antibody administration.

FIG. 5 depicts a graph showing the percentage change from baseline of fasting high density lipoprotein cholesterol levels after L1L3 antibody administration.

FIG. 6 depicts a graph showing the percentage change from baseline of fasting triglyceride lipoprotein cholesterol levels after L1L3 antibody administration.

FIG. 7A depicts a graph showing absolute fasting LDL-C levels in mg/dL after L1L3 antibody administration. FIG. 7B depicts a graph showing the percentage change from baseline of fasting LDL-C levels in mg/dL after L1L3 antibody administration.

FIG. 8 depicts a graph showing the percentage change from baseline of fasting LDL-C levels after L1L3 antibody administration, with or without statin present. X-axis indicates the dose amount of L1L3 in mg/kg of the PCSK9 antagonist antibody.

FIGS. 9A-F depicts simulated time profiles for L1L3 (A-C) and LDL-C (E-F). (A) and (D): L1L3 at 2 mg/kg of the PCSK9 antagonist antibody. (B) and (E): L1L3 at 6 mg/kg of the PCSK9 antagonist antibody. (C) and (F): Placebo. X-axis indicates time in days.

FIG. 10 depicts simulated time profiles for LDL-C after dosing with the indicated L1L3 dose amounts.

FIG. 11 depicts a schematic of the study design for L1L3 monotherapy.

FIG. 12 depicts a graph showing absolute fasting LDL-C levels in mg/dL after L1L3 antibody administration.

FIG. 13 depicts a graph showing the percentage change from baseline of fasting LDL-C levels after L1L3 antibody administration.

FIG. 14 depicts a table showing the mean percentage change from baseline of fasting LDL-C levels after L1L3 antibody administration.

FIG. 15 depicts a graph showing the percent change from baseline of fasting LDL-C levels after L1L3 antibody administration.

FIG. 16 depicts a graph showing the percent change from baseline of fasting LDL-C levels after L1L3 antibody administration, excluding subjects with missed doses.

DETAILED DESCRIPTION

Provided herein are therapeutic regimens for treatment of disorders characterized by marked elevations of LDL particles in the plasma. The subject therapeutic regimens

involve administration of a PCSK9 antagonist antibody, alone or in combination with a statin. The subject therapeutic regimens provide for prolonged reduction of LDL-cholesterol levels in blood, and can be used in the prevention and/or treatment of cholesterol and lipoprotein metabolism disorders, including familial hypercholesterolemia, atherogenic dyslipidemia, atherosclerosis, acute coronary syndrome (ACS), and, more generally, cardiovascular disease.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

An “antibody” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least

one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (i.e., "antigen-binding portion") or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (*e.g.*, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, 2005, Nature Biotechnology 23(9): 1126-1136). An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antigen binding portion" of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., PCSK9). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include Fab; Fab’; $F(ab')_2$; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., 1989, Nature 341:544-546), and an isolated complementarity determining region (CDR).

The term "monoclonal antibody" (Mab) refers to an antibody that is derived from a single copy or clone, including e.g., any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

"Humanized" antibody refers to forms of non-human (*e.g.* murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv,

Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody that can be produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, Nature Biotechnology, 14:309-314; Sheets et al., 1998, Proc. Natl. Acad. Sci. (USA) 95:6157-6162; Hoogenboom and Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581). Human antibodies can also be made by immunization of animals into which human immunoglobulin loci have been transgenically introduced in place of the endogenous loci, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, 1985; Boerner et al., 1991, J. Immunol., 147 (1):86-95; and U.S. Patent No. 5,750,373.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, contribute to the

formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonincal class as the subject variable region (Chothia and Lesk, J Mol Biol 196(4): 901-917, 1987). When choosing FR to flank subject CDRs, e.g., when humanizing or optimizing an antibody, FRs from antibodies which contain CDR1 and CDR2 sequences in the same canonical class are preferred.

A "CDR" of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the acccumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., 1989, Nature 342:877-883. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment

containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

As known in the art a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

As used herein, the term "PCSK9" refers to any form of PCSK9 and variants thereof that retain at least part of the activity of PCSK9. Unless indicated differently, such as by specific reference to human PCSK9, PCSK9 includes all mammalian species of native sequence PCSK9, e.g., human, canine, feline, equine, and bovine. One exemplary human PCSK9 is found as Uniprot Accession Number Q8NBP7 (SEQ ID NO: 1).

As used herein, a "PCSK9 antagonist antibody" refers to an anti-PCSK9 antibody that is able to inhibit PCSK9 biological activity and/or downstream pathway(s) mediated by PCSK9 signaling, including PCSK9-mediated down-regulation of the LDLR, and PCSK9-mediated decrease in LDL blood clearance. A PCSK9 antagonist antibody encompasses antibodies that block, antagonize, suppress or reduce (to any degree including significantly) PCSK9 biological activity, including downstream pathways mediated by PCSK9 signaling, such as LDLR interaction and/or elicitation of a cellular response to PCSK9. For purpose of the present invention, it will be explicitly understood that the term "PCSK9 antagonist antibody" encompasses all the previously identified terms, titles, and functional states and characteristics whereby the PCSK9 itself, a PCSK9 biological activity (including but not limited to its ability to mediate any aspect of interaction with the LDLR, down regulation of LDLR, and decreased blood LDL clearance), or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, a PCSK9 antagonist antibody binds PCSK9 and prevents interaction with the LDLR. Examples of PCSK9 antagonist antibodies are provided in, e.g., U.S. Patent Application Publication No. 20100068199, which is herein incorporated by reference in its entirety.

As used herein a "full antagonist" is an antagonist which, at an effective concentration, essentially completely blocks a measurable effect of PCSK9. By a partial antagonist is meant an antagonist that is capable of partially blocking a measurable effect, but that, even at a highest concentration is not a full antagonist. By essentially completely is meant at least about 80%, preferably, at least about 90%, more preferably,

at least about 95%, and most preferably, at least about 98% or 99% of the measurable effect is blocked. The relevant “measurable effects” are described herein and include down regulation of LDLR by a PCSK9 antagonist as assayed in Huh7 cells in vitro, in vivo decrease in blood (or plasma) levels of total cholesterol, and in vivo decrease in LDL levels in blood (or plasma).

As used herein, the term “clinically meaningful” means at least a 15% reduction in blood LDL-cholesterol levels in humans or at least a 15% reduction in total blood cholesterol in mice. It is clear that measurements in plasma or serum can serve as surrogates for measurement of levels in blood.

As used herein, the term “dosing regimen" refers to the total course of treatment administered to a patient, e.g., treatment with a PCSK9 antagonist antibody.

As used herein, the term "continuous" in the context of the time in which the mean level of LDL cholesterol in blood is within a specific range of levels, means that the time the mean level is in that specific range is not interrupted by any time in which that mean level is not within that specific range of levels.

As used herein, the term "not continuous" in the context of the time in which the mean level of LDL cholesterol in blood is within a specific range of levels, means that the time the mean level is in that specific range is interrupted by some amount of time (e.g., 15 minutes, 20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4, hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours 18 hours, 20 hours, 24 hours 28 hours, 32 hours, 36 hours, 40 hours, 44 hours, 48 hours, 60 hours, 72 hours, 84 hours, 90 hours, or any range of time of having upper and lower limits of any of above the specifically stated times), in which that mean level is not within that specific range of levels.

The terms “polypeptide”, “oligopeptide”, “peptide” and “protein” are used interchangeably herein to refer to chains of amino acids of any length, preferably, relatively short (e.g., 10-100 amino acids). The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural

amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

As known in the art, "polynucleotide," or "nucleic acid," as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups

include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

As used herein, an antibody "interacts with" PCSK9 when the equilibrium dissociation constant is equal to or less than 20 nM, preferably less than about 6 nM, more preferably less than about 1 nM, most preferably less than about 0.2 nM, as measured by the methods disclosed in Example 2 of U.S. Patent Application Publication No. 20100068199.

An antibody that "preferentially binds" or "specifically binds" (used interchangeably herein) to an epitope is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a PCSK9 epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other PCSK9 epitopes or non-PCSK9 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3.

As used in the art, "Fc receptor" and “FcR” describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976 J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen-binding portion thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen-binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the

binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

By an antibody with an epitope that "overlaps" with another (second) epitope or with a surface on PCSK9 that interacts with the EGF-like domain of the LDLR is meant the sharing of space in terms of the PCSK9 residues that are interacted with. To calculate the percent of overlap, for example, the percent overlap of the claimed antibody's PCSK9 epitope with the surface of PCSK9 which interacts with the EGF-like domain of the LDLR, the surface area of PCSK9 buried when in complex with the LDLR is calculated on a per-residue basis. The buried area is also calculated for these residues in the PCSK9:antibody complex. To prevent more than 100% possible overlap, surface area for residues that have higher buried surface area in the PCSK9:antibody complex than in LDLR:PCSK9 complex is set to values from the LDLR:PCSK9 complex (100%). Percent surface overlap is calculated by summing over all of the LDLR:PCSK9 interacting residues and is weighted by the interaction area.

A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity; phagocytosis; down-regulation of cell surface receptors (e.g., B cell receptor), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably, at least about 90% sequence identity therewith, more preferably, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

As used herein, the terms “atorvastatin”, “cerivastatin”, “fluvastatin”, “lovastatin”, “mevastatin”, “pitavastatin”, “pravastatin”, “rosuvastatin” and “simvastatin” include atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, respectively, and any pharmaceutically acceptable salts, or stereoisomers, thereof. As used herein, the term “pharmaceutically acceptable salt” includes salts that are physiologically tolerated by a patient. Such salts are typically prepared from inorganic acids or bases and/or organic acids or bases. Examples of these acids and bases are well known to those of ordinary skill in the art.

As used herein, “treatment” is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: enhancement of LDL clearance and reducing incidence or amelioration of aberrant cholesterol and/or lipoprotein levels resulting from metabolic and/or eating disorders, or including familial hypercholesterolemia, atherogenic dyslipidemia, atherosclerosis, ACS, and, more generally, cardiovascular disease (CVD).

“Reducing incidence” means any of reducing severity (which can include reducing need for and/or amount of (e.g., exposure to) other drugs and/or therapies generally used for this condition. As is understood by those skilled in the art, individuals may vary in terms of their response to treatment, and, as such, for example, a “method of reducing incidence” reflects administering the PCSK9 antagonist antibody based on a

reasonable expectation that such administration may likely cause such a reduction in incidence in that particular individual.

"Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering a PCSK9 antagonist antibody. "Ameliorating" also includes shortening or reduction in duration of a symptom.

As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing hypercholesterolemia or one or more symptoms of dyslipidemia, atherosclerosis, cardiovascular disease, or coronary heart disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An "individual" or a "subject" is a mammal, more preferably, a human. Mammals also include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats.

As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors

associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing, 2000).

The term "k_{on}", as used herein, refers to the rate constant for association of an antibody to an antigen. Specifically, the rate constants (k_{on} and k_{off}) and equilibrium dissociation constants are measured using Fab antibody fragments (i.e., univalent) and PCSK9.

The term "k_{off} ", as used herein, refers to the rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "K_D", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction.

Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X.” Numeric ranges are inclusive of the numbers defining the range.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, 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. Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The materials, methods, and examples are illustrative only and not intended to be limiting.

Published information related to anti-PCSK9 antibodies includes the following published applications: PCT/IB2009/053990, published March 18, 2010 as WO 2010/029513, and U.S. Patent Application No. 12/558312, published March 18, 2010 as US 2010/0068199, each of which is herein incorporated by reference in its entirety.

Treatment with anti-PCSK9 antibodies

Provided herein are therapeutic regimens for treatment of disorders characterized by marked elevations of LDL particles in the plasma. The subject therapeutic regimens involve administration of a PCSK9 antagonist antibody. In some embodiments, the subject therapeutic regimens involve administration of a PCSK9 antagonist antibody to a patient who has been receiving stable doses of a statin. The therapeutic regimens

disclosed herein provide an effective amount of a PCSK9 antagonist antibody that antagonizes circulating PCSK9 for use in treating or preventing hypercholesterolemia, and/or at least one symptom of dyslipidemia, atherosclerosis, cardiovascular disease, acute coronary syndrome (ACS), or coronary heart disease, in an individual.

Advantageously, the therapeutic regimens disclosed herein result in substantial and durable LDL-C lowering. Preferably, blood cholesterol and/or blood LDL is at least about 10% or 15% lower than before administration. More preferably, blood cholesterol and/or blood LDL is at least about 20, 30, 40, 50, 60, 70 or 80% lower than before administration of the antibody.

Dosing regimens

In some embodiments, a dosing regimen comprises administering an initial dose of about 2 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 2 mg/kg every 4 weeks. In other embodiments, a dosing regimen comprises administering an initial dose of about 4 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 4 mg/kg every 4 weeks. In other embodiments, a dosing regimen comprises administering an initial dose of about 4 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 4 mg/kg every 8 weeks. In other embodiments, a dosing regimen comprises administering an initial dose of about 8 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 8 mg/kg every 8 weeks. In other embodiments, a dosing regimen comprises administering an initial dose of about 12 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 12 mg/kg every 8 weeks.

In other embodiments, a dosing regimen comprises administering a weekly dose of about 0.25 mg/kg of the PCSK9 antibody. In other embodiments, a dosing regimen comprises administering a weekly dose of about 0.5 mg/kg of the PCSK9 antibody. In other embodiments, a dosing regimen comprises administering a weekly dose of about 1 mg/kg of the PCSK9 antibody. In other embodiments, a dosing regimen comprises administering a weekly dose of about 1.5 mg/kg of the PCSK9 antibody.

However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. The progress of this therapy is easily monitored by conventional techniques and assays. In preferred embodiments, the initial dose and the first subsequent and additional subsequent doses

are separated in time from each other by at least four weeks. The dosing regimen (including the PCSK9 antagonist(s) used) can vary over time.

Generally, for administration of PCSK9 antibodies, an initial candidate dosage can be about 0.3 mg/kg to about 18 mg/kg of the PCSK9 antagonist antibody. For the purpose of the present invention, a typical dosage might range from about any of about 3 µg/kg to 30 µg/kg to 300 µg/kg to 3 mg/kg, to 30 mg/kg, to 100 mg/kg or more, depending on the factors mentioned above. For example, dosage of about 0.3 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, about 10 mg/kg, about 10.5 mg/kg, about 11 mg/kg, about 11.5 mg/kg, about 12 mg/kg, about 12.5 mg/kg, about 13 mg/kg, about 13.5 mg/kg, about 14 mg/kg, about 14.5 mg/kg, about 15 mg/kg, about 15.5 mg/kg, about 16 mg/kg, about 16.5 mg/kg, about 17 mg/kg, about 17.5 mg/kg, about 18 mg/kg, about 18.5 mg/kg, about 19 mg/kg, about 19.5 mg/kg, about 20 mg/kg, about 20.5 mg/kg, about 21 mg/kg, about 21.5 mg/kg, about 22 mg/kg, about 22.5 mg/kg, about 23 mg/kg, about 23.5 mg/kg, about 24 mg/kg, about 24.5 mg/kg, and about 25 mg/kg may be used. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved, for example, to reduce blood LDL levels.

An exemplary dosing regimen comprises administering an initial dose of about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, or about 18 mg/kg, followed by a maintenance dose of about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, or about 18 mg/kg of the PCSK9 antibody. In some embodiments, the maintenance dose is administered weekly. In some embodiments, the maintenance dose is administered every other week. In some

embodiments, the maintenance dose is administered about every three weeks. In some embodiments, the maintenance dose is administered about every four weeks. In some embodiments, the maintenance dose is administered about every five weeks. In some embodiments, the maintenance dose is administered about every six weeks. In some embodiments, the maintenance dose is administered about every seven weeks. In some embodiments, the maintenance dose is administered about every eight weeks. In preferred embodiments, the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks. In some embodiments, the maintenance dose is administered monthly.

In other embodiments, a fixed dose may be used. For example, a PCSK9 antagonist antibody dose of about 0.25 mg, about 0.3 mg, about 0.5 mg, about 1 mg, about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, about 15 mg, about 16 mg, about 17 mg, about 18 mg, about 19 mg, about 20 mg, about 21 mg, about 22 mg, about 23 mg, about 24 mg, about 25 mg, about 26 mg, about 27 mg, about 28 mg, about 29 mg, about 30 mg, about 31 mg, about 32 mg, about 33 mg, about 34 mg, about 35 mg, about 36 mg, about 37 mg, about 38 mg, about 39 mg, about 40 mg, about 41 mg, about 42 mg, about 43 mg, about 44 mg, about 45 mg, about 46 mg, about 47 mg, about 48 mg, about 49 mg, about 50 mg, about 51 mg, about 52 mg, about 53 mg, about 54 mg, about 55 mg, about 56 mg, about 57 mg, about 58 mg, about 59 mg, about 60 mg, about 61 mg, about 62 mg, about 63 mg, about 64 mg, about 65 mg, about 66 mg, about 67 mg, about 68 mg, about 69 mg, about 70 mg, about 71 mg, about 72 mg, about 73 mg, about 74 mg, about 75 mg, about 76 mg, about 77 mg, about 78 mg, about 79 mg, about 80 mg, about 81 mg, about 82 mg, about 83 mg, about 84 mg, about 85 mg, about 86 mg, about 87 mg, about 88 mg, about 89 mg, about 90 mg, about 91 mg, about 92 mg, about 93 mg, about 94 mg, about 95 mg, about 96 mg, about 99 mg, about 98 mg, about 99 mg, about 100 mg, about 101 mg, about 102 mg, about 103 mg, about 104 mg, about 105 mg, about 106 mg, about 107 mg, about 108 mg, about 109 mg, about 110 mg, about 111 mg, about 112 mg, about 113 mg, about 114 mg, about 115 mg, about 116 mg, about 117 mg, about 118 mg, about 119 mg, about 120 mg, about 121 mg, about 122 mg, about 123 mg, about 124 mg, about 125 mg, about 126 mg, about 127 mg, about 128 mg, about 129 mg, about 130 mg, about

131 mg, about 132 mg, about 133 mg, about 134 mg, about 135 mg, about 136 mg, about 137 mg, about 138 mg, about 139 mg, about 140 mg, about 141 mg, about 142 mg, about 143 mg, about 144 mg, about 145 mg, about 146 mg, about 147 mg, about 148 mg, about 149 mg, about 150 mg, about 151 mg, about 152 mg, about 153 mg, about 154 mg, about 155 mg, about 156 mg, about 157 mg, about 158 mg, about 159 mg, about 160 mg, about 161 mg, about 162 mg, about 163 mg, about 164 mg, about 165 mg, about 166 mg, about 167 mg, about 168 mg, about 169 mg, about 170 mg, about 171 mg, about 172 mg, about 173 mg, about 174 mg, about 175 mg, about 176 mg, about 177 mg, about 178 mg, about 179 mg, about 180 mg, about 181 mg, about 182 mg, about 183 mg, about 184 mg, about 185 mg, about 186 mg, about 187 mg, about 188 mg, about 189 mg, about 190 mg, about 191 mg, about 192 mg, about 193 mg, about 194 mg, about 195 mg, about 196 mg, about 199 mg, about 198 mg, about 199 mg, about 200 mg, about 250, about 300, about 350, about 400, about 450, or about 500 mg may be used. In some embodiments, the fixed doses is administered subcutaneously or intravenously.

PCSK9 antagonist antibodies can be administered according to one or more dosing regimens disclosed herein to an individual on stable doses of a statin. The stable doses can be, for example without limitation, a daily dose or an every-other-day dose of a statin. A variety of statins known to those of skill in the art, and include, for example without limitation, atorvastatin, simvastatin, lovastatin, pravastatin, rosuvastatin, fluvastatin, cerivastatin, mevastatin, pitavastatin, and statin combination therapies. Non-limiting examples of statin combination therapies include atorvastatin plus amlodipine (CADUET™), simvastatin plus ezetimibe (VYTORIN™), lovastatin plus niacin (ADVICOR™), and simvastatin plus niacin (SIMCOR™).

In some embodiments, an individual has been on stable doses of a statin for at least one, two, three, four, five or six weeks prior to administration of an initial dose of PCSK9 antagonist antibody. Preferably, the individual on stable doses of a statin has a fasting LDL-C greater than or equal to about 70 mg/dL prior to administration of an initial dose of PCSK9 antagonist antibody. In some embodiments, the individual on stable doses of a statin has a fasting LDL-C greater than or equal to about 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200 mg/dL prior to administration of an initial dose of PCSK9 antagonist antibody.

For the purpose of the present invention, a typical statin dose might range from about 1 mg to about 80 mg, depending on the factors mentioned above. For example, a statin dose of about 0.3 mg, about 0.5 mg, about 1 mg, about 2.5 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, about 15 mg, about 16 mg, about 17 mg, about 18 mg, about 19 mg, about 20 mg, about 21 mg, about 22 mg, about 23 mg, about 24 mg, about 25 mg, about 26 mg, about 27 mg, about 28 mg, about 29 mg, about 30 mg, about 30 mg, about 31 mg, about 32 mg, about 33 mg, about 34 mg, about 35 mg, about 36 mg, about 37 mg, about 38 mg, about 39 mg, about 40 mg, about 41 mg, about 42 mg, about 43 mg, about 44 mg, about 45 mg, about 46 mg, about 47 mg, about 48 mg, about 49 mg, about 50 mg, about 51 mg, about 52 mg, about 53 mg, about 54 mg, about 55 mg, about 56 mg, about 57 mg, about 58 mg, about 59 mg, about 60 mg, about 61 mg, about 62 mg, about 63 mg, about 64 mg, about 65 mg, about 66 mg, about 67 mg, about 68 mg, about 69 mg, about 70 mg, about 71 mg, about 72 mg, about 73 mg, about 74 mg, about 75 mg, about 76 mg, about 77 mg, about 78 mg, about 79 mg, or about 80 mg may be used.

In preferred embodiments, a dose of 40 mg or 80 mg atorvastatin is used. In other embodiments, a dose of 20 mg or 40 mg rosuvastatin is used. In other embodiments, a dose of 40 mg or 80 mg simvastatin is used.

In some embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 2 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 2 mg/kg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 3 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 3 mg/kg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 4 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 4 mg/kg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 5 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 5 mg/kg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 4 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 4 mg/kg every 8 weeks. In other embodiments, a dosing

regimen comprises administering to a subject on stable doses of a statin an initial dose of about 6 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 6 mg/kg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 8 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 8 mg/kg every 8 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 12 mg/kg of the PCSK9 antibody, followed by a maintenance dose of about 12 mg/kg every 8 weeks.

In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 200 mg of the PCSK9 antibody subcutaneously, followed by a maintenance dose of about 200 mg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 300 mg of the PCSK9 antibody, followed by a maintenance dose of about 300 mg about every 4 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 50 mg of the PCSK9 antibody, followed by a maintenance dose of about 50 mg about every 2 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 100 mg of the PCSK9 antibody, followed by a maintenance dose of about 100 mg about every 2 weeks. In other embodiments, a dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 150 mg of the PCSK9 antibody, followed by a maintenance dose of about 150 mg about every 2 weeks.

Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 0.25 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 0.25 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 0.5 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 0.5 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 1 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly

maintenance dose of about 1 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 1.5 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 1.5 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 2 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 2 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 3 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 4 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 4 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 5 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 5 mg/kg of the PCSK9 antagonist antibody. Another exemplary dosing regimen comprises administering to a subject on stable doses of a statin an initial dose of about 6 mg/kg of the PCSK9 antagonist antibody. In some embodiments, the dosing regimen further comprises administering a monthly maintenance dose of about 6 mg/kg of the PCSK9 antagonist antibody.

However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. The progress of this therapy is easily monitored by conventional techniques and assays. In preferred embodiments, the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least four weeks. The dosing regimen (including the PCSK9 antagonist(s) used) can vary over time.

PCSK9 antagonist antibodies

A description follows as to an exemplary technique for the production of the antibodies used in accordance with the present invention. The PCSK9 antigen to be

used for production of antibodies may be, e.g. full-length human PCSK9, full length mouse PCSK9, and various peptides fragments of PCSK9. Other forms of PCSK9 useful for generating antibodies will be apparent to those skilled in the art.

Monoclonal antibodies were generated by immunizing PCSK9 null mice with recombinant full-length PCSK9 protein. This manner of antibody preparation yielded antagonist antibodies that show complete blocking of PCSK9 binding to LDLR, complete blocking of PCSK9-mediated lowering of LDLR levels in Huh7 cells, and lowering of LDL cholesterol levels in vivo including in mice to levels comparable to that seen in PCSK9 - /- mice, as shown in Example 7 of U.S. Patent Application No. 12/558312.

As will be appreciated, antibodies for use in the present invention may be derived from hybridomas but can also be expressed in cell lines other than hybridomas. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of suitable mammalian or nonmammalian host cells. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (e.g., Hep G6). Non-mammalian cells can also be employed, including bacterial, yeast, insect, and plant cells. Site directed mutagenesis of the antibody CH6 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The glutamine synthase system of expression is discussed in whole or part in connection with European Patents 616 846, 656 055, and 363 997 and European Patent Application 89303964.4. Further, a dihydrofolate reductase (DHFR) expression system, including those known in the art, can be used to produce the antibody.

In some embodiments, the invention is practiced using the PCSK9 antagonist antibody L1L3. In some embodiments, the invention is practiced using an antibody that recognizes an epitope of PCSK9 that is the same as the epitope that is recognized by antibody L1L3.

In some embodiments, the invention is practiced using an antibody comprising three CDRS from a heavy chain variable region having the amino acid sequence shown

in SEQ ID NO: 11 and three CDRS from a light chain variable region having the amino acid sequence shown in SEQ ID NO: 12.

In some embodiments, the invention is practiced using an antibody that specifically binds PCSK9 comprising a VH complementary determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 2 (SYYMH), SEQ ID NO: 13 (GYTFTSY), or SEQ ID NO: 14 (GYTFTSYYMH); a VH CDR2 having the amino acid sequence shown in SEQ ID NO: 3 (EISPFGGRTNYNEKFKS) or SEQ ID NO: 15 (ISPFGGR), and/or VH CDR3 having the amino acid sequence shown in SEQ ID NO: 4 (ERPLYASDL), or a variant thereof having one or more conservative amino acid substitutions in said sequences of CDR1, CDR2, and/or CDR3, wherein the variant retains essentially the same binding specificity as the CDR defined by said sequences. Preferably, the variant comprises up to about ten amino acid substitutions and, more preferably, up to about four amino acid substitutions.

In some embodiments, the invention is practiced using an antibody comprising a VL CDR1 having the amino acid sequence shown in SEQ ID NO: 5 (RASQGISSALA), a CDR2 having the amino acid sequence shown in SEQ ID NO: 6 (SASYRYT), and/or CDR3 having the amino acid sequence shown in SEQ ID NO: 7 (QQRYSLWRT), or a variant thereof having one or more conservative amino acid substitutions in said sequences of CDR1, CDR2, and/or CDR3, wherein the variant retains essentially the same binding specificity as the CDR1 defined by said sequences. Preferably, the variant comprises up to about ten amino acid substitutions and, more preferably, up to about four amino acid substitutions.

In some embodiments, the invention is practiced using an antibody having a heavy chain sequence comprising or consisting of SEQ ID NO: 8 or 10 and a light chain sequence comprising or consisting of SEQ ID NO: 9.

In some embodiments, the invention is practiced using an antibody having a heavy chain variable region comprising or consisting of the amino acid sequence shown in SEQ ID NO: 11 and a light chain variable region comprising or consisting of the amino acid sequence shown in SEQ ID NO: 12.

In some embodiments, the invention is practiced using an antibody that recognizes an epitope on human PCSK9 comprising amino acid residues 153-155, 194, 195, 197, 237-239, 367, 369, 374-379 and 381 of the PCSK9 amino acid sequence of SEQ ID NO: 1. Preferably, the antibody epitope on human PCSK9 does not comprise

one or more of amino acid residues 71, 72, 150-152, 187-192, 198-202, 212, 214-217, 220-226, 243, 255-258, 317, 318, 347-351, 372, 373, 380, 382, and 383 of the PCSK9 amino acid sequence of SEQ ID NO: 1.

In some embodiments, the invention is practiced using an antibody that recognizes a first epitope of PCSK9 that is the same as or overlaps with a second epitope that is recognized by a monoclonal antibody selected from the group consisting of 5A10, which is produced by a hybridoma cell line deposited with the American Type Culture Collection and assigned accession number PTA-8986; 4A5, which is produced by a hybridoma cell line deposited with the American Type Culture Collection and assigned accession number PTA-8985; 6F6, which is produced by a hybridoma cell line deposited with the American Type Culture Collection and assigned accession number PTA-8984, and 7D4, which is produced by a hybridoma cell line deposited with the American Type Culture Collection and assigned accession number PTA-8983. In preferred embodiments, the invention is practiced using the PCSK9 antagonist antibody L1L3 (see, PCT/IB2009/053990, published March 18, 2010 as WO 2010/029513, and U.S. Patent Application No. 12/558312, published March 18, 2010 as US 2010/0068199).

Preferably, the variant comprises up to about twenty amino acid substitutions and more preferably, up to about eight amino acid substitutions. Preferably, the antibody further comprises an immunologically inert constant region, and/or the antibody has an isotype that is selected from the group consisting of IgG_2, IgG_4, $IgG_{2\Delta a}$, $IgG_{4\Delta b}$, $IgG_{4\Delta c}$, IgG_4 S228P, $IgG_{4\Delta b}$ S228P and $IgG_{4\Delta c}$ S228P. In another preferred embodiment, the constant region is aglycosylated Fc.

The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a domain antibody), human antibodies, humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies).

In some embodiments, the PCSK9 antagonist antibody is a monoclonal antibody. The PCSK9 antagonist antibody can also be humanized. In other embodiments, the antibody is human.

In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert, that is, having a reduced potential for provoking an immune response. In some embodiments, the constant region is modified as described in Eur. J. Immunol., 1999, 29:2613-2624; PCT Publ. No. WO99/58572; and/or UK Patent Application No. 9809951.8. The Fc can be human IgG_2 or human IgG_4. The Fc can be human IgG_2 containing the mutation A330P331 to S330S331 ($IgG_{2\Delta a}$), in which the amino acid residues are numbered with reference to the wild type IgG2 sequence. Eur. J. Immunol., 1999, 29:2613-2624. In some embodiments, the antibody comprises a constant region of IgG_4 comprising the following mutations (Armour et al., 2003, Molecular Immunology 40 585-593): E233F234L235 to P233V234A235 ($IgG_{4\Delta c}$), in which the numbering is with reference to wild type IgG4. In yet another embodiment, the Fc is human IgG_4 E233F234L235 to P233V234A235 with deletion G236 ($IgG_{4\Delta b}$). In another embodiment the Fc is any human IgG_4 Fc (IgG_4, $IgG_{4\Delta b}$ or $IgG_{4\Delta c}$) containing hinge stabilizing mutation S228 to P228 (Aalberse et al., 2002, Immunology 105, 9-19). In another embodiment, the Fc can be aglycosylated Fc.

In some embodiments, the constant region is aglycosylated by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the glycosylation recognition sequence in the constant region. In some embodiments, the constant region is aglycosylated for N-linked glycosylation enzymatically. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

In some embodiments, more than one antagonist antibody may be present. At least one, at least two, at least three, at least four, at least five different, or more antagonist antibodies and/or peptides can be present. Generally, those PCSK9 antagonist antibodies or peptides may have complementary activities that do not adversely affect each other. A PCSK9 antagonist antibody can also be used in conjunction with other PCSK9 antagonists or PCSK9 receptor antagonists. For example, one or more of the following PCSK9 antagonists may be used: an antisense molecule directed to a PCSK9 (including an anti-sense molecule directed to a nucleic acid encoding PCSK9), a PCSK9 inhibitory compound, and a PCSK9 structural analog. A

PCSK9 antagonist antibody can also be used in conjunction with other agents that serve to enhance and/or complement the effectiveness of the agents.

With respect to all methods described herein, reference to PCSK9 antagonist antibodies also include compositions comprising one or more additional agents. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art. The present invention can be used alone or in combination with other conventional methods of treatment.

The PCSK9 antagonist antibody can be administered to an individual via any suitable route. It should be apparent to a person skilled in the art that the examples described herein are not intended to be limiting but to be illustrative of the techniques available. Accordingly, in some embodiments, the PCSK9 antagonist antibody is administered to an individual in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, transdermal, subcutaneous, intra-articular, sublingually, intrasynovial, via insufflation, intrathecal, oral, inhalation or topical routes. Administration can be systemic, e.g., intravenous administration, or localized. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, PCSK9 antagonist antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

In one embodiment, a PCSK9 antagonist antibody is administered via site-specific or targeted local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the PCSK9 antagonist antibody or local delivery catheters, such as infusion catheters, indwelling catheters, or needle catheters, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct application. See, e.g., PCT Publ. No. WO 00/53211 and U.S. Patent No. 5,981,568.

Various formulations of a PCSK9 antagonist antibody may be used for administration. In some embodiments, the PCSK9 antagonist antibody may be administered neat. In some embodiments, PCSK9 antagonist antibody and a pharmaceutically acceptable excipient may be in various formulations. Pharmaceutically

acceptable excipients are known in the art, and are relatively inert substances that facilitate administration of a pharmacologically effective substance. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing (2000).

These agents can be combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history.

Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and may comprise buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Liposomes containing the PCSK9 antagonist antibody are prepared by methods known in the art, such as described in Epstein, et al., 1985, Proc. Natl. Acad. Sci. USA 82:3688; Hwang, et al., 1980, Proc. Natl Acad. Sci. USA 77:4030; and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-

PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing (2000).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or 'poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic PCSK9 antagonist antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Suitable emulsions may be prepared using commercially available fat emulsions, such as Intralipid™, Liposyn™, Infonutrol™, Lipofundin™ and Lipiphysan™. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g., soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (e.g., egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil,

for example, between 5 and 20%. The fat emulsion can comprise fat droplets between 0.1 and 1.0 μm, particularly 0.1 and 0.5 μm, and have a pH in the range of 5.5 to 8.0.

The emulsion compositions can be those prepared by mixing a PCSK9 antagonist antibody with Intralipid™ or the components thereof (soybean oil, egg phospholipids, glycerol and water).

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Polynucleotides encoding the heavy and light chain variable regions of antibody L1L3 were deposited in the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 90110, U.S.A., on August 25, 2009. The L1L3 heavy chain variable region polynucleotide deposit was assigned ATCC Accession No. PTA-10302, and the L1L3 light chain variable region polynucleotide deposit was assigned ATCC Accession No. PTA-10303. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules

pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Examples

The following examples are meant to illustrate the methods and materials of the present invention. Suitable modifications and adaptations of the described conditions and parameters normally encountered in the art that are obvious to those skilled in the art are within the spirit and scope of the present invention.

Example 1: Treatment with a humanized PCSK9 antagonist antibody L1L3 is effective for reducing in serum cholesterol and LDL cholesterol levels

This example illustrates efficacy of a humanized PCSK9 antagonist antibody, L1L3, in reducing serum cholesterol and LDL cholesterol levels in animal models.

L1L3 is a humanized (<5% murine residues) monoclonal antibody that binds to secreted PCSK9, effectively prevents its down-regulation of LDLR, leading to improved LDL clearance in serum and reduction of LDL-C.

When 10 mg/kg of L1L3 was administered as a single intraperitoneal (IP) dose to *C57BL/6* mice fed a normal diet (n=10), serum cholesterol levels were reduced to 47 mg/dL (37% reduction) compared to 75 mg/dL in saline treated controls 48 hours post treatment and 44 mg/dL (47% reduction) compared to 83 mg/dL in control animals 4 days post-treatment. Serum cholesterol levels recovered to 69 mg/dL by day 7 post-treatment.

L1L3 was administered as a single IP dose at 0, 0.1, 1, 10 and 80 mg/kg (n=6/group) in a dose-response experiment in Sprague-Dawley rats fed a normal diet. Serum cholesterol levels were dose-dependently reduced, with maximum effect of 50% seen at 10 and 80 mg/kg 48 hours post dosing. The duration of the cholesterol repression was also dose dependent, ranging from 1 to 21 days. Both the magnitude

and duration of the cholesterol-lowering effect of L1L3 correlated with drug exposure. Non-fasting serum triglyceride levels also dose-dependently increased, with a maximum increase of approximately three fold at 80 mg/kg, and a time course correlated with drug exposure. Since similar effects of L1L3 on serum triglyceride levels were not observed in other species such as mice and non-human primate (see below), and changes in blood triglyceride levels were not reported in humans harboring PCSK9 mutations (Abifadel et al., 2003, Nat. Genet., 34:154-156; Cohen et al., 2005, Nat. Genet. 37:161-165; Zhao et al., 2006, Am. J. Hum. Genet. 79:514-523), the increase in serum triglyceride levels caused by L1L3 treatment appears to be a species-specific phenomenon in rat.

In cynomolgus monkeys, fed a normal diet, L1L3 was administered as a single IV dose at 0.1, 1, 3 and 10 mg/kg (n=4/group). Administration of 0.1 mg/kg L1L3 caused a transient 50% drop in LDL-C levels at day 2 and quickly recovered by day 5. One (1) mg/kg dosing reached a maximum effect of 71% reduction in LDL-C on day 5, and began to recover immediately thereafter, reaching pre-dose levels by day 14. Three (3) mg/kg dosing reached a maximum effect of 72% reduction in LDL-C by day 7, levels began to recover by day 13, and returned to baseline by day 22. Ten (10) mg/kg dosing maintained the 70% reduction in LDL-C levels until day 21 post-dosing, and animals fully recovered by day 31. Both the magnitude and duration of the LDL -C lowering effect of L1L3 correlated with drug exposure. HDL-C levels were not affected by L1L3 treatment in all dose groups.

The monkeys in the 3 mg/kg dose group (n=4) were also given two additional IV doses of 3 mg/kg L1L3 on study days 42 and 56 (2-weeks apart). These two additional doses again lowered LDL-C and kept LDL-C levels below 50% for 4 weeks. LDL-C levels returned to normal two weeks later. Serum HDL-C levels remained unchanged.

PK studies were conducted by a single bolus i.v. injection of 0.1, 1.0, 3.0, 10.0 and 100.0 mg/kg of L1L3 in cynomolgus monkeys and total antibody concentration was measured. The estimated β-phase half-life for L1L3 was 0.67 days at a single dose of 0.1mg/kg, and increased to 1.91, 2.33, 3.49 and 5.25 days at 1.0, 3.0, 10.0 and 100.0 mg/kg, respectively. Thus, in cynomolgus monkeys, L1L3 demonstrated a dose-dependent and non-linear shortening of half-life consistent with antigen mediated degradation and seen with antibody therapeutics having membrane-associated antigens.

In summary, L1L3 binds to and antagonizes serum PCSK9 function, resulting in rapid and significant reduction in serum cholesterol and LDL cholesterol levels in animal models.

Example 2: Pharmacokinetics and pharmacodynamics following single, escalating, intravenous doses of PCSK9 antagonist antibody L1L3

This example illustrates a clinical trial study to evaluate pharmacokinetics and pharmacodynamics following single, escalating, intravenous doses of a humanized PCSK9 antagonist antibody, L1L3, in otherwise healthy human subjects who were candidates for cholesterol lowering therapy. Administration of L1L3 resulted in a lowering of LDL-C in all dosage groups evaluated.

The study entailed a randomized, placebo-controlled, ascending, single dose study of L1L3. The subjects, investigator, and site personnel (except site personnel responsible for drug preparation) were blinded to treatment assignments, as was the CRO designee; while the Sponsor clinical research team was unblinded. The study was conducted in 6 planned cohorts of 8 subjects per cohort in an effort to seek a maximum tolerated dose or MTD (total of approximately 48 subjects). Within each cohort subjects were randomized to either L1L3 or placebo (3:1 allocation ratio). Doses were administered following an overnight fast as an intravenous infusion over 60 minutes. Infusion rates were carefully controlled by an infusion device per protocol. Infusions will be administered as a single infusion over 60 minutes.

Dosing was as illustrated below in Table 1:

Table 1

Cohort	Dose L1L3	Number of Subjects Dosed
1	0.3 mg/kg Placebo	6 2
2	1.0 mg/kg Placebo	6 2
3	3.0 mg/kg Placebo	6 2
4	6.0 mg/kg Placebo	6 2
5	12 mg/kg Placebo	6 2
6	18 mg/kg Placebo	6 2

The dosing schedule was adjusted to allow administration of lower, intermediate, or higher doses to obtain a maximum tolerated dose and no effect dose. Each subject enrolled into the study, regardless of cohort assignment, received only one dose of study drug during their study participation. All patients were observed for safety for an additional 21 days (total 28 days) prior to their study completion.

The primary PK endpoints of the study were $AUC_{(0\text{-}t[last])}$, T_{max}, and C_{max} of L1L3. Secondary PK endpoints included terminal elimination half-life ($T_{1/2}$), Clearance (CL), Volume in steady state (Vss), and $AUC_{(0\text{-}\infty)}$ of L1L3. Change in serum lipids (total cholesterol, LDL, HDL, Triglycerides, Non-HDL-C and Apoprotein B) were assessed.

Screening occurred within 28 days of the dose for each subject. Subjects received a single dose of L1L3 on Day 0, with daily PK and safety assessments through confinement period (study Days -1, 0, and 1) as well as on days 4, 7, 14, 21, 28 and, depending upon initial PK findings, after day 28.

Inclusion criteria for the study were as follows: healthy, ambulatory, males and/or females (females will be women of non-childbearing potential) between the ages of 18 and 70 years, inclusive; baseline total cholesterol ≥ 200 mg/dl, baseline LDL ≥ 130 mg/dl; body mass index (BMI) of 18.5 to 35 kg/m^2 BMI 18.5 to 35, and body weight ≤ 150kg, inclusive; evidence of a personally signed and dated informed consent document indicating that the subject (or a legally acceptable representative) has been informed of all pertinent aspects of the trial; and willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other trial procedures.

Exclusion criteria for the study were as follows: evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing); secondary hyperlipidemia; subjects should not have taken other prescription medications for at least 1 week prior to dosing. If patients have received lipid lowering medications these drugs should have been discontinued for an adequate period of time to allow return of serum lipids to pretreatment levels; history of febrile illness within 5 days prior to dosing; history of stroke or transient ischemic attack; history of myocardial infarction within the past year; a positive urine drug screen; history of regular alcohol consumption exceeding 7 drinks/week for females or 14 drinks/week for men (1 drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of hard liquor)

within 6 months of screening; treatment with an investigational drug within 30 days or 5 half-lives (whichever is longer) preceding the first dose of trial medication; 12-lead ECG demonstrating QTc >450 msec at screening; pregnant or nursing females; women of childbearing potential; blood donation of approximately 1 pint (500 mL) within 56 days prior to dosing; history of sensitivity to heparin or heparin-induced thrombocytopenia (if heparin is used to flush intravenous catheters; other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the subject inappropriate for entry into this study.

Subjects were randomized into the study provided they have satisfied all subject selection criteria. A computer-generated randomization schedule was used to assign subjects to the treatment sequences.

For dose escalation, the decision to proceed to a higher dose of L1L3 was made by the Sponsor and the Investigator after review of the available safety and tolerability data from all cohort subjects followed for at least 7 days following administration of the previous dose level.

L1L3 drug product (100 mg) was provided in sterile, liquid form at a concentration of 10 mg/mL in a glass vial for intravenous (IV) administration, with a rubber stopper and aluminum seal. Each vial contained 10 mL (extractable volume) of L1L3 at a concentration of 10 mg/mL and a pH of 5.5. L1L3 and placebo were prepared according to the Dosage and Administration Instructions in the Pharmacy Manual that will be provided to the site. Drug was prepared by qualified unblinded site personnel and dispensed in a blinded fashion to the patient and immediate study staff. L1L3 was administered by rate controlled intravenous infusion over approximately 60 minutes in accordance with the Dosage Administration Instructions (DAI) located in the Pharmacy Manual and Study Reference Guide.

Study protocol

Day -1: Subjects were assigned a randomization number and admitted to the Clinical Research Unit at least 12 hours prior to the start of Day 0 activities and were required to remain in the Clinical Research Unit (CRU) until completion of procedures on Day 1. Subject began fasting in the evening at least 10 hours prior to scheduled Lipid Panel for Day 0. The following procedures were completed: reviewed changes in

medical history since screening; reviewed changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”; physical examination, including weight; urine drug screen; obtained supine vital signs; obtained triplicate 12-lead ECGs approximately 2-4 minutes apart

Day 0: Prior to dosing, the following procedures were completed: collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected sample for pre-dose PK; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; reviewed changes in concomitant medications since screening; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”; obtained supine vital signs; administered Study Drug Infusion according to Pharmacy Manual Instructions.

After dosing, the following procedures were completed: obtained triplicate 12-lead ECGs approximately 2-4 minutes apart beginning within 10 minutes of end of infusion (EOI); obtained supine vital signs at EOI; collected blooded sample for PK analysis at EOI, and the following timepoints post infusion (i.e. EOI + the following timepoints): 60 minutes, 120 min., and 360 min.

Day 1: The following procedures were completed: collected blood sample for PK analysis at 1440 min (24 hours) +/- 30 min post dose; performed abbreviated physical exam; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected sample for PCSK9 levels/PD markers of interest; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as “How do you feel?”; reviewed changes in concomitant medications since screening; obtained supine vital signs; discharged from CRU.

Day 4: The following procedures were completed: collected samples for routine laboratory tests: hematology; chemistry; and urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for

PCSK9 levels/PD markers of interest; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed changes in concomitant medications since screening; obtained supine vital signs

Day 7: The following procedures were completed: performed abbreviated physical exam; collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; obtained supine vital signs; obtained triplicate 12-lead ECGs approximately 2-4 minutes apart.

Day 14: The following procedures were completed: performed abbreviated physical exam; collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; obtained supine vital signs.

Day 21: The following procedures were completed: performed abbreviated physical exam; collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed

changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; obtained supine vital signs.

Day 28: The following procedures were completed: performed full physical exam; obtained subject's weight; collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; obtained supine vital signs; obtained triplicate 12-lead ECGs approximately 2-4 minutes apart.

Additional Follow-up for Prolonged PK: The following procedures were completed when applicable: performed abbreviated physical ; collected samples for routine and additional laboratory tests: hematology; chemistry; coagulation, amylase; urinalysis; collected fasting lipid profile after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides); collected single blood sample for PK analysis; collected sample for PCSK9 levels/PD markers of interest; collected sample for Anti-L1L3 antibodies; assessed symptoms by spontaneous reporting of adverse events and by asking the subjects to respond to a non-leading question such as "How do you feel?"; reviewed changes in concomitant medications since screening; reviewed history of drug, alcohol, and tobacco use since screening; obtained supine vital signs; obtained triplicate 12-lead ECGs approximately 2-4 minutes apart.

Total blood sampling volume for individual patients was approximately 183-210 mL. Plasma samples for analysis of L1L3 levels were collected before dosing on Day 0, at termination of infusion, and at 60, 120, 360 and 1440 minutes (24-hours) after infusion ends. In addition, single PK samples were obtained on Days 4, 7, 14, 21, 28 and additional PK follow-up visit (if applicable). One sample was drawn at each time point.

Blood samples for assessment of PCSK9 levels and other experimental pharmcaodynamic markers of interest were obtained pre-dose on Day 0 and Days 1, 4, 7, 14, 21, 28 and additional follow-up visit if applicable.

Collection of fasting lipid profile was performed after at least a 10-hour fast (total cholesterol, LDL, HDL, Non-HDL Cholesterol, Apo B and triglycerides).

Study results

L1L3 PK NCA Results: The median half-life of L1L3 administered at 0.3 mg/kg was 2.71 days. The median half-life of L1L3 administered at 1 mg/kg was 4.77 days. The median half-life of L1L3 administered at 3 mg/kg was 8.1 days. The median half-life of L1L3 administered at 6 mg/kg was 7.75 days. The median half-life of L1L3 administered at 12 mg/kg was 12.24 days. The median half-life of L1L3 administered at 18 mg/kg was 11.76 days. The L1L3 PK concentration-time profiles were multi-phasic and consistent with target-mediated drug disposition. However, the half-life of L1L3 in human subjects is unexpectedly and significantly longer than the half-life of L1L3 in cynomologus monkeys (i.e., 1.91, 2.33, 3.49 and 5.25 days at 1.0, 3.0, 10.0 and 100.0 mg/kg, respectively, in cynomologus monkeys (see, Example 1)). The mean rate of drug clearance (Cl) for L1L3 administered at 0.3, 1, 3, 6, 12 and 18 mg/kg was 8.70, 6.58, 4.54, 4.33, 3.28 and 3.85 mL/Day/kg, respectively. The PK NCA results from this study are summarized in Table 2 below. In columns 2-7 of the table, the top value indicates the mean, and the bottom value is the median.

Table 2: PK NCA Results

DOSE (mg/kg)	**Cmax** (ng/mL)	**Tmax** (Day)	**Half-life** (Day)	**Cl** (mL/Day/kg)	**Vss** (mL/kg)	**$AUC_{(0-\infty)}$** (Day•ng/mL)
0.3	10319.67 10537.50	0.083 0.06	2.74 2.71	8.70 8.92	31.77 30.74	34997.88 33748.15
1	29251.83 28231.50	0.063 0.06	4.80 4.77	6.58 6.00	41.59 42.34	156399.94 166736.58
3	96711.50 100620.5	0.049 0.04	8.74 8.1	4.54 4.12	49.06 48.94	709485.10 728278.54
6	175854.33 177485	0.056 0.04	8.36 7.75	4.33 4.65	60.45 61.33	1446945.71 1289916.44
12	353960.17 357671.00	0.090 0.08	20.53 12.24	3.28 3.36	72.25 57.52	3768691.17 3599992.39
18	532449.17 560463.50	0.090 0.08	12.97 11.76	3.85 3.71	65.46 60.83	4812012.99 4857618.28

Treatment with L1L3 resulted in substantial and durable dose-dependent fasting LDL-cholesterol (LDL-C) lowering. The LDL-C vs. time profiles are shown in FIG. 1. The baseline fasting LDL-C was about 145 mg/dL. At day 7 post-dosing, LDL-C levels in subjects treated with a single 0.3, 1, 3, 6, 12, or 18 mg/kg dose of L1L3 were between 50 and 100 mg/dL. In contrast, LDL-C levels in subjects administered placebo remained generally about baseline. By day 14 post-dosing, LDL-C levels in subjects treated with 1, 3, 6, 12, or 18 mg/kg L1L3 were about 70 mg/dL or lower. By day 14 post-dosing, subjects treated with 6 mg/kg or 12 mg/kg L1L3 had LDL-C levels of about 55 mg/dL, and subjects treated with 18 mg/kg L1L3 had LDL-C levels of about 20 mg/dL. LDL-C levels in subjects treated with a single 12 mg/kg dose of L1L3 remained at or below about 60 mg/dL until at least about 57 days post-dosing (end of study). LDL-C levels in subjects treated with a single 18 mg/kg dose of L1L3 remained below 50 mg/dL until at least about 57 days post-dosing. LDL-C levels in subjects treated with a single 6 mg/kg dose of L1L3 remained below 50 mg/dL for about 42 days post-dosing and below 100 mg/dL until at least about 57 days post-dosing. LDL-C levels in subjects treated with a single 3 mg/kg dose of L1L3 were about 70 mg/dL at day 14 post-dosing, about 60 mg/dL at day 21 post-dosing, and remained below 100 mg/dL until about 36 days post-dosing. LDL-C levels in subjects treated with a single 1 mg/kg dose of L1L3 were about 65 mg/dL at day 14 post-dosing, and remained below 100 mg/dL until about 21 days post-dosing. LDL-C levels in subjects treated with a single 0.3 mg/kg dose of L1L3 were about 85 mg/dL at day 7 post-dosing, and remained below 100 mg/dL until about 10 days post-dosing.

The percentage change from baseline of fasting LDL-C levels in blood is shown in FIG. 2 (data shown are mean +/- SE) and summarized in Table 3 below. In the table, “N” indicates the number of subjects, “mean” indicates the mean percentage change from baseline of fasting LDL-C levels, and “PBO” is placebo.

Table 3

Visit day	PBO		L1L3											
			0.3 mg/kg		1 mg/kg		3 mg/kg		6 mg/kg		12 mg/kg		18 mg/kg	
	N	mean	N	mean	N	mean	N	mean	N	mean	N	mean	N	mean
1	12	0.000	6	0.000	6	0.000	6	0.000	6	0.000	6	0.000	6	0.000
2	12	3.26	6	-15.8	6	-0.33	6	-1.97	6	-1.44	6	-8.26	4	-11.52
3	12	-0.5	6	-14.13	6	-9.79	6	-13.20	6	-9.23	6	-14.05	6	-22.43
4	12	2.25	6	-30.14	6	-19.14	6	-19.10	6	-18.80	5	-23.23	6	-34.36
8	11	8.32	6	-42.86	6	-33.33	6	-39.78	6	-43.77	5	-37.96	5	-43.70
15	11	-3.24	6	-23.68	6	-50.50	6	-57.93	6	-61.52	5	-66.25	5	-82.89
22	11	6.26	6	-11.36	6	-22.40	6	-65.09	6	-68.92	5	-59.79	6	-72.97
29	11	11.87	6	-9.12	6	-3.36	6	-64.77	6	-64.19	6	-74.67	6	-67.40
36	5	17.38							6	-67.70	5	-65.23	4	-61.47
43	5	12.14					3	-27.56	6	-64.18	4	-69.31	3	-80.21
50	4	3.67							6	-49.17	4	-56.08		
57	4	12.08							6	-36.12	3	-63.10		

LDL-C levels in subjects dosed with placebo remained generally at or above baseline, indicated as “0” in FIG. 2. As noted above, the baseline fasting LDL-C was about 145 mg/dL. Administration of 18 mg/kg L1L3 resulted in a percentage change from baseline of up to about 83% (FIG. 2). A single 18 mg/kg L1L3 dose maintained LDL-C levels lower than about 65% below baseline for at least up to 57 days post administration. A single 6 mg/kg or 12 mg/kg L1L3 dose maintained LDL-C levels lower than about 60% below baseline up to 43 days post administration. A single 3 mg/kg L1L3 dose maintained LDL-C levels lower than about 60% below baseline up to 29 days post administration, and lower than 20% below baseline up to 50 days post administration.

Treatment with L1L3 resulted in substantial and durable dose-dependent fasting total cholesterol (TC) lowering. The percentage change from baseline of fasting TC levels in blood is shown in FIG. 3 (data shown are mean +/- 2 SE). The baseline fasting TC was about 230 mg/dL; baseline is indicated as “0” in FIG. 3. By about day 9 after dosing, TC levels in subjects dosed with a single dose of 12 or 18 mg/kg L1L3 were reduced to about 30% below baseline or lower; the TC lowering effect lasted at least to

day 57 post-dosing (end of study). TC levels in subjects dosed with a single dose of 6 mg/kg L1L3 were reduced to about 30% below baseline or lower by about day 9 after dosing until about day 52 post-dosing. TC levels in subjects dosed with a single dose of 3 mg/kg L1L3 were reduced to about 30% below baseline by about day 9 after dosing, and about 40% below baseline by about day 22 after dosing. TC levels in subjects dosed with a single dose of 3 mg/kg L1L3 were reduced to about 40% below baseline by about day 22 after dosing. TC levels in subjects dosed with a single dose of 1 mg/kg L1L3 were reduced to about 36% below baseline by about day 15 after dosing. TC levels in subjects dosed with a single dose of 0.3 mg/kg L1L3 were reduced to about 25% by about day 9 after dosing. By day 15 post-dosing, a number of subjects had TC levels lower than 50% below baseline after dosing with a single dose of 12 or 18 mg/kg L1L3. By day 30 post-dosing, a number of subjects had TC levels lower than 50% below baseline after dosing with a single dose of 6 mg/kg L1L3. TC levels in subjects dosed with placebo remained at or above 2% below baseline for the duration of the study.

Treatment with L1L3 resulted in substantial and durable dose-dependent fasting apolipoprotein B (apo B) lowering. The percentage change from baseline of fasting apo B levels in blood is shown in FIG. 4. Data shown are mean +/- 2 SE. The baseline fasting apo B level was about 119 mg/dL; baseline is indicated as “0” in FIG. 4. Apo B levels in subjects dosed with placebo remained about baseline for the duration of the study. Apo B levels in subjects dosed with 12 or 18 mg/kg L1L3 were reduced to about 50% below baseline by day 14, and remained at about 50% below baseline or lower for the remainder of the study. Apo B levels in subjects dosed with 6 mg/kg L1L3 were reduced to about 40% below baseline by day 14, about 50% below baseline by day 21, and generally below about 30% below baseline for the remainder of the study. Apo B levels in subjects dosed with 3 mg/kg L1L3 were reduced to about 40% below baseline by day 14, about 50% below baseline by day 28. Apo B levels in subjects dosed with 1 mg/kg L1L3 were reduced to about 40% below baseline by day 14. Apo B levels in subjects dosed with 0.3 mg/kg L1L3 were reduced to about 25% below baseline by day 7.

As shown in FIG. 5, high density lipoprotein cholesterol (HDL-C) levels did not change significantly after treatment with L1L3. Data shown in FIG. 5 are mean +/- 2 SE. The baseline fasting HDL-C level was about 49 mg/dL; baseline is indicated as “0” in FIG. 5. HDL-C levels in subjects dosed with placebo remained about baseline for the

duration of the study. Fasting triglyceride (TGs) levels remained unchanged during the study. The percentage change from baseline of fasting TG levels in blood is shown in FIG. 6. Data shown are mean +/- 2 SE. The baseline fasting TG level was 173 mg/dL; baseline is indicated as "0" in FIG. 6.

In the study, no serious adverse events occurred, and there were no subjects discontinued due to treatment emergent adverse events (TEAEs). The majority of TEAEs were mild in intensity; none were severe.

In summary, administration of L1L3 resulted in a lowering of LDL-C in all dosage groups evaluated. In general, maximum percentage LDL-C lowering occurred in measurements taken on Day 15 or Day 22. The lowering effects were seen as early as Day 3. The extent and duration of LDL-C lowering was dose-dependent. The results demonstrate L1L3 has a long duration of action, i.e., with maximum effect for 7 and 14 days, for doses of 0.3 mg/kg and 1.0 mg/kg, respectively, for up to 4 weeks for a 3.0 mg/kg dose, and for more than 6 weeks, at doses of 6 mg/kg, 12 mg/kg, and 18 mg/kg L1L3 antibody. These duration effects were unexpected based upon the T½ data for L1L3.

Example 3: Pharmacokinetics and pharmacodynamics of a single dose of PCSK9 antagonist antibody L1L3 in combination with statin

This example illustrates a clinical trial study to evaluate pharmacokinetics and pharmacodynamics of a single dose of PCSK9 antagonist antibody (L1L3) in human subjects on stable doses of atorvastatin.

In the study, human subjects on stable doses of atorvastatin were administered a single dose of L1L3 antibody at either 0.5 mg/kg or 4 mg/kg of the PCSK9 antagonist antibody. L1L3 was administered as a single infusion over approximately 60 minutes. Infusion rates were carefully controlled by an infusion device per protocol. Atorvastatin (40 mg daily) was administered as described below in the study protocol. Subjects self-administered atorvastatin during their participation in this study except from Days 1 through 7 during their confinement to the clinic where the same dose was administered by qualified site personnel.

L1L3 Injection, 10 mg/mL, was presented as a sterile solution for intraveneous (IV) administration. Each vial contained 100 mg of L1L3 in 10 mL of aqueous buffered

solution, and was sealed with a coated stopper and an aluminum seal. Atorvastatin (40 mg) is a white tablet coded “PD 157” on one side and “40” on the other.

Screening took place within 28 days of the dose for each subject. Subjects were on stable dosages of atorvastatin for at least 45 days prior to screening. Subjects received a single dose of L1L3 on Day 4, with multiple PK and safety assessments through the confinement period (study Days -1, 1-7). The subjects returned to the clinical research unit for subsequent visits.

Key inclusion criteria for the subjects were: on stable doses of atorvastatin (40 mg daily) for 45 days prior to Day 1, body mass index (BMI) of 18.5 to 40 kg/m2 inclusive, and body weight equal or lower than 150 kg. Key exclusion criteria for the subjects were: history of a cardiovascular event (e.g., myocardial infarction (MI)) during the past year; poorly controlled Type 1 or Type 2 Diabetes mellitus (definition: uncontrolled diabetes is defined as HBIAc >9%); and poorly controlled hypertension (uncontrolled hypertension is defined as a systolic blood pressure greater than 140 mm Hg or a diastolic blood pressure greater than 90 mm Hg, even with treatment). Subjects who have hypertension and are controlled on stable dosages of anti-hypertensive medications could be included. The study included both genders, with a minimum age limit of 18 and a maximum age limit of 80.

Pharmacokinetics parameter estimates of L1L3 antibody in the presence of atorvastatin and of atorvastatin were evaluated after a single dose of 0.5 or 4 mg/kg L1L3 antibody. The absolute and percent change from baseline of fasting LDL cholesterol (LDL-C) were measured after L1L3 antibody administration. In the study, the incidence of subjects meeting toxicity or intolerable dose criteria was measured. Incidence of treatment emergent adverse events (TEAEs) categorized by severity and causal relationship to study drug was also be measured. The timeframe for measurement of each of the above outcomes was two months.

Study Protocol

Day -1: Subjects were admitted to the clinical research unit (CRU), and the following were completed: reviewed and update inclusion and exclusion criteria; reviewed and update medical history; reviewed and update history of all prescription or nonprescription drugs, and dietary supplements taken within 28 days prior to the planned first dose; brief physical examination; vitals sign measurements (blood pressure,

pulse rate, body temperature) supine and standing; collected blood and urine specimens for safety laboratory tests (serum chemistry; hematology, urinalysis, coagulation, lipase, amylase, CRP) following a 10-hour fast; urine drug and alcohol screen test; urine pregnancy test (females of childbearing potential); collected blood sample for immunogenicity analysis (Anti-L1L3 Antibody); collected blood sample for pharmacodynamic analysis (PCSK9 and Lipid Particle); collected blood sample for pharmacogenomics (optional, subject's consent required); triplicate, supine ECG; assessed alcohol, caffeine and tobacco use; assessed baseline symptoms/adverse events; and randomized subject.

Day 1: Prior to dosing, the following were completed: triplicate, supine ECG (prior to inserting IV catheter, if applicable); vital signs measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 1, 0 hr.) blood sample for PK (atorvastatin); subjects took the sponsor-provided atorvastatin dose (40 mg); post dosing, blood samples for PK (atorvastatin) were collected at the following time points for Day 1: .25, .5, 1, 2, 3, 4, 6, 8 and 12 hours. The following were completed: assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects fasted at least 10 hours prior to the lipid panel blood sample on Day 2.

Day 2: Prior to dosing, the following were completed: vital signs measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 2, 0 hr) blood sample for PK (atorvastatin); collected lipid panel following a 10-hour fast; subjects took the sponsor-provided atorvastatin dose (40 mg). The following were completed: assessed baseline symptoms/adverse events and reviewed concomitant medications.

Day 3: Prior to dosing, the following were completed: collected Day 3, 0 hr) blood sample for PK (atorvastatin); vitals signs measurements (blood pressure, pulse rate, body temperature) supine and standing; subjects took the sponsor-provided atorvastatin dose (40 mg). The following were completed: assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects fasted at least 10 hours prior to the lipid panel blood sample on Day 4.

Day 4: Prior to dosing with atorvastatin and L1L3, the following were completed: triplicate, supine ECG; vital signs measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 4, 0 hr.) blood samples for

atorvastatin PK; collected (Day 4, 0 hr) blood samples for L1L3 PK; collected blood and urine specimens for safety laboratory tests (serum chemistry; hematology, urinalysis, lipase, amylase, CRP) following a 10-hour fast; weight; collected lipid panel following a 10-hour fast; collected blood sample for pharmacodynamic analyses (PCSK9 and Lipid Particle); collected blood sample for immunogenicity (Anti-L1L3 Antibodies). Dose Administration: subjects took sponsor-provided atorvastatin (40 mg). L1L3 was administered by rate controlled intravenous infusion over approximately 60 minutes. Post dose administrations, the following were completed: collected blood samples for PK (atorvastatin) for Day 4 at .25, .5, 1, 2, 3, 4, 6, 8, and 12 hours post atorvastatin dose; collected blood samples for PK (L1L3) for Day 4 at 1, 4, 8, and 12 hours from start of infusion; triplicate, supine ECG 1 hour post dose; vital signs measurements (blood pressure, pulse rate, body temperature) supine and standing at 1 and 4 hours from start of the L1L3 infusion; and assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects fasted at least 10 hours prior to the lipid panel blood sample on Days 5 and 6.

Days 5 and 6: Prior to dosing, the following were completed: vital signs measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 5, 0 hr.) blood sample for PK (atorvastatin); collected (Day 5) blood sample for PK (L1L3) ; collected lipid panel following a 10-hour fast. Day 5 only: collected blood sample for pharmacodynamic analyses (PCSK9 and Lipid Particle). Subjects took the sponsor-provided atorvastatin dose (40 mg). The following were completed: assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects fasted at least 10 hours prior to the lipid panel blood sample on Day 7.

Day 7: Prior to dosing, the following were completed: triplicate, supine ECG; vitals sign measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 7) blood sample for PK (atorvastatin); collected (Day 7) blood sample for PK (L1L3); collected lipid panel following a 10-hour fast; collected blood sample for pharmacodynamic analysis (PCSK9 and Lipid Particle); collected blood and urine specimens for safety laboratory tests (serum chemistry; hematology, urinalysis, coagulation, lipase, amylase, CRP) following a 10-hour fast. Subjects took the last sponsor-provided atorvastatin dose (40 mg). Prior to discharge from the unit, the following were completed: brief physical examination; assessed baseline

symptoms/adverse events; reviewed concomitant medications. Subjects were reminded to return to the clinic and to fast at least 10 hours prior to the lipid panel blood sample on Day 15. Subjects continued taking their prescribed atorvastatin medication throughout the remainder of the study.

Day 15 (± 1 day): The following were completed: brief physical examination; compliance check for atorvastatin; standard, supine ECG; vitals sign measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 15) blood sample for PK (L1L3); collected lipid panel following a 10-hour fast; collected blood sample for immunogenicity (Anti-L1L3 Antibodies) ; collected blood sample for pharmacodynamic analysis (PCSK9 and Lipid Particle); collected blood and urine specimens for safety laboratory tests (serum chemistry, hematology, urinalysis, CRP) following a 10-hour fast; assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects were reminded to return to the clinic and to fast at least 10 hours prior to the lipid panel blood sample on Day 22.

Day 22 (± 1 day): The following were completed: brief physical examination; compliance check for atorvastatin; vitals sign measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 22) blood sample for PK (L1L3); collected lipid panel following a 10-hour fast; collected blood and urine specimens for safety laboratory tests (serum chemistry, hematology, urinalysis, CRP) following a 10-hour fast; assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects were reminded to return to the clinic and to fast at least 10 hours prior to the lipid panel blood sample on Day 29.

Day 29 (± 1 day): The following were completed: complete physical examination; compliance check for atorvastatin; vitals sign measurements (blood pressure, pulse rate, body temperature) supine and standing; collected (Day 29) blood sample for PK (L1L3); collected blood sample for pharmacodynamic analyses (PCSK9 and Lipid Particle); collected blood sample for immunogenicity (Anti-L1L3 Antibodies); collected lipid panel following a 10-hour fast; triplicate, supine ECG; collected blood and urine specimens for safety laboratory tests (serum chemistry, hematology, urinalysis, coagulation, lipase, amylase) following a 10-hour fast, urine drug and alcohol screen test; serum pregnancy test (females of childbearing potential); assessed baseline symptoms/adverse events; reviewed concomitant medications. Subjects were reminded

to return to the clinic and to fast at least 10 hours prior to the lipid panel blood sample on Day 36.

Days 36, 43, 50, 57, and 64 (Termination Visit): The following were completed: brief physical examination; compliance check for atorvastatin; standard, supine ECG; vitals sign measurements (blood pressure, pulse rate, body temperature) supine and standing; collected blood sample for PK (L1L3); collected blood sample for immunogenicity (Anti-L1L3 Antibodies); collected lipid panel following a 10-hour fast; collected blood and urine specimens for safety laboratory tests (serum chemistry, hematology, urinalysis, lipase, amylase, CRP) following a 10-hour fast; assessed baseline symptoms/adverse events; reviewed concomitant medications. Day 64 Only: urine pregnancy test (females of childbearing potential); coagulation Panel; weight; collected blood sample for pharmacodynamic analyses (PCSK9 and Lipid Particle).

Day 78 and 92: In some instances, two visits were added, Day 78 and 92, pending the pharmacokinetic results from Day 57. In this event, the procedures for Day 57 were followed for Day 78, and the procedures for Day 64 were followed for Day 92. Day 92 became the termination visit.

Results

There were no discontinued subjects in the study. There was one serious adverse event (SAE), i.e. worsening of migraine headache, which was not drug-related. The TEAEs were generally nonspecific, and none were severe in intensity. In addition, the TEAEs were transient, with greater than 3x ULN alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), without clinical signs/symptoms, and all were resolved within one week.

Table 4 summarizes the L1L3 PK parameters of this study.

Table 4: L1L3 PK Parameters: Geometric Mean (CV%)

Parameter	4 mg/kg L1L3 + Atorvastatin	0.5 mg/kg L1L3 + Atorvastatin
N, n	12,12	7,7
AUC_{inf} (ng.day/mL)	777167 (13)	46338 (28)
AUC_t (ng.day/mL)	726337 (17)	38310 (35)
C_{max} (ng/mL)	105048 (16)	13827 (9)
T_{max} (day)	0.1 (0.04 – 0.50)	0.17 (0.04 – 0.33)
$t_{1/2}$ (day)	7.3 (33)	2.6 (34)
CL (mL/day/kg)	5.2 (15)	10.8 (29)
Vss (mL/kg)	52.3 (16)	40.2 (14)

Table 5 summarizes the results from this clinical trial study to evaluate pharmacokinetics and pharmacodynamics of a single dose of L1L3 in human subjects on stable doses of atorvastatin. The mean percent change from baseline of fasting LDL-C levels after L1L3 antibody administration is provided (Table 4).

Table 5. Mean (SD) LDL-C vs Time Data

Day	0.5 mg/kg L1L3 (n=12)		4 mg/kg L1L3 (n=12)	
	Mean	SD	Mean	SD
0	0.0	0.0	0.0	0.0
1	-28.8	20.2	-20.9	18.5
2	-48.5	26.3	-38.4	13.0
3	-66.7	28.2	-43.3	18.1
11	-34.4	27.0	-64.6	26.0
18	9.0	39.7	-73.2	21.2
25	23.3	43.5	-70.8	20.4
32	14.8	37.0	-69.9	14.8
39	21.2	36.7	-45.1	16.9
46	17.0	37.2	-19.2	16.4
53	27.9	42.7	-3.6	25.4
60	30.1	39.3	7.1	25.8

Treatment with L1L3 in the presence of atorvastatin (dose = 40 mg) resulted in substantial and durable dose-dependent fasting LDL-C lowering. The baseline fasting LDL C was about 72.5 mg/dL. FIG. 7A depicts absolute fasting LDL-C levels after L1L3 antibody administration. FIG. 7B depicts the percent change from baseline of fasting LDL-C levels after L1L3 antibody administration. Baseline is indicated as "0" in FIG. 7B. With an L1L3 dose of 0.5 mg/kg, the maximum LDL-C lowering effect was observed on day 3 following L1L3 administration. With an L1L3 dose of 4 mg/kg, the maximum LDL-C lowering effect was observed through day 32 following L1L3 administration. The dose-dependent response in LDL-C lowering is shown in FIG. 8. As shown in FIG. 8, L1L3 lowered LDL-C in patients on stable doses of statin at every dose administered. Furthermore, the LDL-C lowering effect in patients on stable doses of statin was greater than the effect in patients dosed with L1L3 alone (FIG. 8).

Example 4: PK-PD modeling and simulated time profiles

Based on the data provided in the studies described above, simulated serum L1L3-time profiles and LDL-C-time profiles were generated. FIGS. 9A-F depict graphs of simulated time profiles for L1L3 (top panel) and LDL-C (bottom panel) after administration of L1L3 at the indicated doses, or placebo. The simulated profiles were generated for dosing with 2 mg/kg L1L3 (left) or 6 mg/kg L1L3 (middle) compared to placebo (right). L1L3 or placebo was administered at Day 0 and Day 29, i.e., two doses four weeks apart. FIG. 10 depicts the simulated LDL-C-time profiles after administration of following L1L3 dose amounts: 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg and 6 mg/kg, each administered at Day 0, Day 29 and Day 56 (FIG. 10). The simulated L1L3-time profiles and LDL-C-time profiles demonstrate that low doses of L1L3 administered once every four weeks produces sustained LDL-C lowering.

Example 5: Pharmacokinetics and pharmacodynamics following multiple doses of L1L3

This example illustrates a clinical trial study to evaluate pharmacokinetics and pharmacodynamics following multiple intravenous doses of PCSK9 antagonist antibody (L1L3) in human subjects.

This study was a randomized, multi-center, double-blind, placebo control, parallel designed trial with a 28 day screening period, 4 week treatment period and 8 week follow-up period (Figure 11). In the study, human Japanese subjects were administered

L1L3 antibody at 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, or 1.5 mg/kg of the PCSK9 antagonist antibody. For each subject, the study consisted of 3 periods: screening, treatment, and follow-up. The treatment period lasted up to approximately 28 days with 4 single I.V. doses of either L1L3 or placebo administered on Days 1, 8, 15, and 22. The follow-up period will lasted approximately 8 weeks, from approximately Day 29 to the last visit (Day 78). Subjects were seen periodically in the clinic for safety assessments and collection of blood for routine laboratory tests, lipid profiles, PK, PD, and immunogenicity samples.

Weekly treatment with L1L3 at all doses tested resulted in sustained, substantial and durable dose-dependent fasting LDL-C lowering. The baseline fasting LDL-C was about 155 mg/dL. FIG. 12 depicts absolute fasting LDL-C levels after L1L3 antibody administration. FIG. 13 depicts the percent change from baseline of fasting LDL-C levels after L1L3 antibody administration. Baseline is indicated as "0" in FIG. 13.

The table in FIG. 14 summarizes the results from this clinical trial study to evaluate pharmacokinetics and pharmacodynamics following multiple doses of L1L3 in human subjects on stable doses of atorvastatin. The mean percent change from baseline of fasting LDL-C levels after L1L3 antibody administration is provided ("Mean") (FIG. 14).

Example 6: Pharmacokinetics and pharmacodynamics following multiple doses of L1L3 in combination with statin

This example illustrates a clinical trial study to evaluate pharmacokinetics and pharmacodynamics following multiple intravenous doses of PCSK9 antagonist antibody (L1L3) in human subjects on atorvastatin, simvastatin or rosuvastatin.

This study was a randomized, multi center, double blind, placebo control, parallel designed trial with a 3 week screening period, 12 week treatment period and 8 week follow up period.

Subjects enrolled in the study met all of the following criteria: men and women subjects greater than equal to age of 18; body mass index of 18.5 to 40 kg/m^2; total body weight greater than 50 kg (110 lbs) and less than 150 kg (330 lbs); on a stable daily dose of a statin, defined as atorvastatin 40 or 80 mg, rosuvastatin 20 or 40 mg or simvastatin 40 or 80 mg for a minimum of 45 days prior to Day 1; lipids meet the

following criteria at two qualifying visits (screening and Day -7): fasting LDL-C greater than or equal to 100 mg/dL, ;

Subjects were seen periodically in the clinic for safety assessments and collection of blood for safety labs, lipid profiles, PK, PD, and immunogenicity samples. Telephone contacts were made prior to each visit to remind them of the 10-hour fasting requirements, during screening and on Day 3 to assess adverse events and document the contact in the subject's source document. Subjects received one infusion of 1 mg/kg L1L3, 3 mg/kg L1L3, 6 mg/kg L1L3, or placebo on Days 1, 29 and 57 with multiple efficacy, safety and PK assessments throughout the treatment and follow-up periods. Infusion rates were carefully controlled by an infusion device per protocol. Infusions were administered as a single infusion over approximately 60 minutes.

Results

The 3 mg/kg dosage regimen and 6 mg/kg dosage regimen both achieved statistical significance and exceeded the target value of 30% change in LDL-C from baseline. No effect of L1L3 on triglycerides was observed. A slight elevation of HDL up to 9% was seen. The treatment groups and enrollment are shown in Table 6.

Table 6

	Placebo (N=19)		L1L3 0.25 mg/kg (N=19)		L1L3 1 mg/kg (N=18)		L1L3 3 mg/kg (N=18)		L1L3 6 mg/kg (N=18)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
#of Subjects:										
Atorvastatin	6	(31.6)	6	(31.6)	6	(33.3)	6	(33.3)	6	(33.3)
Rosuvastatin	6	(31.6)	5	(26.3)	6	(33.3)	5	(27.8)	5	(27.8)
Simvastatin	7	(36.8)	6	(31.6)	6	(33.3)	7	(38.9)	6	(33.3)

The pre-specified primary efficacy endpoint was the percentage change from baseline of LDL-C at Day 85 analyzed using an ANCOVA model. The final ANCOVA model contained terms for baseline LDL-C and treatment. To preserve the overall type I error rate at a level of 0.05 for the primary endpoint analysis, a Haybittle-Peto, boundary with 0.001 alpha spent was employed.

A strong treatment effect with a clear dose response was observed with variation in LDL-C for the 3 and 6 mg/kg treatment groups driven by the missing-ness of doses

(FIGS. 15 and 16). The LDL-C data were subsequently analyzed using mixed model repeated measures to estimate both the treatment by time and empirical dose-response profiles.

The pre-determined target value of additional 30% LDL-C when added to statins was the proof-of-concept criterion of success. This target level of 30% of LDL-C lowering or more, when added to statin therapy, was clearly achieved with the 3 and 6 mg/kg doses given every 4 weeks (FIGS. 15 and 16). The graph in FIG. 15 shows the percent change from baseline by study day and treatment, and the graph in FIG. 16 shows the percent change from baseline by study day and treatment excluding the subjects with missed doses. The 3 mg/kg L1L3 dosing regimen in patients on a stable daily dose of a statin achieved LDL-C lowering to about 50% below baseline by Day 29 (FIG. 15). The 6 mg/kg L1L3 dosing regimen in patients on a stable daily dose of a statin achieved LDL-C lowering to about 65% below baseline by Day 29 (FIG. 15). With both the 3 mg/kg and 6 mg/kg dosing regimens, greater than 30% LDL-C lowering persisted for 28 days (FIG. 16). A statistical summary of the placebo adjusted treatment effects at Day 85 is provided in Table 7. In Table 7, the baseline of lipid profile is defined as the average of values observed at Days -7 and 1.

Table 7: Summary of Statistical Analysis (MMRM) of Percentage Changes from Baseline for LDL-C Data on Day 85

Comparison (Test vs. Reference)	Difference in LS means (Test – Reference)	Standard Error	95% CI	*P-value
L1L3 0.25 mg/kg vs. Placebo on Day 85	2.67	10.252	(-17.87, 23.20)	0.7958
L1L3 1 mg/kg vs. Placebo on Day 85	0.83	10.013	(-19.23, 20.89)	0.9340
L1L3 3 mg/kg vs. Placebo on Day 85	-38.92	9.721	(-58.39, -19.46)	0.0002
L1L3 6 mg/kg vs. Placebo on Day 85	-50.14	10.266	(-70.70, -29.57)	<0.0001

A summary of L1L3 Cmax and trough concentrations is shown in Table 8.

Table 8: L1L3 Pharmacokinetics

Dosage (mg/kg)	After 1st Dose		After 3rd Dose	
	C_{max} (µg/mL)	C_{trough}(µg/mL)	C_{max} (µg/mL)	C_{trough}(µg/mL)
0.25	10.9 ± 13.0 (n=17)	0.109 ± 0.406 (n=14)	6.95 ± 1.55 (n=13)	0.122 ± 0.226 (n=8)
1	28.3 ± 6.6 (n=17)	0.256 ± 0.310 (n=14)	37.3 ± 26.4 (n=11)	0 ± 0 (n=9)
3	92.2 ± 22.6 (n=18)	3.16 ± 2.30 (n=13)	86.5 ± 15.0 (n=12)	3.04 ± 4.42 (n=7)
6	182 ± 64 (n=17)	17.4 ± 11.6 (n=14)	179 ± 66 (n=8)	15.6 ± 15.3 (n=7)

Monthly treatment with L1L3 at 3 and 6 mg/kg in patients on a stable daily dose of a statin resulted in greater than 30% lowering of blood LDL-C levels from baseline. Minor elevations (up to 9%) in HDL levels and little effects of L1L3 on triglycerides were observes. L1L3 was generally safe and well-tolerated. Changes in LFTs, CK, ECGs, and BP were transient, mild in nature and in most cases were considered not related to treatment. No subject had positive ADA.

Example 7: Pharmacokinetics and pharmacodynamics following multiple doses of L1L3 in combination with statin

This example illustrates a clinical trial study to evaluate LDL-C levels following multiple subcutaneous doses of PCSK9 antagonist antibody (L1L3) in human subjects on a statin.

This study is a randomized, multi center, double blind, placebo control, parallel group, dose-ranging study designed trial to assess the efficacy, safety and tolerability of L1L3 following monthly and twice monthly subcutaneous dosing for six months in hypercholesterolemic subjects on a statin. A total of 7 dose groups in two dosing schedules (Q28d or Q14d), with 50 subjects per dose group are planned. Protocol design is set forth in Table 9.

Table 9

Arms	Assigned Interventions
Experimental: Q28d Dosing Arm Q28d dose groups will receive subcutaneous administration of L1L3 antibody or Placebo once a month.	**Group 1:** Placebo, Q28d **Group 2:** L1L3 200 mg, Q28d **Group 3:** L1L3 300 mg, Q28d
Experimental: Q14d Dosing Arm Q14d dose groups will receive subcutaneous administration of L1L3 antibody or Placebo every 2 weeks.	**Group 4:** Placebo, Q14d **Group 5:** L1L3 50mg, Q14d **Group 6:** L1L3 100 mg, Q14d **Group 7:** L1L3 150 mg, Q14d

Eligibility: ages 18 years or older.

Inclusion criteria: subjects should be receiving stable doses (at least 6 weeks) of any statin and continue on same dose of statin for the duration of this trial. Lipids should meet the following criteria on a background treatment with a statin at 2 screening visits that occur at screening and at least 7 days prior to randomization on Day 1: fasting LDL-C greater than or equal to 80 mg/dL (2.31 mmol/L); fasting TG less than or equal to 400 mg/dL (4.52 mmol/L); subject's fasting LDL-C must be greater than or equal to 80 mg/dL (2.31 mmol/L at the initial screen visit, and the value at the second visit within 7 days of randomization must be not lower than 20% of this initial value to meet eligibility criteria for this trial.

The primary outcome measure will be the absolute change from baseline in LDL-C at the end of week 12 following randomization. Secondary outcome measures include the following: LDL-C will be assessed as change and % change from baseline at the end of week 12 following randomization; plasma steady-state L1L3 pharmacokinetic parameters; proportion of subjects having LDL-C less than specified limits (<100 mg/dL, <70 mg/dL, <40 mg/dL, <25 mg/dL); total cholesterol will be assessed as change and % change from baseline at the end of week 12 following randomization; ApoB will be assessed as change and % change from baseline at the end of week 12 following randomization; ApoA1 will be assessed as change and % change from baseline at the end of week 12 following randomization; lipoprotein (a) will be assessed as change and % change from baseline at the end of week 12 following randomization; HDL-

cholesterol will be assessed as change and % change from baseline at the end of week 12 following randomization; very low density lipoprotein-cholesterol will be assessed as change and % change from baseline at the end of week 12 following randomization; triglycerides will be assessed as change and % change from baseline at the end of week 12 following randomization; and non-HDL-cholesterol will be assessed as change and % change from baseline at the end of week 12 following randomization.

Although the disclosed teachings have been described with reference to various applications, methods, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. A proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody for use in the treatment of a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, wherein the PCSK9 antagonist antibody is administered as an initial dose of at least about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, or about 6 mg/kg; and administered as a plurality of subsequent doses in an amount that is about the same as or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks.

2. A proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody for use in the treatment of a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, wherein the PCSK9 antagonist antibody is administered as an initial dose of at least about 200 mg or about 300 mg; and administered as a plurality of subsequent doses in an amount that is about the same as or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks.

3. A proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody for use in the treatment of a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, wherein the PCSK9 antagonist antibody is administered as an initial dose of at least about 50 mg, about 100 mg or about 150 mg; and administered as a plurality of subsequent doses in an amount that is about the same as or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about two weeks.

4. The PCSK9 antagonist antibody of any one of claims 1 to 3, wherein a statin has been administered prior to the initial dose of the PCSK9 antagonist antibody.

5. The PCSK9 antagonist antibody of claim 4, wherein a daily dose of a statin is administered.

6. The PCSK9 antagonist antibody of claim 4 or 5, wherein stable doses of the statin have been administered for at least about two, three, four, five or six weeks prior to the initial dose of PCSK9 antibody.

7. The PCSK9 antagonist antibody of any one of claims 4 to 6, wherein the statin is atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or or any pharmaceutically acceptable salts, or stereoisomers, thereof.

8. The PCSK9 antagonist antibody of claim 5, wherein the daily statin dose is selected from the group consisting of 40 mg atorvastatin, 80 mg atorvastatin, 20 mg rosuvastatin, 40 mg rosuvastatin, 40 mg simvastatin, and 80 mg simvastatin.

9. The PCSK9 antagonist antibody of any one of claims 1 to 8, wherein the disorder is hypercholesterolemia, dyslipidemia, atherosclerosis, cardiovascular disease, or acute coronary syndrome (ACS).

10. The PCSK9 antagonist antibody of any one of claims 1 to 19, wherein the antibody comprises three CDRs from a heavy chain variable region having the amino acid sequence shown in SEQ ID NO: 11 and three CDRs from a light chain variable region having the amino acid sequence shown in SEQ ID NO: 12.

11. The PCSK9 antagonist antibody of claim 10, wherein the antibody is L1L3.

12. The PCSK9 antagonist antibody of any one of claims 1 to 11, wherein the antibody is administered subcutaneously or intravenously.

13. The PCSK9 antagonist antibody of any one of claims 1 to 12, wherein the antibody is administered about once a month.

14. A method for the treatment of a patient susceptible to or diagnosed with a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, comprising:

administering to the patient an initial dose of at least about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, or about 6 mg/kg of a proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody; and

administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same as or less than the initial dose, wherein the

initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks.

15. A method for the treatment of a patient susceptible to or diagnosed with a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, comprising:

administering to the patient an initial dose of at least about 200 mg or about 300 mg of a proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody; and

administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same as or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about four weeks.

16. A method for the treatment of a patient susceptible to or diagnosed with a disorder characterized by an elevated low-density lipoprotein cholesterol (LDL-C) level in the blood, comprising:

administering to the patient an initial dose of at least about 50 mg, about 100 mg or about 150 mg of a proprotein convertase subtilisin kexin type 9 (PCSK9) antagonist antibody; and

administering to the patient a plurality of subsequent doses of the antibody in an amount that is about the same as or less than the initial dose, wherein the initial dose and the first subsequent and additional subsequent doses are separated in time from each other by at least about two weeks.

17. The method of any one of claims 14 to 16, wherein the patient is being treated with a statin.

18. The method of claim 17, wherein the patient is being treated with a daily dose of a statin.

19. The method of claim 17 or 18, wherein the patient has been receiving stable doses of the statin for at least about two, three, four, five or six weeks prior to the initial dose of PCSK9 antibody.

20. The method of any one of claims 17 to 19, wherein the statin is atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or or any pharmaceutically acceptable salts, or stereoisomers, thereof.

21. The method of claim 18, wherein the daily statin dose is selected from the group consisting of 40 mg atorvastatin, 80 mg atorvastatin, 20 mg rosuvastatin, 40 mg rosuvastatin, 40 mg simvastatin, and 80 mg simvastatin.

22. The method of any one of claims 14 to 21, wherein the disorder is hypercholesterolemia, dyslipidemia, atherosclerosis, cardiovascular disease, or acute coronary syndrome (ACS).

23. The method of any one of claims 14 to 22, wherein the patient has a fasting total cholesterol level of about 70 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody.

24. The method of any one of claims 14 to 23, wherein the patient has a fasting LDL cholesterol level of about 130 mg/dL or greater prior to administration of the initial dose of PCSK9 antagonist antibody.

25. The method of any one of claims 14 to 24, wherein the antibody comprises three CDRs from a heavy chain variable region having the amino acid sequence shown in SEQ ID NO: 11 and three CDRs from a light chain variable region having the amino acid sequence shown in SEQ ID NO: 12.

26. The method of claim 25, wherein the antibody is L1L3.

27. The method of any one of claims 14 to 26, wherein the antibody is administered subcutaneously or intravenously.

28. The method of any one of claims 14 to 27, wherein the antibody is administered about once a month.

29. An article of manufacture, comprising a container, a composition within the container comprising a PCSK9 antagonist antibody, and a package insert containing instructions to administer an initial dose of PCSK9 antagonist antibody of at least about 3 mg/kg, about 6 mg/kg, about 200 mg, or about 300 mg, and at least one subsequent

dose that is the same amount or less than the initial dose, wherein administration of the initial dose and subsequent doses are separated in time by at least about four weeks.

30. The article of manufacture of claim 29, wherein the package insert includes instructions for administration of the PCSK9 antagonist antibody to an individual being treated with a statin.

31. The article of manufacture of claim 30, wherein the statin is atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or any pharmaceutically acceptable salts, or stereoisomers, thereof.

32. The article of manufacture of any one of claims 29 to 31, wherein the instructions are for administration of an initial dose by intravenous or subcutaneous injection and at least one subsequent dose by intravenous or subcutaneous injection.

33. The article of manufacture of any one of claims 29 to 32, wherein a plurality of subsequent doses are administered.

34. The article of manufacture of any one of claims 29 to 33, further comprising a label on or associated with the container that indicates that the composition can be used for treating a condition characterized by an elevated low-density lipoprotein cholesterol level in the blood.

35. The article of manufacture of any one of claims 29 to 34, wherein the label indicates that the composition can be used for the treatment of hypercholesterolemia, atherogenic dyslipidemia, atherosclerosis, cardiovascular disease, or acute coronary syndrome (ACS).

36. The article of manufacture of any one of claims 29 to 35, wherein the antibody is L1L3.

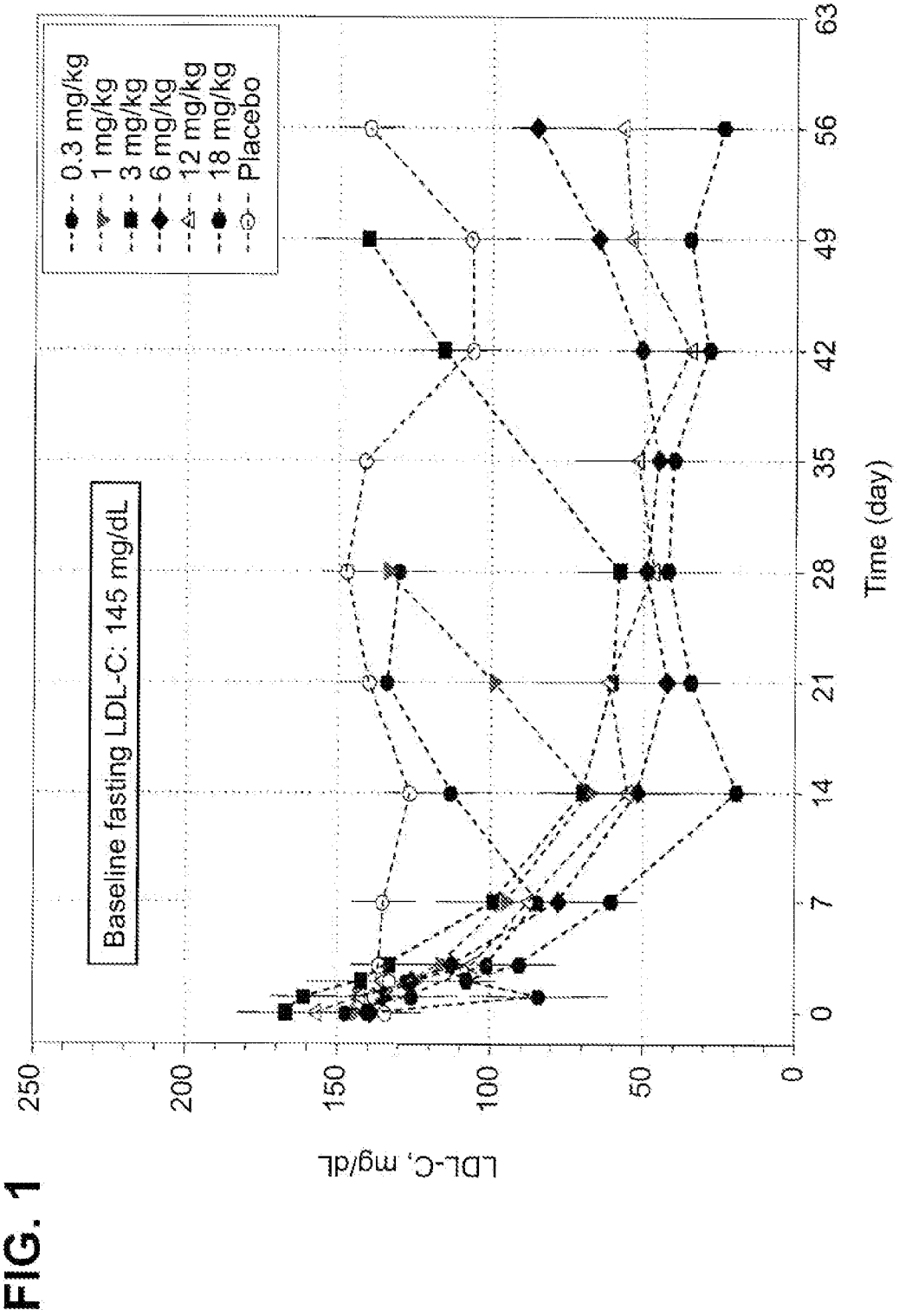

0.3 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
12 mg/kg
18 mg/kg
Placebo
Baseline fasting LDL-C: 145 mg/dL
LDL-C, mg/dL
250
200
150
100
50
0
0
7
14
21
28
35
42
49
56
63
Time (day)

FIG. 1

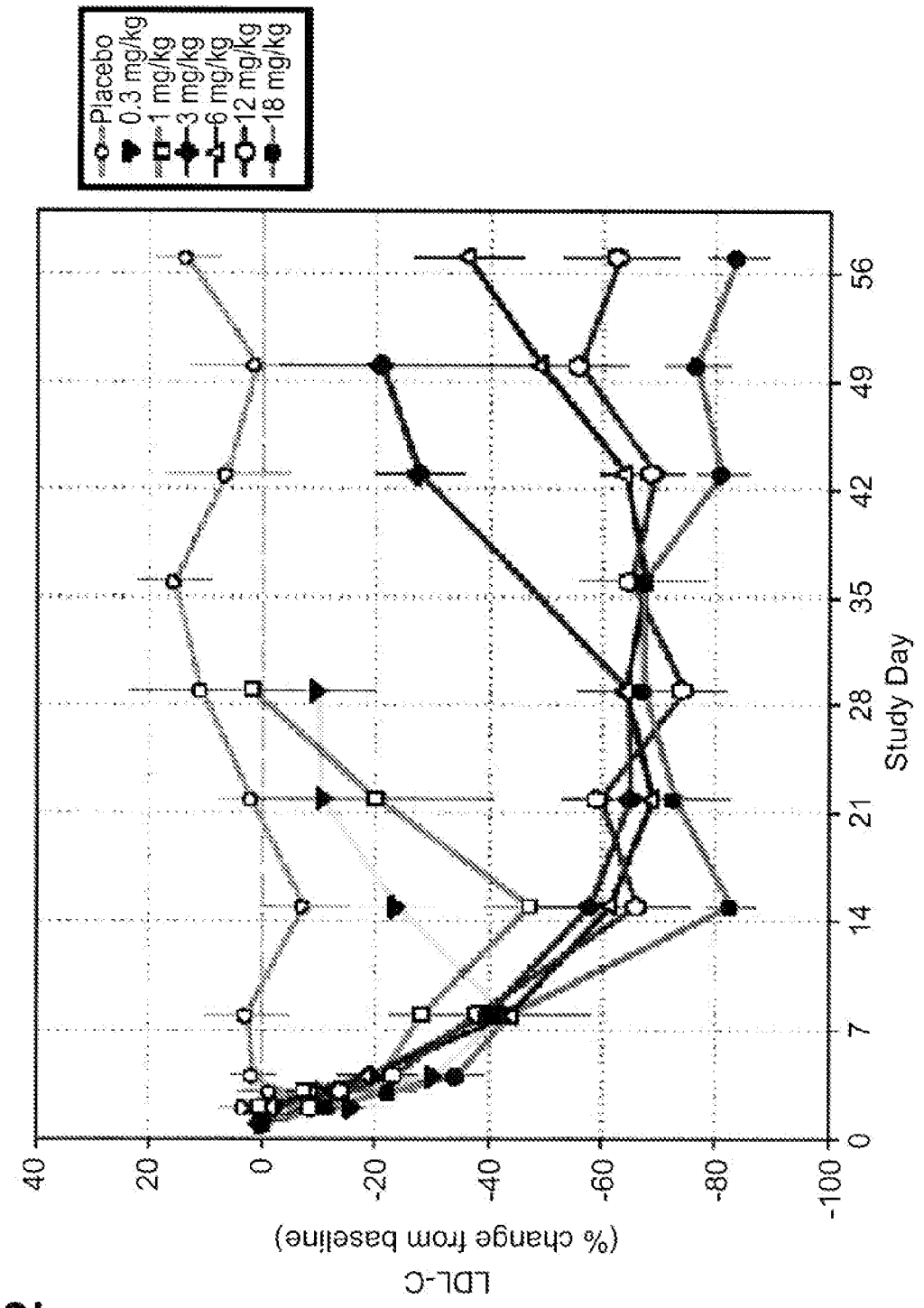

Placebo
0.3 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
12 mg/kg
18 mg/kg
LDL-C
(% change from baseline)
40
20
0
-20
-40
-60
-80
-100
0
7
14
21
28
35
42
49
56
Study Day

FIG. 2

FIG. 3

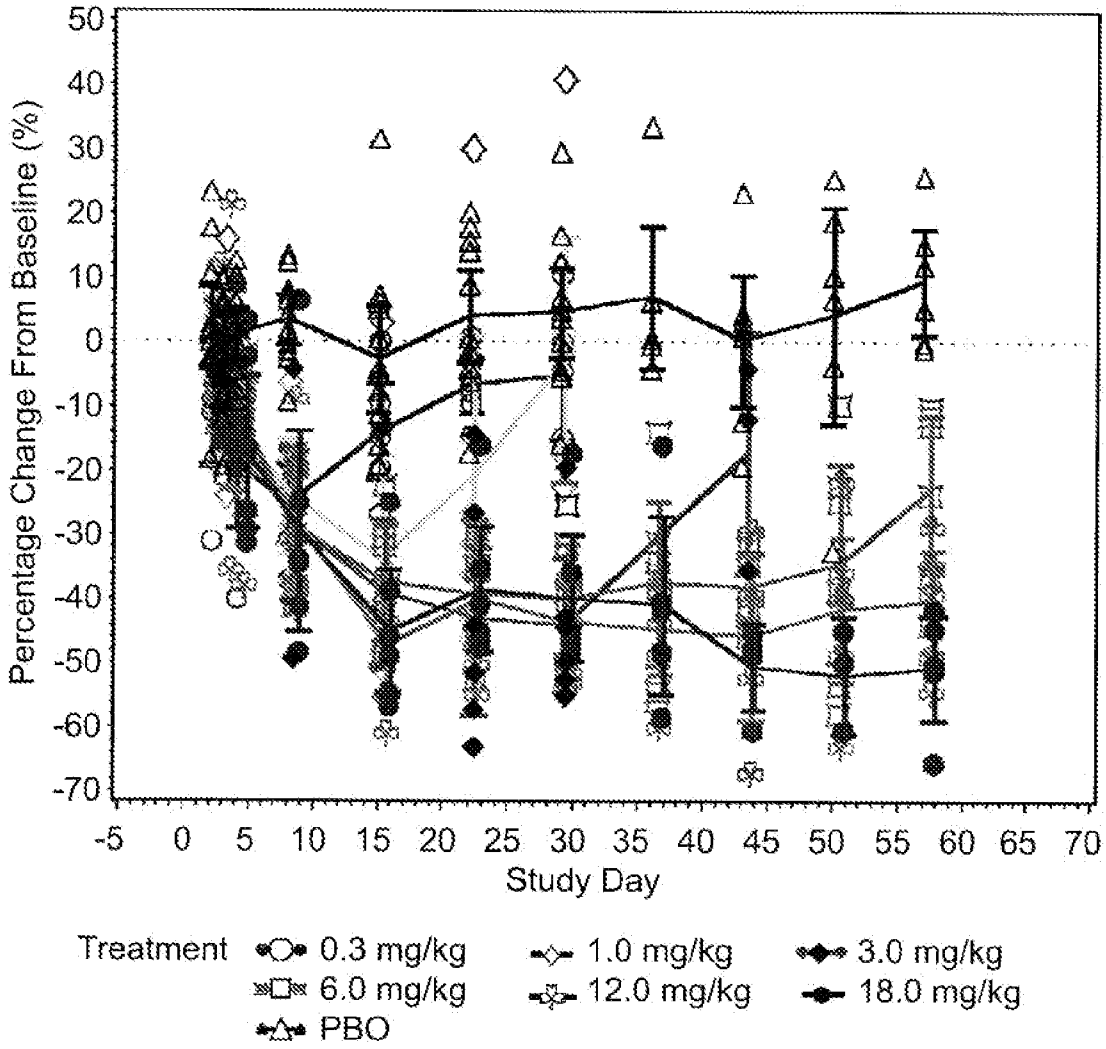

Percentage Change From Baseline (%)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Study Day
Treatment
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

FIG. 4

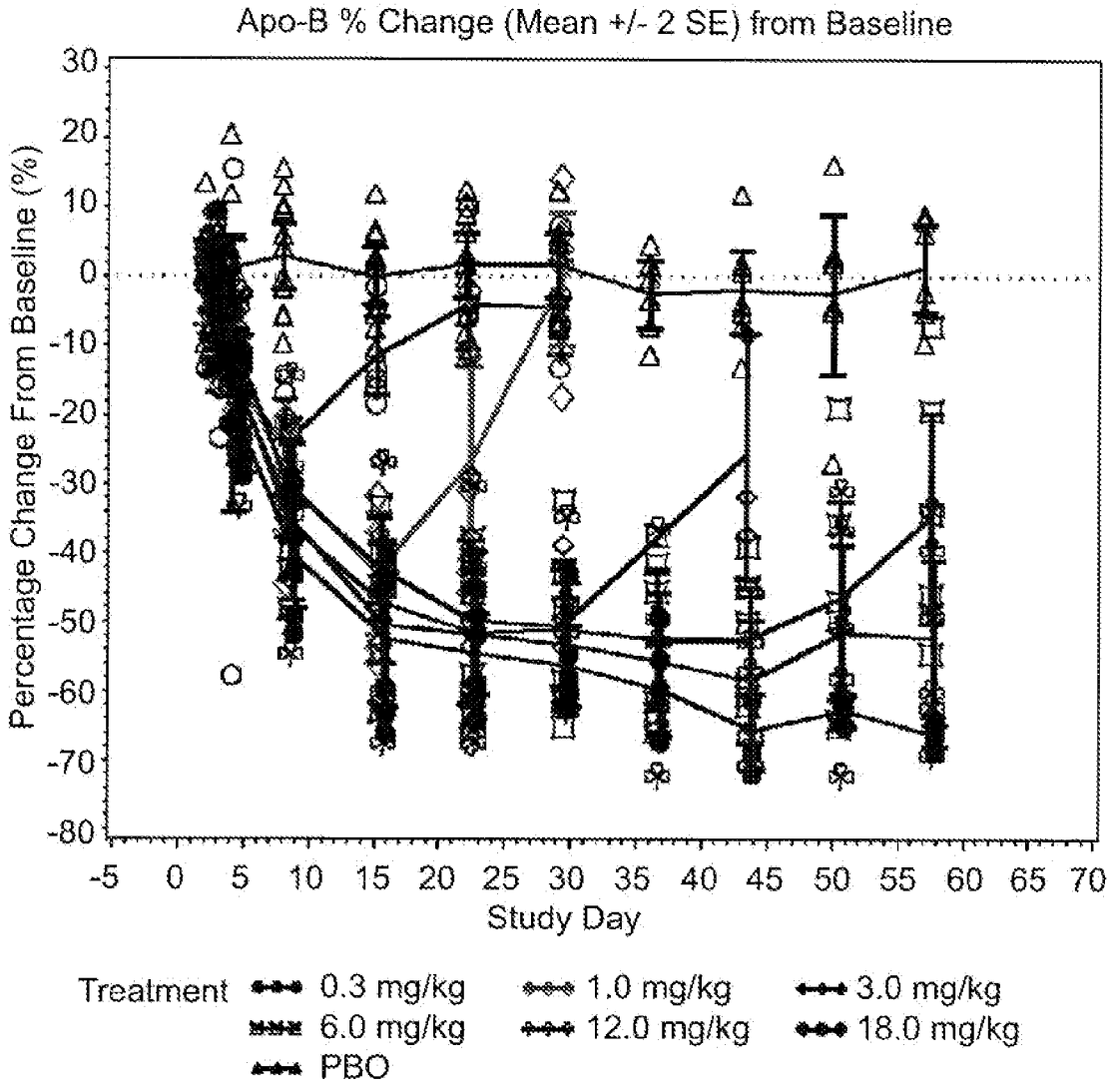

Apo-B % Change (Mean +/- 2 SE) from Baseline
Percentage Change From Baseline (%)
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Study Day
Treatment
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

FIG. 5

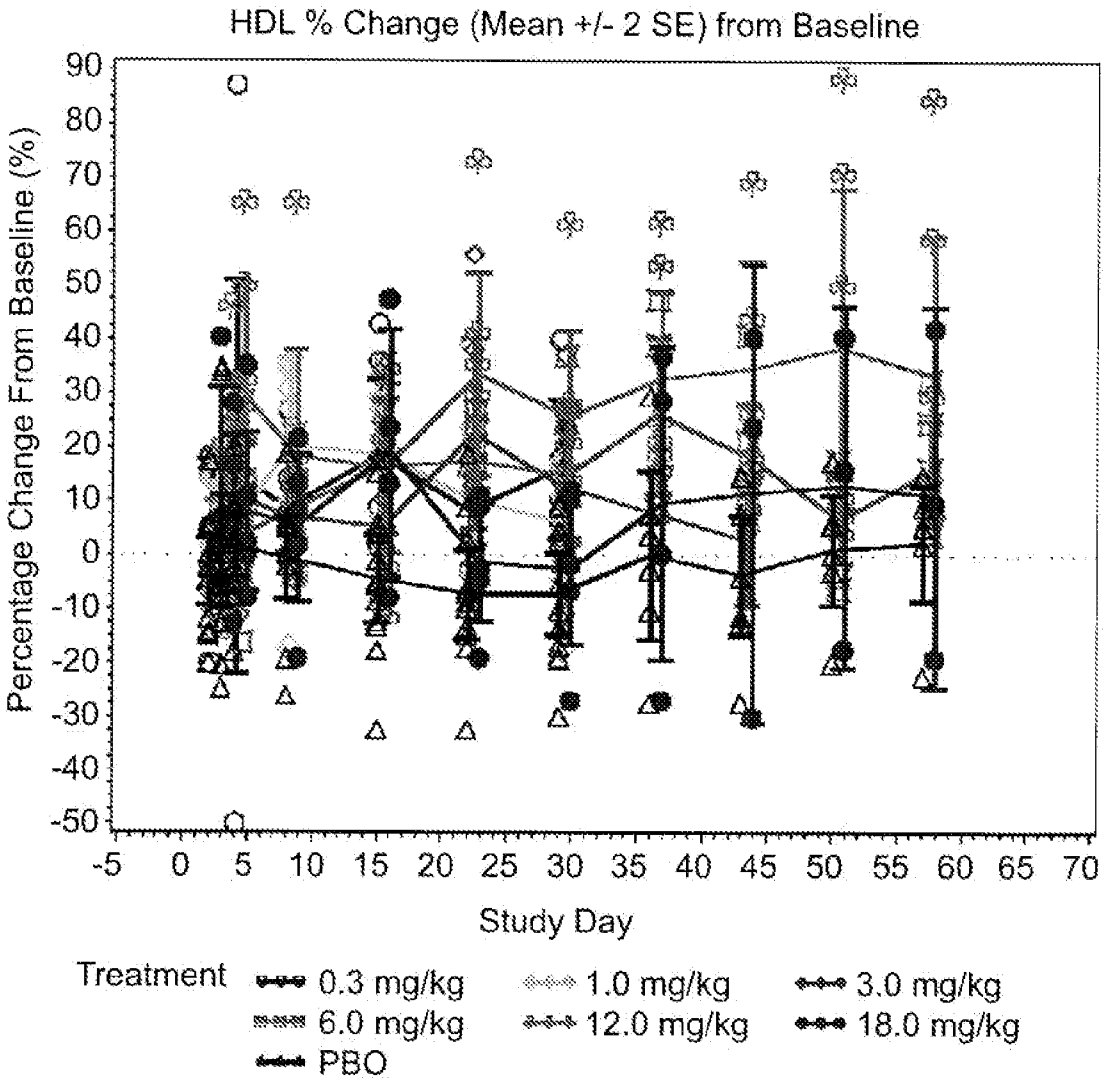

HDL % Change (Mean +/- 2 SE) from Baseline
Percentage Change From Baseline (%)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Study Day
Treatment
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

FIG. 6

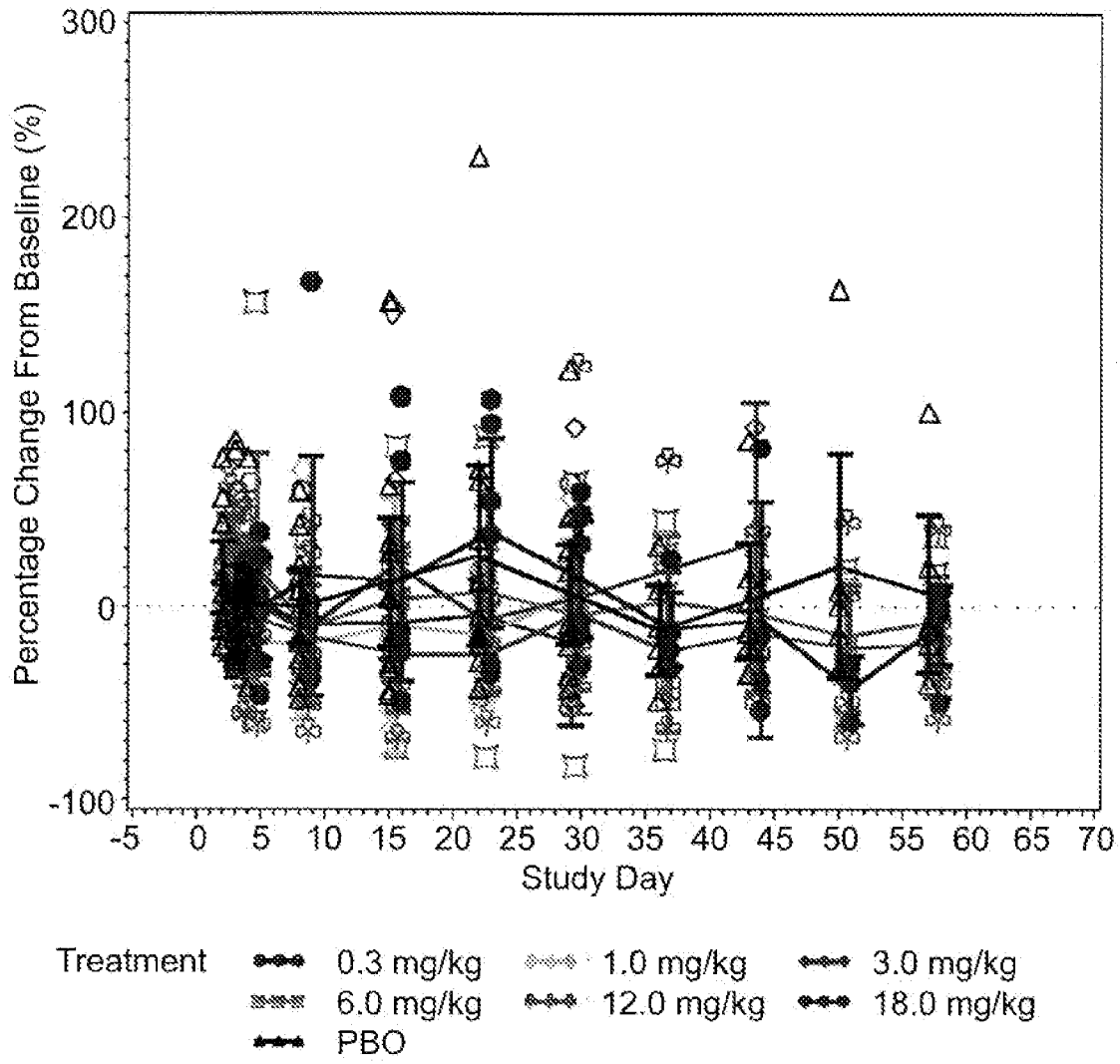

Percentage Change From Baseline (%)
300
200
100
0
-100
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Study Day
Treatment
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

FIG. 7A

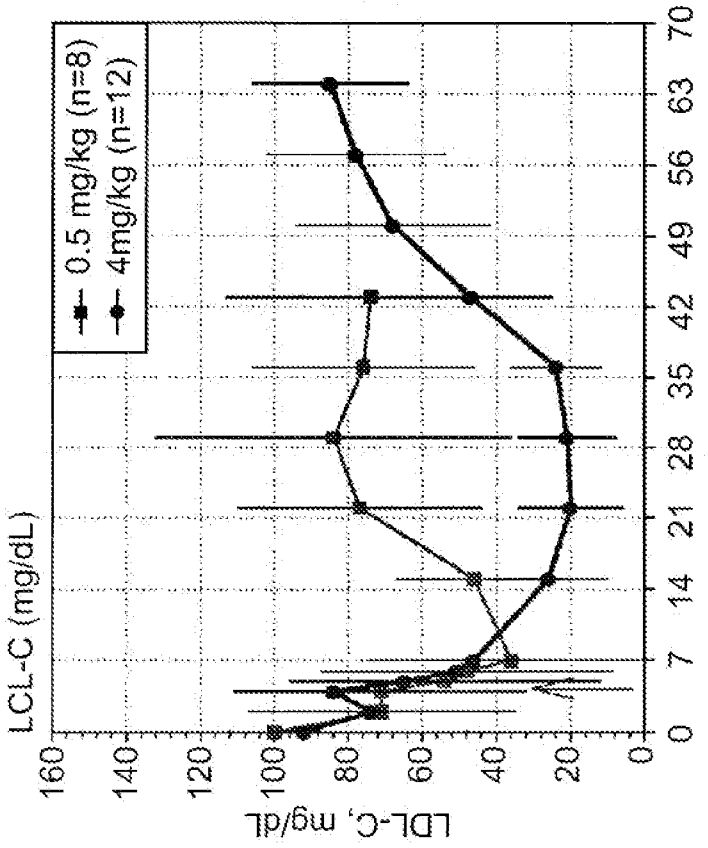
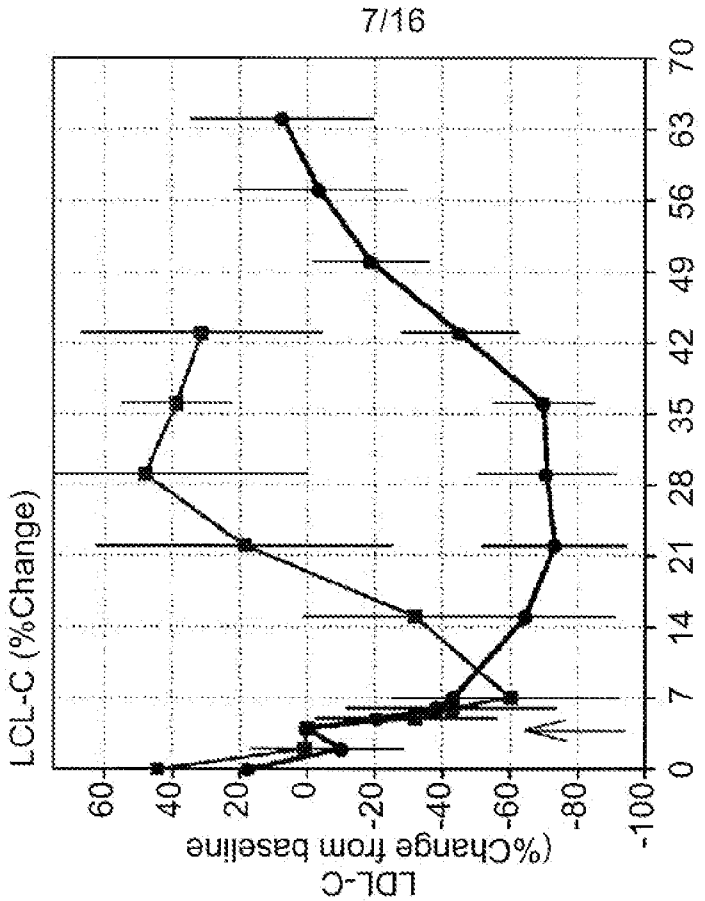
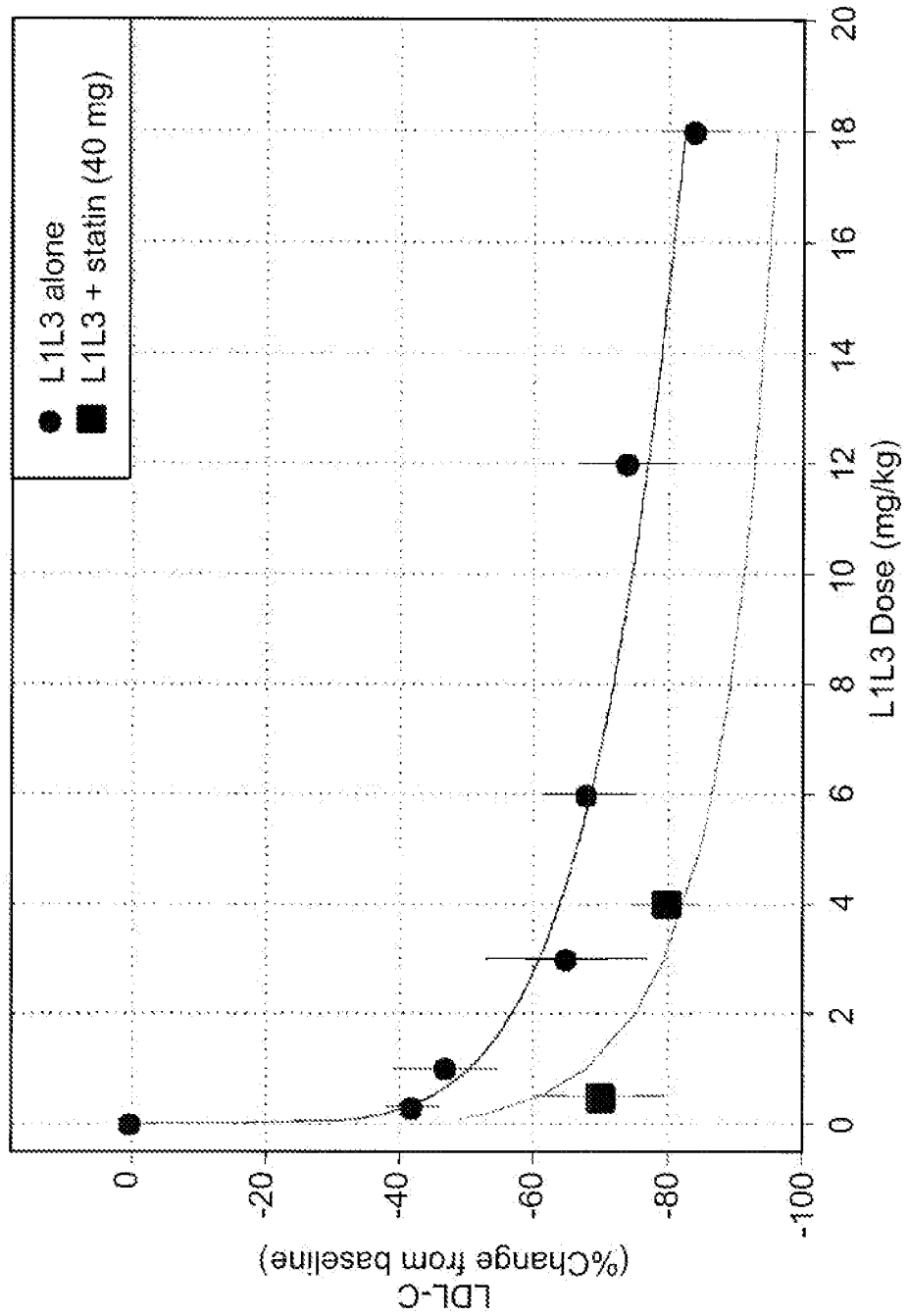


FIG. 7B



Note: Data shown are mean (±SD)

FIG. 8


L1L3 alone
L1L3 + statin (40 mg)
LDL-C
(%Change from baseline)
0
-20
-40
-60
-80
-100
0
2
4
6
8
10
12
14
16
18
20
L1L3 Dose (mg/kg)

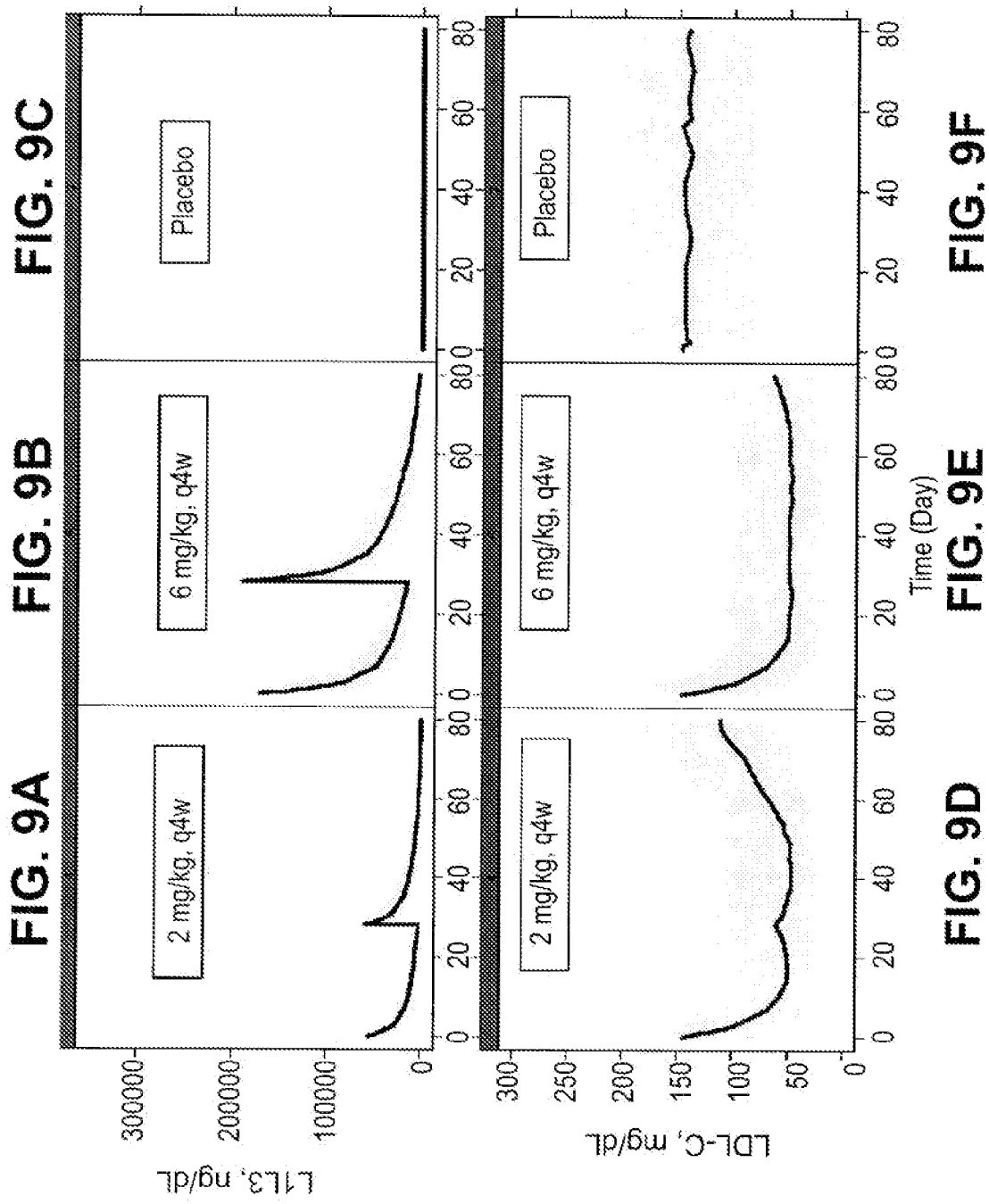


FIG. 9A FIG. 9B FIG. 9C

FIG. 9D FIG. 9E FIG. 9F

FIG. 10

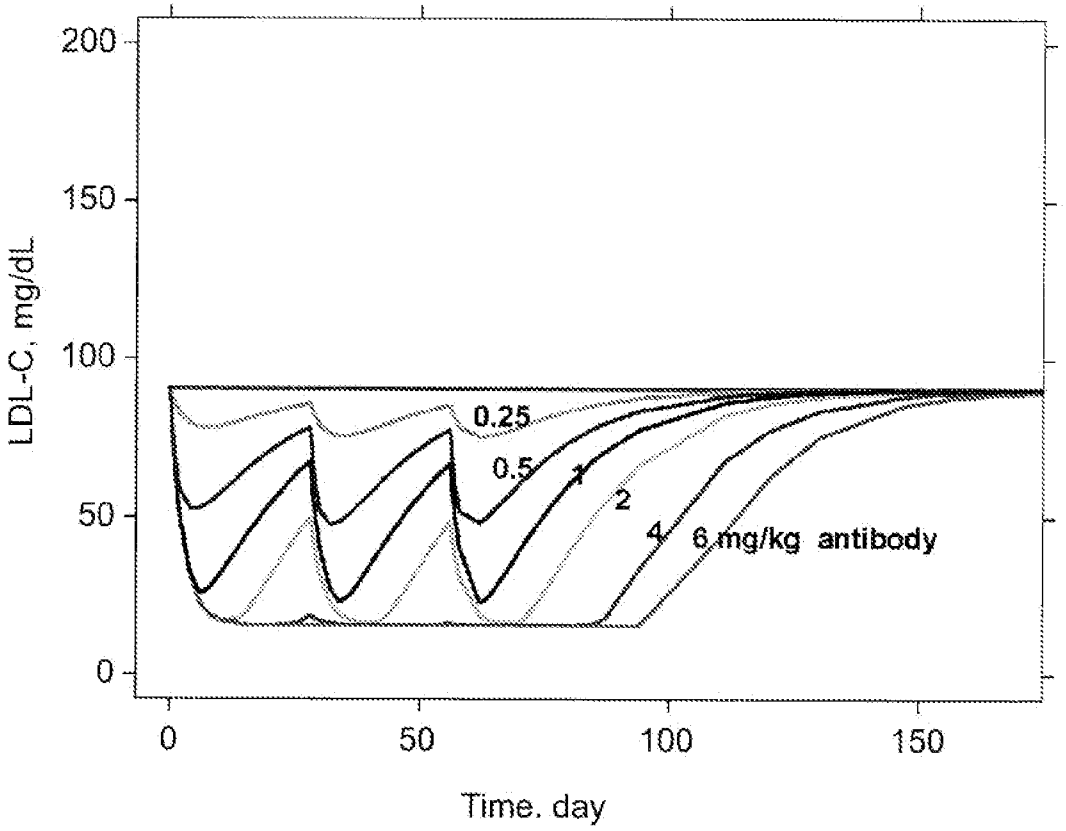
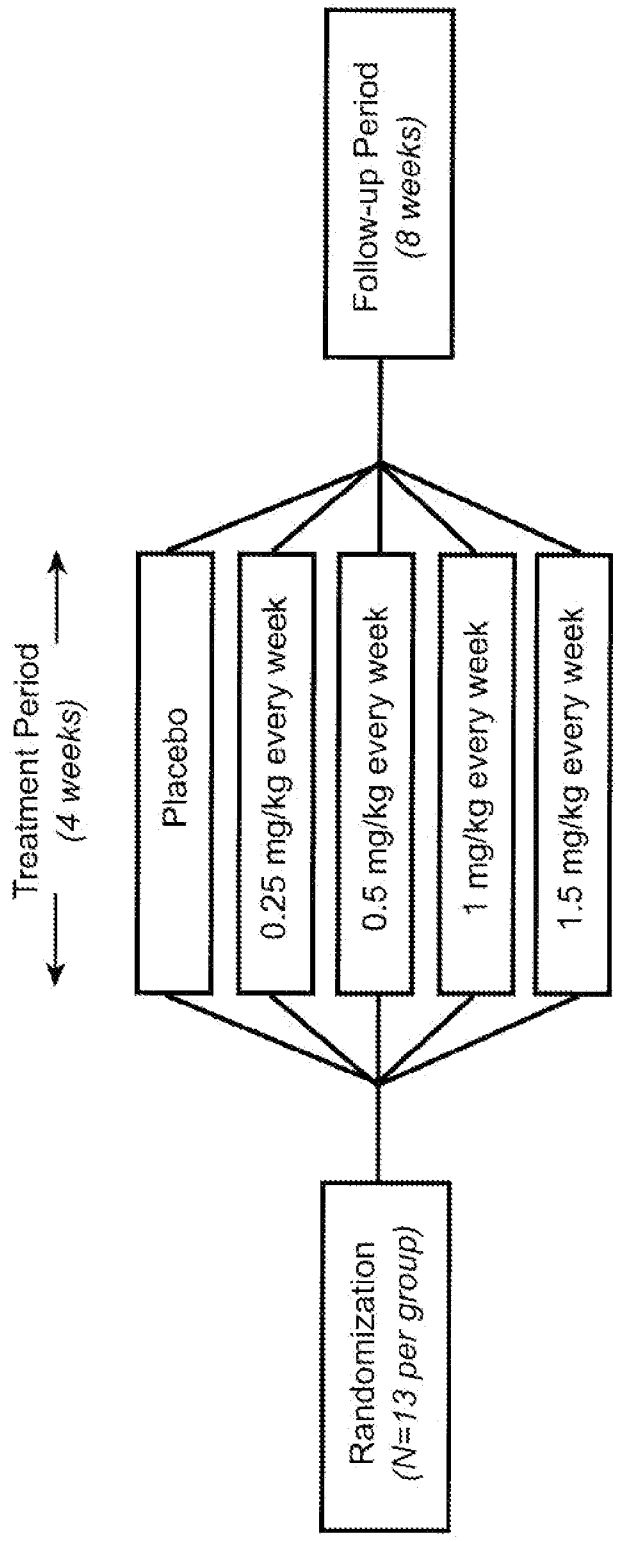

200
150
100
50
0
LDL-C, mg/dL
0.25
0.5
1
2
4
6 mg/kg antibody
0
50
100
150
Time. day

FIG. 11


Randomization
(N=13 per group)
Placebo
0.25 mg/kg every week
0.5 mg/kg every week
1 mg/kg every week
1.5 mg/kg every week
Treatment Period
(4 weeks)
Follow-up Period
(8 weeks)

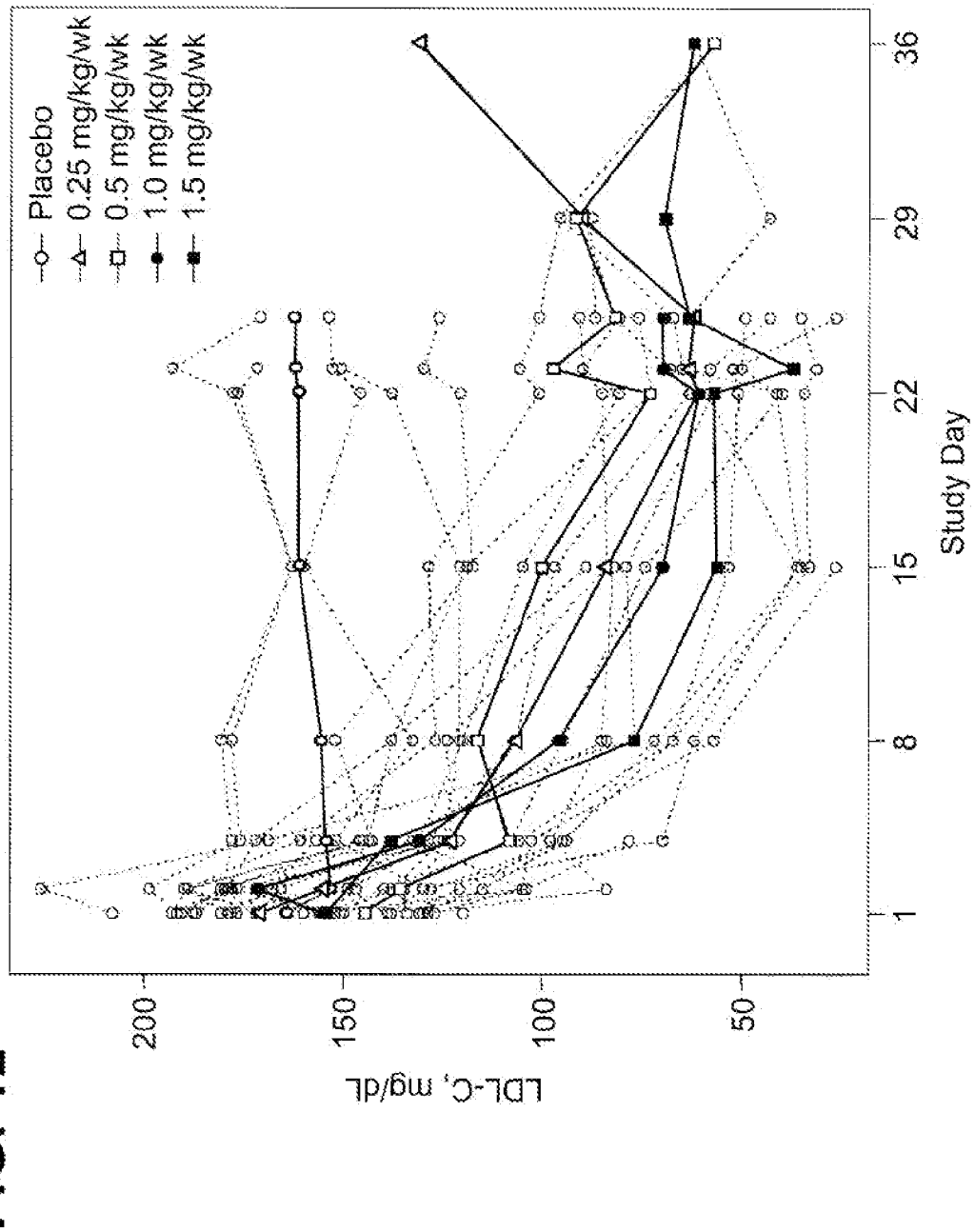

Placebo
0.25 mg/kg/wk
0.5 mg/kg/wk
1.0 mg/kg/wk
1.5 mg/kg/wk
LDL-C, mg/dL
200
150
100
50
1
8
15
22
29
36
Study Day

FIG. 12

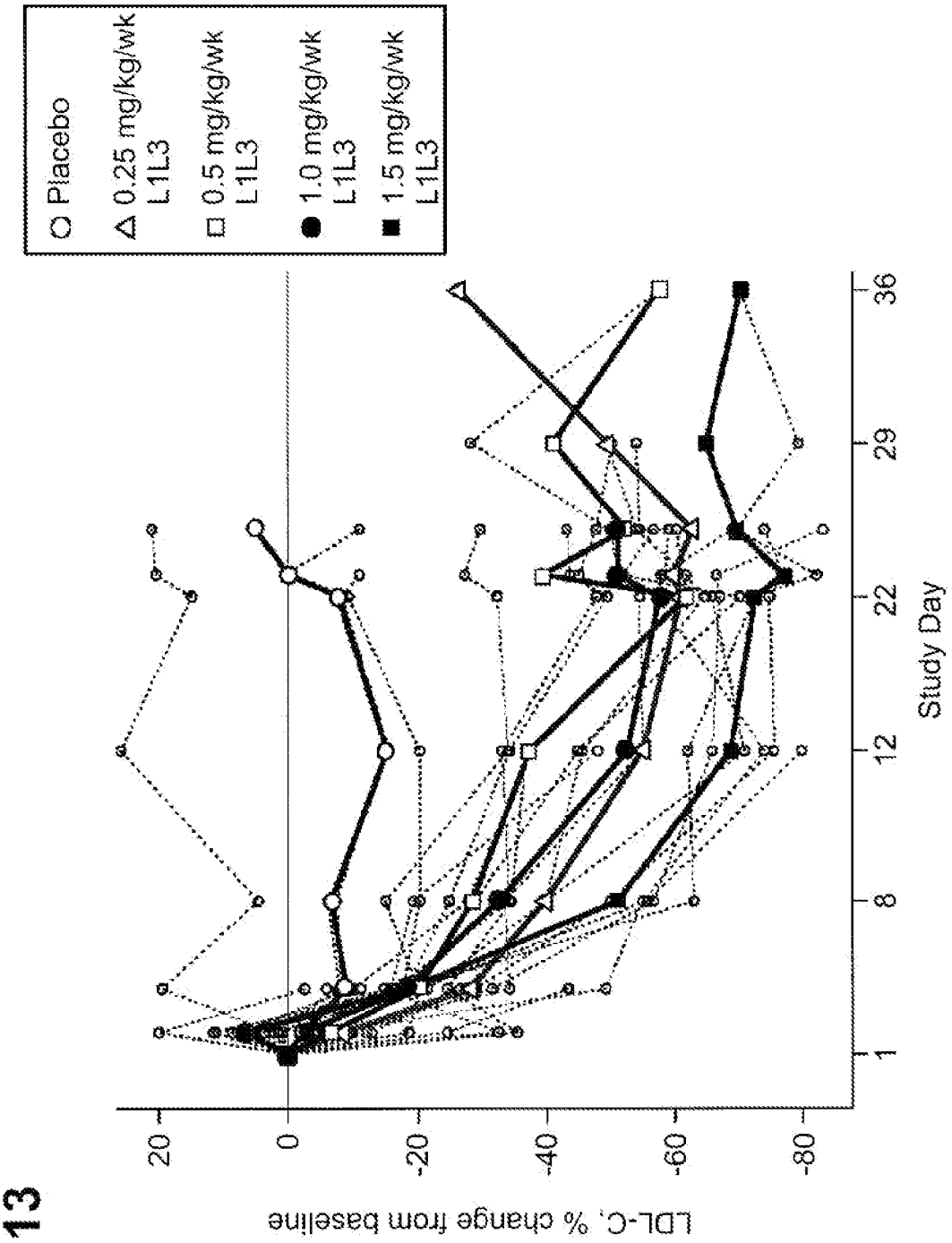

Placebo
0.25 mg/kg/wk L1L3
0.5 mg/kg/wk L1L3
1.0 mg/kg/wk L1L3
1.5 mg/kg/wk L1L3
LDL-C, % change from baseline
20
0
-20
-40
-60
-80
1
8
12
22
29
36
Study Day

FIG. 13

FIG. 14

Mean (SD) LDL-C vs Time Data

Day	Placebo			0.25 mg/kg/week			0.5 mg/kg/week			1 mg/kg/week			1.5 mg/kg/week		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	12	0.0	0.0	14	0.0	0.0	14	0.0	0.0	13	0.0	0.0	14	0.0	0.0
1	12	4.3	11.4	14	-1.3	7.1	14	-0.5	6.9	12	1.2	10.0	14	-2.9	15.7
2	12	3.4	11.8	14	-6.8	10.3	14	-5.8	12.5	12	-1.7	13.3	13	-2.3	18.8
4	12	0.0	10.3	14	-27.9	11.5	14	-23.1	12.8	12	-21.2	10.5	13	-20.9	14.7
8	11	-2.5	9.1	14	-38.9	12.2	13	-32.3	10.1	13	-44.3	17.2	13	-40.5	16.8
15	12	-2.7	10.5	14	-51.0	14.3	13	-46.0	16.9	12	-59.5	19.5	14	-57.8	18.1
22	12	-3.4	10.0	13	-54.8	9.3	13	-58.6	13.7	12	-64.7	13.7	14	-62.5	18.9
23	12	-0.7	10.3	13	-53.7	10.0	13	-55.2	13.7	12	-63.5	13.9	14	-62.1	21.1
25	10	-6.6	23.2	12	-60.1	11.7	13	-55.9	13.7	12	-60.1	22.3	14	-63.3	18.3
29	12	-6.8	12.2	13	-53.2	11.3	12	-54.3	14.3	12	-65.8	16.8	13	-63.3	17.0
36	11	-6.6	13.4	13	-23.6	13.7	12	-55.6	11.9	12	-65.7	17.3	12	-62.3	18.7
43	8	-11.9	22.8	8	-12.7	8.1	9	-36.3	8.7	8	-51.5	27.5	9	-69.5	10.1
50	6	13.3	17.4	7	-0.6	12.7	6	-25.4	13.2	6	-34.2	10.6	7	-67.6	9.7
57	6	5.1	17.7	7	-4.8	18.4	5	-18.4	8.9	6	-14.8	28.8	7	-66.6	8.5
64	4	2.3	7.2	5	1.8	8.0	4	-9.7	17.0	2	-17.6	27.0	4	-69.8	10.6
71	4	-5.7	3.2	4	-6.3	9.0	3	-12.2	4.5	2	-18.5	5.7	3	-59.9	18.9
78	2	-1.4	3.7	4	-19.6	23.6	2	-8.0	19.6	3	-23.6	44.6	3	-58.2	33.8

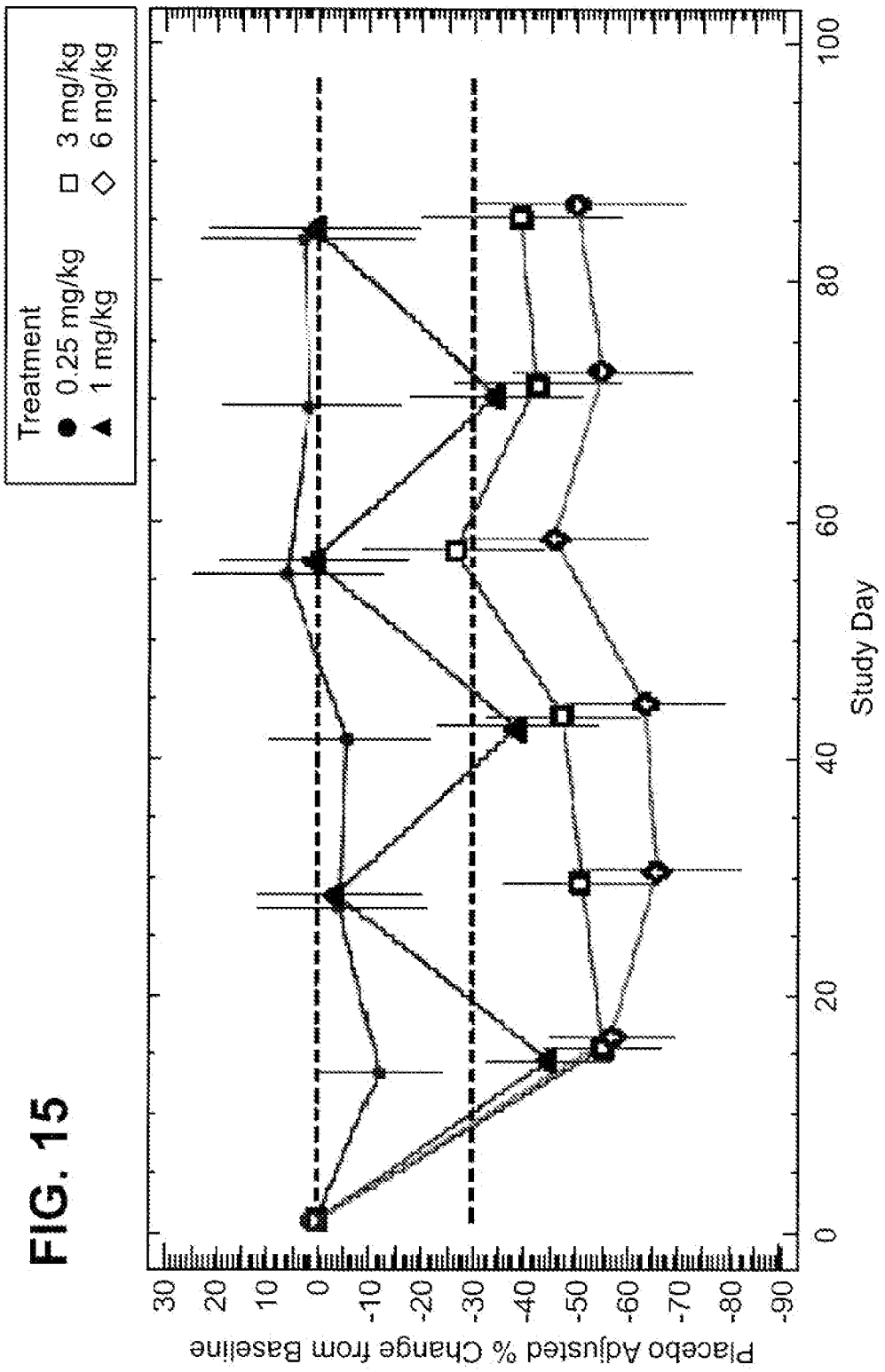

Treatment
0.25 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
Placebo Adjusted % Change from Baseline
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
0
20
40
60
80
100
Study Day

FIG. 15

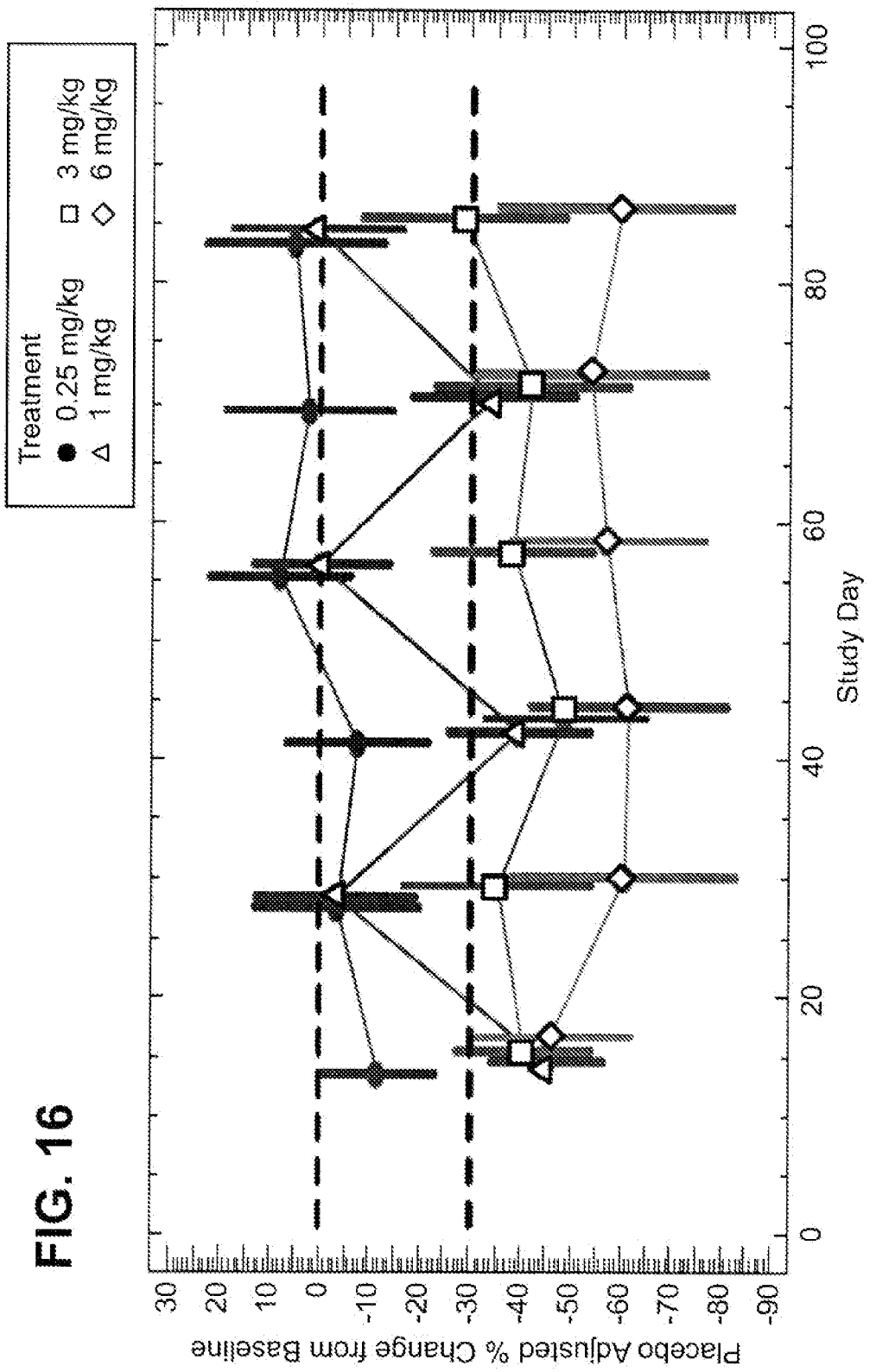

Treatment
0.25 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
Placebo Adjusted % Change from Baseline
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
0
20
40
60
80
100
Study Day

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/053534

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/40
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/029513 A2 (RINAT NEUROSCIENCE CORP [US]; LIANG HONG [US]; ABDICHE YASMINA NOUBIA) 18 March 2010 (2010-03-18) Claim 13; SEQ ID NO 54; page 24, line 7 - line 25; example 7 page 6, line 22 - line 28; figure 9 page 24 page 61, paragraph 4 - page 62, paragraph 2 page 83; claims 1-24 ----- -/--	1-36

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2012	13/11/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Klee, Barbara

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/053534

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2011/111007 A2 (RINAT NEUROSCIENCE CORP [US]; PFIZER [US]; PONS JAUME [US]; CHABOT JEF) 15 September 2011 (2011-09-15) Sequence 5 from Patent WO2011111007 equal to SEQ ID NO 11; 98,3% identity and similarity. SEQ 3 from Patent WO2011111007 equal to SEQ ID NO 12 93,5% identity; 97, 2% similarity; page 25, line 27 - page 26, line 12 page 8, line 9 - line 11; table 4 page 59, line 11 - page 60, line 7 -----	2,3, 9-11,13, 15,16, 22,25, 26,28-36
A	US 2011/142849 A1 (RUE SARAH [US] ET AL) 16 June 2011 (2011-06-16) paragraph [0229] - paragraph [0247] paragraph [0099] - paragraph [0103]; claims 1-50 -----	1-36

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/053534

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010029513 A2	18-03-2010	AR 073292 A1	28-10-2010
		AU 2009290438 A1	18-03-2010
		CA 2736349 A1	18-03-2010
		CN 102333542 A	25-01-2012
		CO 6351752 A2	20-12-2011
		EP 2344194 A2	20-07-2011
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		KR 20110056317 A	26-05-2011
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		US 2012014951 A1	19-01-2012
		US 2012015435 A1	19-01-2012
		WO 2010029513 A2	18-03-2010
WO 2011111007 A2	15-09-2011	AR 080511 A1	11-04-2012
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		CA 2781050 A1	16-06-2011
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		US 2011142849 A1	16-06-2011
		WO 2011072263 A1	16-06-2011

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2014.10.08

(21)申请号 201280044688.2
(22)申请日 2012.07.10
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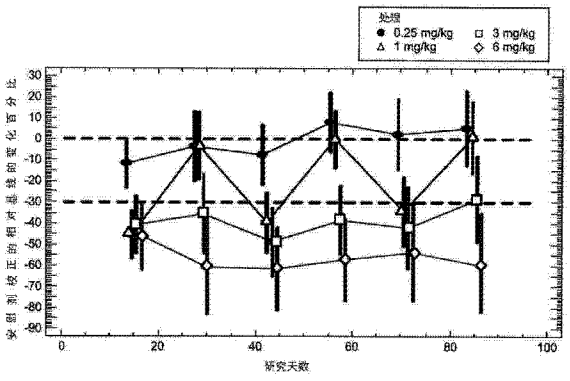
(54)发明名称

使用抗 PCSK9 抗体的治疗

(57)摘要

本发明涉及单独用 PCSK9 拮抗抗体或者与他汀类药物组合治疗易感于或经诊断患有特征在于血浆中低密度脂蛋白颗粒显著升高的疾病的人类患者的给药方案。



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1. 前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，用于治疗特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病，其中所述 PCSK9 拮抗抗体以初始施用剂量为至少大约 3mg/kg、大约 4mg/kg、大约 5mg/kg 或者大约 6mg/kg 施用；随后施用多个后续剂量，所述后续剂量与初始剂量大约相同或低于初始剂量，其中所述初始剂量与第一个后续及额外的后续剂量彼此间隔至少大约 4 周。

2. 前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，用于治疗特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病，其中所述 PCSK9 拮抗抗体以初始施用剂量为至少大约 200mg 或大约 300mg 施用；随后施用多个后续剂量，所述后续剂量与初始剂量大约相同或低于初始剂量，其中所述初始剂量与第一个后续及额外的后续剂量彼此间隔至少大约 4 周。

3. 前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，用于治疗特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病，其中所述 PCSK9 拮抗抗体以初始施用剂量为至少大约 50mg、大约 100mg 或大约 150mg 施用；随后施用多个后续剂量，所述后续剂量与初始剂量大约相同或低于初始剂量，其中所述初始剂量与第一个后续及额外的后续剂量彼此间隔至少大约 2 周。

4. 权利要求 1-3 任一项的 PCSK9 拮抗抗体，其中在施用初始剂量的 PCSK9 拮抗抗体之前已经施用了他汀类药物。

5. 权利要求 4 的 PCSK9 拮抗抗体，其中施用日剂量的他汀类药物。

6. 权利要求 4 或 5 的 PCSK9 拮抗抗体，其中在施用初始剂量的 PCSK9 抗体之前已经施用稳定剂量的他汀类药物至少大约 2、3、4、5 或 6 周。

7. 权利要求 4-6 任一项的 PCSK9 拮抗抗体，其中所述他汀类药物是阿托伐他汀、西立伐他汀、氟伐他汀、洛伐他汀、美伐他汀、匹伐他汀、普伐他汀、罗苏伐他汀、辛伐他汀、或者其任何药物可接受的盐或者立体异构体。

8. 权利要求 5 的 PCSK9 拮抗抗体，其中他汀类药物的日剂量选自 40mg 阿托伐他汀、80mg 阿托伐他汀、20mg 罗苏伐他汀、40mg 罗苏伐他汀、40mg 辛伐他汀、及 80mg 辛伐他汀。

9. 权利要求 1-8 任一项的 PCSK9 拮抗抗体，其中所述疾病是高胆固醇血症、血脂异常、动脉粥样硬化、心血管疾病或者急性冠脉综合征 (ACS)。

10. 权利要求 1-19 任一项的 PCSK9 拮抗抗体，其中所述抗体包含来自具有 SEQ ID NO:11 所示氨基酸序列的重链可变区的三个 CDR，及来自具有 SEQ ID NO:12 所示氨基酸序列的轻链可变区的三个 CDR。

11. 权利要求 10 的 PCSK9 拮抗抗体，其中所述抗体是 L1L3。

12. 权利要求 1-11 任一项的 PCSK9 拮抗抗体，其中所述抗体经皮下或者静脉内施用。

13. 权利要求 1-12 任一项的 PCSK9 拮抗抗体，其中所述抗体大约每月施用一次。

14. 治疗易感于或诊断为患有特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病的患者的方法，包括：

给患者施用初始剂量的前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，所述初始剂量为至少大约 3mg/kg、大约 4mg/kg、大约 5mg/kg，或者大约 6mg/kg；

给患者施用多个后续剂量的所述抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中所述初始剂量与第一次后续剂量及额外的后续剂量彼此间隔至少大约 4 周。

15. 治疗易感于或诊断为患有特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病的患者的方法，包括：

给患者施用初始剂量的前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，所述初始剂量为至少大约 200mg 或者大约 300mg，及

给患者施用多个后续剂量的所述抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中所述初始剂量与第一次后续剂量及额外的后续剂量彼此间隔至少大约 4 周。

16. 治疗易感于或诊断为患有特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平的疾病的患者的方法，包括：

给患者施用初始剂量的前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，所述初始剂量为至少大约 50mg、大约 100mg 或者大约 150mg，及

给患者施用多个后续剂量的所述抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中所述初始剂量与第一次后续剂量及额外的后续剂量彼此间隔至少大约 2 周。

17. 权利要求 14-16 任一项的方法，其中所述患者正在用他汀类药物进行治疗。

18. 权利要求 17 的方法，其中所述患者正在用日剂量的他汀类药物治疗。

19. 权利要求 17 或 18 的方法，其中所述患者在施用初始剂量的 PCSK9 抗体之前已经接受稳定剂量的他汀类药物至少大约 2、3、4、5 或 6 周。

20. 权利要求 17-19 任一项的方法，其中所述他汀类药物是阿托伐他汀、西立伐他汀、氟伐他汀、洛伐他汀、美伐他汀、匹伐他汀、普伐他汀、罗苏伐他汀、辛伐他汀、或者其任何药物可接受的盐或者立体异构体。

21. 权利要求 18 的方法，其中他汀类药物的日剂量选自 40mg 阿托伐他汀、80mg 阿托伐他汀、20mg 罗苏伐他汀、40mg 罗苏伐他汀、40mg 辛伐他汀及 80mg 辛伐他汀。

22. 权利要求 14-21 任一项的方法，其中所述疾病是高胆固醇血症、血脂异常、动脉粥样硬化、心血管疾病或者急性冠脉综合征 (ACS)。

23. 权利要求 14-22 任一项的方法，其中所述患者在施用初始剂量的 PCSK9 拮抗抗体之前的空腹总胆固醇水平为大约 70mg/dL 或更高。

24. 权利要求 14-23 任一项的方法，其中所述患者在施用初始剂量的 PCSK9 拮抗抗体之前的空腹 LDL 胆固醇水平为大约 130mg/dL 或更高。

25. 权利要求 14-24 任一项的方法，其中所述抗体包含来自具有 SEQ ID NO:11 所示氨基酸序列的重链可变区的三个 CDR，及来自具有 SEQ ID NO:12 所示氨基酸序列的轻链可变区的三个 CDR。

26. 权利要求 25 的方法，其中所述抗体是 L1L3。

27. 权利要求 14-26 任一项的方法，其中所述抗体经皮下或静脉内施用。

28. 权利要求 14-27 任一项的方法，其中所述抗体大约每月施用一次。

29. 一种制造品，其包含容器，置于所述容器内的包含 PCSK9 拮抗抗体的组合物，及含有施用 PCSK9 拮抗抗体的说明的包装插页，说明施用的初始剂量为至少大约 3mg/kg、大约 6mg/kg、大约 200mg 或者大约 300mg，并施用至少一个后续剂量，所述后续剂量与初始剂量相同或低于初始剂量，其中初始剂量与后续剂量的施用彼此间隔至少大约 4 周。

30. 权利要求 29 的制造品，其中所述包装插页包括给正用他汀类药物治疗的个体施用 PCSK9 拮抗抗体的说明。

31. 权利要求 30 的制造品，其中所述他汀类药物是阿托伐他汀、西立伐他汀、氟伐他汀、洛伐他汀、美伐他汀、匹伐他汀、普伐他汀、罗苏伐他汀、辛伐他汀、或者其药物可接受的盐或者立体异构体。

32. 权利要求 29-31 任一项的制造品，其中所述说明指明通过静脉内或皮下注射施用初始剂量，并通过静脉内或皮下注射施用至少一个后续剂量。

33. 权利要求 29-32 任一项的制造品，其中施用多个后续剂量。

34. 权利要求 29-33 任一项的制造品，进一步包含在所述容器上或与其关联的标签，所述标签指明所述组合物可用于治疗特征在于血液中升高的低密度脂蛋白胆固醇水平的病症。

35. 权利要求 29-34 任一项的制造品，其中所述标签指明所述组合物可用于治疗高胆固醇血症、致动脉粥样硬化性血脂异常、动脉粥样硬化、心血管疾病或者急性冠脉综合征 (ACS)。

36. 权利要求 29-35 任一项的制造品，其中所述抗体是 L1L3。

使用抗 PCSK9 抗体的治疗

[0001] 相关申请

[0002] 本申请要求 2011 年 7 月 14 日申请的美国临时申请号 61/507,865、2012 年 3 月 22 日申请的美国临时申请号 61/614,312 及 2012 年 5 月 4 日申请的美国临时申请号 61/643,063 的权益，所有文献全文引入本文。

发明领域

[0003] 本发明涉及治疗特征在于血浆中低密度脂蛋白 (LDL) 颗粒显著升高的疾病的治疗方案。所述治疗方案包括施用抗前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 抗体，其单独施用或者与他汀类药物组合施用。所述治疗方案增强了血液中 LDL- 胆固醇水平的降低，可用于预防和 / 或治疗胆固醇和脂蛋白代谢疾病，包括家族性高胆固醇血症、致动脉粥样硬化性血脂异常、动脉粥样硬化、急性冠脉综合征及更普遍地，心血管疾病。

[0004] 发明背景

[0005] 在美国，数百万人有心脏病及因此发生的心脏事件的风险。尽管可针对其多个风险因素进行治疗，心血管疾病及潜在的动脉粥样硬化在所有人群中仍是致死的主要原因。动脉粥样硬化是一种动脉疾病，是工业化国家中与很多死亡相关的冠心病的原因。目前已经鉴定了一些冠心病的风险因素 :血脂异常、高血压、糖尿病、吸烟、不良饮食、不运动及压力。临床上最相关且常见的血脂异常的特征在于伴随高胆固醇血症的 β- 脂蛋白 (极低密度脂蛋白 (VLDL) 和 LDL) 升高，不伴随或伴随高甘油三酯血症。Fredrickson et al.,1967,N Engl J Med.276:34-42,94-103,148-156,215-225 及 273-281。尽管用他汀类药物治疗 (目前动脉粥样硬化的标准治疗)，但是长期以来，仍明显不能满足治疗心血管疾病的需要，所述心血管疾病的 60-70% 为心血管事件、心脏病发作和中风。此外，新的指南建议应达到更低的 LDL 水平以保护高危患者，使其免于过早发生心血管疾病 (National Cholesterol Education Program(NCEP)2004)。

[0006] 已经表明 PCSK9 是血浆低密度脂蛋白胆固醇 (LDL-C) 的主要调节因子，并且已经有希望用以预防和治疗冠心病 (CHD)。Hooper et al.,2011,Expert Opin Ther Targets15(2):157-68。人类遗传学研究鉴定了功能获得型突变，其与升高的血清 LDL-C 水平及 CHD 的提前和发生相关，而功能缺失型突变与低 LDL-C 水平及 CHD 危险降低相关。Abifadel,2003,Nat Genet.43(2):154-6 ;Cohen,2005,Nat Genet.37(2):161-5 ;Cohen,2006,N Engl J Med.354(12):1264-72 ;Kotowski,2006,Am J Hum Genet.78(3):410-22。在人体中，PCSK9 的完全缺失导致低血清 LDL-C<20mg/dL，在健康对象中则否。Hooper,2007,193(2):445-8 ;Zhao,2006,Am J Hum Genet.79(3):514-523。

[0007] PCSK9 属于丝氨酸蛋白酶的枯草杆菌蛋白酶家族，是由一个 N- 末端前结构域 (prodomain)、一个枯草杆菌蛋白酶样催化结构域和一个 C- 末端富半胱氨酸 / 组氨酸结构域 (CHRD) 组成。PCSK9 在肝脏中高度表达，其在前结构域自催化裂解后分泌，所述前结构域保留与催化结构域的非共价结合。PCSK9 的催化结构域在血清 pH7.4(血清 pH 值) 与低密度脂蛋白受体 (LDLR) 的表皮生长因子样重复 A(EGF-A) 结构域结

合，在大约 pH5.5-6.0（内涵体 pH 值）具有更高的亲和性。Bottomley,2009,J Biol Chem.284(2):1313-23。C- 末端结构域参与 LDLR-PCSK9 复合物的内化，而不与催化结构域结合。Nassoury,2007,Traffic8(7):950 ;Ni,2010,J Biol Chem.285(17):12882-91 ; Zhang,2008,Proc Natl Acad Sci USA,2008,105(35):13045-50。PCSK9 的这两种功能都是靶向进行溶酶体降解和降低 LDL-C 的 LDLR-PCSK9 复合物所需的，这与功能缺失型和功能获得型相关的两个结构域中的突变一致。Lambert,2009,Atherosclerosis203(1):1-7。

[0008] 目前正在开发各种治疗方法以抑制 PCSK9，包括通过 siRNA 或反义寡核苷酸的基因沉默以及通过抗体破坏 PCSK9-LDLR 相互作用。Brautbar et al.,2011,Nature Reviews Cardiology8,253-265。例如，Chan,2009 和 Ni,2011 均报道了在小鼠和非人灵长类动物中具有降低 LDL-C 的活性的抗 -PCSK9 单克隆抗体 ；据报道当施用 3mg/kg 的 PCSK9 拮抗抗体时，每种抗体在非人灵长类动物中的半衰期分别为大约 61 小时和 77 小时。Chan,2009,Proc Natl Acad Sci USA106(24):9820-5 ;Ni,2011,J Lipid Res.52(1):78-86。已经报道了 PSCK9 拮抗抗体 7D4 有效降低食蟹猕猴 (cynomolgus monkeys) 血清胆固醇水平 ；在施用单剂 10mg/kg 的 PCSK9 拮抗抗体时，7D4 在食蟹猴中的半衰期低于 2 天。PCT 专利申请公开号 WO2010/029513。

[0009] 从本领域且在本发明之前可获得的信息中，仍不清楚低剂量、非频繁施用 PCSK9 拮抗抗体在人类患者中是否有效降低高胆固醇血症及相关 CVD 发生，及如果如此，为了这种体内有效性需要什么样的给药方案。

[0010] 发明概述

[0011] 本发明涉及通过抑制 PCSK9 活性及 PCSK9 对 LDL-C 血浆水平的相应作用而延长血液中 LDL-C 水平降低的时间的治疗方案。

[0012] 在一些实施方案中，本发明提供了治疗易感于或被诊断患有疾病的疾病的人类患者的方法，该疾病特征在于血液中升高的低密度脂蛋白胆固醇 (LDL-C) 水平，所述方法包括给患者施用初始剂量的前蛋白转化酶枯草杆菌蛋白酶 kexin9 型 (PCSK9) 拮抗抗体，初始剂量为至少大约 0.25mg/kg、0.5mg/kg、1mg/kg、1.5mg/kg、2mg/kg、3mg/kg、4mg/kg、5mg/kg、6mg/kg、8mg/kg、12mg/kg、50mg、100mg、150mg、200mg、250mg、300mg、350mg 或者 400mg ；以及给患者施用多个后续剂量的抗体，所述后续剂量与初始剂量大约相同或低于初始剂量，其中所述初始剂量与第一次后续剂量及再后续剂量的施用彼此间隔至少大约 1、2、3 或 4 周。本发明可以使用例如 PCSK9 拮抗抗体 L1L3 实施。在一些实施方案中，本发明可以使用这样的抗体实施，所述抗体包含来自具有 SEQ ID NO:11 所示氨基酸序列的重链可变区的三个 CDR 及来自具有 SEQ ID NO:12 所示氨基酸序列的轻链可变区的三个 CDR。

[0013] 在一些实施方案中，初始剂量可以是大约 0.25mg/kg、大约 0.5mg/kg、大约 1mg/kg 或者大约 1.5mg/kg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔大约 1 周。

[0014] 在另一些实施方案中，初始剂量可以是大约 2mg/kg、大约 4mg/kg、大约 8mg/kg 或者大约 12mg/kg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔至少大约 2 周。

[0015] 在另一些实施方案中，初始剂量可以是大约 50mg、大约 100mg、大约 150mg 或者大约 175mg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔至少大约 2 周。

[0016] 在另一些实施方案中，初始剂量可以是大约 3mg/kg 或者大约 6mg/kg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔至少大约 4 周。在另一些实施方案中，初始剂量可以是大约 200mg 或者大约 300mg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔至少大约 4 周。在一些实施方案中，PCSK9 拮抗抗体是经皮下施用。在一些实施方案中，PCSK9 拮抗体是经静脉内施用。

[0017] 在一些实施方案中，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔大约 4 周。在一些实施方案中，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔大约 8 周。多次后续剂量都可以与初始剂量大约相同或者低于初始剂量。

[0018] 在一些实施方案中，所述疾病可以是高胆固醇血症、血脂异常、动脉粥样硬化、心血管疾病、冠心病或者急性冠脉综合征 (ACS)。在初次施用 PCSK9 拮抗抗体之前，人类患者的空腹总胆固醇水平可为，例如大约 600mg/dL 或者更高。在初次施用 PCSK9 拮抗抗体之前，人类患者的空腹 LDL 胆固醇水平可例如是大约 130mg/dL 或者更高。在一些实施方案中，在初次施用 PCSK9 拮抗抗体之前，人类患者的空腹 LDL 胆固醇水平可以是大约 145mg/dL 或者更高。

[0019] 在一些实施方案中，患者正在用他汀类药物治疗。在一些实施方案中，患者在用日剂量的他汀类药物治疗。在一些实施方案中，在初次施用 PCSK9 拮抗抗体之前，可以对人类患者施用有效量的他汀类药物。在一些实施方案中，在初次施用 PCSK9 抗体之前，给患者施用稳定剂量的他汀类药物。所述稳定剂量可以是，例如日剂量或者隔日剂量。在一些实施方案中，在初次施用 PCSK9 拮抗抗体之前，对人类患者每日施用稳定剂量的他汀类药物，施用至少大约 2、3、4、5 或者 6 周。在一些实施方案中，在初次施用 PCSK9 拮抗抗体之前，施用稳定剂量的他汀类药物的人类患者的空腹 LDL 胆固醇水平为，例如大约 70 或 80mg/dL 或者更高水平。

[0020] 在一些实施方案中，所述方法进一步包括施用有效量的他汀类药物。

[0021] 在一些实施方案中，PCSK9 拮抗抗体的初始剂量可以是大约 3mg/kg 或者大约 6mg/kg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔大约 4 周或者大约 1 个月。在一些实施方案中，PCSK9 拮抗抗体的初始剂量可以是大约 200mg 或者大约 300mg，初始剂量与第一次后续剂量及再后续剂量的施用彼此可间隔大约 4 周或者大约 1 个月。

[0022] 所述他汀类药物可以是，例如阿托伐他汀 (atorvastatin)、西立伐他汀 (cerivastatin)、氟伐他汀 (fluvastatin)、洛伐他汀 (lovastatin)、美伐他汀 (mevastatin)、匹伐他汀 (pitavastatin)、普伐他汀 (pravastatin)、罗苏伐他汀 (rosuvastatin)、辛伐他汀 (simvastatin)，或者选自如下的组合治疗：辛伐他汀加依泽替米贝 (ezetimibe)，洛伐他汀加烟酸，阿托伐他汀 (atorvastin) 加氨氯地平 (amlodipine)，以及辛伐他汀加烟酸。在一些实施方案中，所述他汀类药物剂量可以是，例如 40mg 阿托伐他汀、80mg 阿托伐他汀、20mg 罗苏伐他汀、40mg 罗苏伐他汀、40mg 辛伐他汀或者 80mg 辛伐他汀。

[0023] 在一些实施方案中，所述方法包括给患者施用初始剂量的 PCSK9 拮抗抗体 L1L3，所述初始剂量为至少大约 3mg/kg 或者大约 6mg/kg ；以及给患者施用多个后续剂量的抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中初始剂量与第一次后续剂量及再后续剂量的施用彼此间隔至少大约 4 周，其中所述患者正在用稳定的日剂量的他汀类药

物治疗。在一些实施方案中，他汀类药物的稳定的日剂量可以是 40mg 阿托伐他汀、80mg 阿托伐他汀、20mg 罗苏伐他汀、40mg 罗苏伐他汀、40mg 辛伐他汀或者 80mg 辛伐他汀。

[0024] 在一些实施方案中，所述方法包括给患者施用初始剂量的 PCSK9 拮抗抗体 L1L3，所述初始剂量为至少大约 200mg 或者大约 300mg ；及给患者施用多个后续剂量的抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中初始剂量与第一次后续剂量及再后续剂量的施用彼此间隔至少大约 4 周，其中所述患者正在用稳定的日剂量的他汀类药物治疗。在一些实施方案中，所述方法包括给患者施用初始剂量的 PCSK9 拮抗抗体 L1L3，所述初始剂量为至少大约 50mg、大约 100mg、大约 150mg 或者大约 175mg ；及给患者施用多个后续剂量的抗体，所述后续剂量与初始剂量大约相同或者低于初始剂量，其中初始剂量与第一次后续剂量及再后续剂量的施用彼此间隔至少大约 2 周，其中所述患者正在用稳定的日剂量的他汀类药物治疗。在一些实施方案中，他汀类药物的稳定的日剂量可以是 40mg 阿托伐他汀、80mg 阿托伐他汀、20mg 罗苏伐他汀、40mg 罗苏伐他汀、40mg 辛伐他汀或者 80mg 辛伐他汀。

[0025] 在一些实施方案中，所述 PCSK9 拮抗抗体是经皮下或者静脉内施用的。

[0026] 本发明还提供了制造品，其包含容器，在该容器内包含 PCSK9 拮抗抗体的组合物，及含有用法说明的包装插页，所述用法说明涉及施用初始剂量及至少一次与初始剂量相同或低于其的后续剂量的 PCSK9 拮抗抗体，所述初始剂量为至少大约 0.25mg/kg、0.5mg/kg、1mg/kg、1.5mg/kg、2mg/kg、3mg/kg、4mg/kg、6mg/kg、8mg/kg、12mg/kg、50mg、100mg、150mg、200mg、250mg、300mg、350mg 或者 400mg。在一些实施方案中，本发明可以使用这样的抗体实施，所述抗体包含来自具有 SEQ ID NO:11 所示氨基酸序列的重链可变区的三个 CDR 以及来自具有 SEQ ID NO:12 所示氨基酸序列的轻链可变区的三个 CDR。在一些实施方案中，本发明可以使用 PCSK9 拮抗抗体 L1L3 实施。

[0027] 初始剂量与后续剂量的施用可以间隔例如至少大约 1、2、3、4、5、6、7 或者 8 周。在一些实施方案中，用法说明可以是，例如通过静脉内注射施用初始剂量，通过静脉内或皮下注射施用至少一次后续剂量。在另一些实施方案中，用法说明可以是，例如通过皮下注射施用初始剂量，及通过静脉内或者皮下注射施用至少一次后续剂量。

[0028] 在一些实施方案中，可以施用多个后续剂量。多次后续剂量的施用可以间隔例如至少 2、3、4、5、6、7 或者 8 周。

[0029] 在一些实施方案中，包装插页可进一步包括将 PCSK9 拮抗抗体施用正在用他汀类药物治疗的患者的用法说明。所述他汀类药物可以是，例如阿托伐他汀、西立伐他汀、氟伐他汀、洛伐他汀、美伐他汀、匹伐他汀、普伐他汀、罗苏伐他汀、辛伐他汀，或者选自如下的组合治疗 ：辛伐他汀加依泽替米贝，洛伐他汀加烟酸，阿托伐他汀加氨氯地平，及辛伐他汀加烟酸。

[0030] 在一些实施方案中，所述制造品可进一步包含容器上或与容器相关的标签，该标签指明所述组合物可用于治疗特征在于血液中升高的低密度脂蛋白胆固醇水平的疾病。所述标签可指明所述组合物可用于治疗，例如高胆固醇血症、致动脉粥样硬化性血脂异常、动脉粥样硬化、心血管疾病和 / 或急性冠脉综合征 (ACS)。

附图说明

[0031] 图 1 描述在施用 L1L3 抗体后的绝对空腹 LDL-C 水平 (mg/dL)。

[0032] 图 2 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的变化百分比。

[0033] 图 3 描述在施用 L1L3 抗体后的空腹总胆固醇水平相对基线的变化百分比。

[0034] 图 4 描述在施用 L1L3 抗体后的空腹阿朴脂蛋白 B 水平相对基线的变化百分比。

[0035] 图 5 描述在施用 L1L3 抗体后的空腹高密度脂蛋白胆固醇水平相对基线的变化百分比。

[0036] 图 6 描述在施用 L1L3 抗体后的空腹甘油三酯脂蛋白胆固醇水平相对基线的变化百分比。

[0037] 图 7A 描述在施用 L1L3 抗体后绝对空腹 LDL-C 水平 (mg/dL)。图 7B 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的变化百分比。

[0038] 图 8 描述在存在或不存在他汀类药物条件下施用 L1L3 抗体后空腹 LDL-C 水平相对基线的变化百分比。X- 轴表示 PCSK9 拮抗抗体 L1L3 的剂量 (mg/kg)。

[0039] 图 9A-F 描述 L1L3(A-C) 和 LDL-C(E-F) 的模拟时间谱。(A) 和 (D) :PCSK9 拮抗抗体 L1L3,2mg/kg。(B) 和 (E) :PCSK9 拮抗抗体 L1L3,6mg/kg。(C) 和 (F) :安慰剂。X- 轴表示时间 (天)。

[0040] 图 10 描述施用指定剂量 L1L3 之后的 LDL-C 模拟时间图。

[0041] 图 11 描述 L1L3 单一治疗 (monotherapy) 的研究设计示意图。

[0042] 图 12 描述在施用 L1L3 抗体后的绝对空腹 LDL-C 水平 (mg/dL)。

[0043] 图 13 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的变化百分比。

[0044] 图 14 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的平均变化百分比的表。

[0045] 图 15 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的变化百分比图表。

[0046] 图 16 描述在施用 L1L3 抗体后的空腹 LDL-C 水平相对基线的变化百分比图表,排除有剂量漏服的对象。

[0047] 发明详述

[0048] 本发明提供了治疗特征在于血浆中 LDL 颗粒明显升高的疾病的治疗方案。所述治疗方案包括施用 PCSK9 拮抗抗体,其是单独施用或者与他汀类药物组合施用。本发明的治疗方案可以延长血液中 LDL- 胆固醇水平降低的时间,可用于预防和 / 或治疗胆固醇和脂蛋白代谢性疾病,包括家族性高胆固醇血症、致动脉粥样硬化性血脂异常、动脉粥样硬化、急性冠脉综合征 (ACS),及更普遍地,心血管疾病。

[0049] 通用技术

[0050] 除非特别指出,实施本发明将使用分子生物学 (包括重组技术)、微生物学、细胞生物学、生物化学和免疫学的常规技术,这些技术在本领域技术范围内。这些技术已经在文献中充分解释,如 Molecular Cloning:A Laboratory Manual,second edition(Sambrook et al.,1989)Cold Spring Harbor Press ;Oligonucleotide Synthesis(M.J.Gait,ed.,1984) ;Methods in Molecular Biology,Humana Press ;Cell Biology:A Laboratory Notebook(J.E.Cellis,ed.,1998)Academic Press ;Animal Cell Culture(R.I.Freshney,ed.,1987) ;Introduction to Cell and Tissue Culture(J.P.Mather and P.E.Roberts,1998)Plenum Press ;Cell and Tissue Culture:Laboratory Procedures(A.

Doyle,J.B.Griffiths,and D.G.Newell,eds.,1993-1998)J.Wiley and Sons;Methods in Enzymology(Academic Press,Inc.);Handbook of Experimental Immunology(D.M.Weir and C.C.Blackwell,eds.);Gene Transfer Vectors for Mammalian Cells(J.M.Miller and M.P.Calos,eds.,1987);Current Protocols in Molecular Biology(F.M.Ausubel et al.,eds.,1987);PCR:The Polymerase Chain Reaction,(Mullis et al.,eds.,1994);Current Protocols in Immunology(J.E.Coligan et al.,eds.,1991);Short Protocols in Molecular Biology(Wiley and Sons,1999);Immunobiology(C.A.Janeway and P.Travers,1997);Antibodies(P.Finch,1997);Antibodies:a practical approach(D.Catty.,ed.,IRL Press,1988-1989);Monoclonal antibodies:a practical approach(P.Shepherd and C.Dean,eds.,Oxford University Press,2000);Using antibodies:a laboratory manual(E.Harlow and D.Lane(Cold Spring Harbor Laboratory Press,1999);The Antibodies(M.Zanetti and J.D.Capra,eds.,Harwood Academic Publishers,1995)。

[0051] 定义

[0052] “抗体”是一种免疫球蛋白分子,其能通过位于免疫球蛋白分子可变区内的至少一个抗原识别位点特异性结合靶,例如碳水化合物、多核苷酸、脂质、多肽等。如本文所用,术语“抗体”不仅涵盖了完整的多克隆或单克隆抗体,也包含任何抗原结合片段(即“抗原结合部分”)或者其单链,包含抗体的融合蛋白,以及包含抗原识别位点的免疫球蛋白分子的任何其它修饰的构型,例如但不限于:scFv、单域抗体(如鲨鱼和骆驼抗体)、大抗体(maxibodies)、微抗体(minibodies)、细胞内抗体(intrabodies)、双价抗体(diabodies)、三价抗体(triabodies)、四价抗体(tetrabodies)、v-NAR和bis-scFv(见例如Hollinger and Hudson,2005,Nature Biotechnology23(9):1126-1136)。抗体包括任何类型的抗体,如IgG、IgA或IgM(或者其亚类),并且不必是任何特定类型的抗体。根据抗体重链恒定区的氨基酸序列,免疫球蛋白可以被分为不同类型。有五种主要类型的免疫球蛋白:IgA、IgD、IgE、IgG和IgM,其中一些可以进一步分为亚类(同种型),例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。相应于不同类型免疫球蛋白的重链恒定区分别称作α、δ、ε、γ和μ。本领域熟知不同类型免疫球蛋白的亚基结构和三维构型。

[0053] 如本文所用,术语抗体的“抗原结合部分”是指完整抗体的一或多个片段,其保留特异性结合指定抗原(如PCSK9)的能力。抗体的抗原结合功能可以通过完整抗体的片段完成。术语抗体的“抗原结合部分”所涵盖的结合片段的实例包括Fab;Fab';$F(ab')_2$;由VH和CH1结构域组成的Fd片段;由抗体一个臂的VL和VH结构域组成的Fv片段;单域抗体(dAb)片段(Ward et al.,1989,Nature341:544-546),以及分离的互补决定区(CDR)。

[0054] 术语“单克隆抗体(Mab)”是指衍生自单拷贝或克隆的抗体,而不是根据其生产方法而定,所述单拷贝或克隆包括例如任何真核、原核或噬菌体克隆。优选地,本发明的单克隆抗体存在于同种或基本同种群体中。

[0055] “人源化”抗体是指非人(例如鼠)抗体的形式,其是嵌合的含有衍生自非人免疫球蛋白的最小序列的免疫球蛋白、免疫球蛋白链或者其片段(如抗体的Fv、Fab、Fab'、$F(ab')_2$或者其它抗原结合亚序列)。优选地,人源化抗体是人免疫球蛋白(受者抗体),其中来自受者的互补决定区(CDR)的残基由具有所希望的特异性、亲和性和能力(capacity)

的来自非人物种如小鼠、大鼠或兔的CDR(供者抗体)的残基置换。

[0056] 如本文所用,“人抗体”是指具有特定的氨基酸序列的抗体,该氨基酸序列相应于可由人产生的抗体和/或使用本领域技术人员已知的或者本发明公开的产生人抗体的技术产生的抗体的氨基酸序列。这个人抗体的定义包括包含至少一个人重链多肽或者至少一个人轻链多肽的抗体。一个这样的实例是包含鼠轻链和人重链多肽的抗体。人抗体可以使用本领域已知的各种技术产生。在一个实施方案中,人抗体选自噬菌体文库,其中所述噬菌体文库表达人抗体(Vaughan et al.,1996,Nature Biotechnology,14:309-314;Sheets et al.,1998,Proc.Natl.Acad.Sci.(USA)95:6157-6162;Hoogenboom and Winter,1991,J.Mol.Biol.,227:381;Marks et al.,1991,J.Mol.Biol.,222:581)。人抗体也可以通过免疫动物而产生,所述动物中已经转基因导入了人免疫球蛋白基因座以置换内源基因座,例如其中内源免疫球蛋白基因已经部分或完全失活的小鼠。这种方法在美国专利号5,545,807、5,545,806、5,569,825、5,625,126、5,633,425和5,661,016中进行了描述。或者,人抗体可以通过使产生针对抗靶抗原的抗体的人B淋巴细胞永生化而制备(这种B淋巴细胞可以回收自个体或者可以已经在体外免疫)。见例如Cole et al.Monoclonal Antibodies and Cancer Therapy,Alan R.Liss,p.77,1985;Boerner et al.,1991,J.Immunol.,147(1):86-95;以及美国专利号5,750,373。

[0057] 抗体的“可变区”是指单独或者组合形式的抗体轻链的可变区或者抗体重链的可变区。如本领域公知的,重链和轻链的可变区均由四个构架区(FR)组成,所述框架区通过三个互补决定区(CDR)连接。互补决定区也称作超变区,参与抗体的抗原结合位点的形成。如果希望一个所述可变区的变体,特别是具有位于CDR区之外(即在构架区内)的氨基酸残基的替换,可以鉴定合适的氨基酸替换,优选保守氨基酸替换,上述鉴定通过对比所述可变区与其它抗体的可变区而进行,所述其它抗体含有与所述可变区相同标准类型(canonincal class)的CDR1和CDR2序列(Chothia and Lesk,J Mol Biol196(4):901-917,1987)。当选择FR位于对象CDR的侧面时,例如当人源化或者优化抗体时,优选来自含有相同规范类型的CDR1和CDR2序列的抗体的FR。

[0058] 可变结构域的“CDR”是根据Kabat、Chothia、Kabat与Chothia二者的累加、AbM、接触和/或构象定义或者本领域熟知的任何CDR确定方法鉴定的可变区内的氨基酸残基。抗体CDR最初根据Kabat等的定义可被鉴定为超变区。见例如Kabat et al.,1992,Sequences of Proteins of Immunological Interest,5th ed.,Public Health Service,NIH,Washington D.C。CDR的位置最初根据Chothia及其它人所述也可以被鉴定为结构环结构。见例如Chothia et al.,1989,Nature342:877-883。其它CDR鉴定方法包括“AbM定义”,其是Kabat与Chothia所述方法的折中,使用Oxford Molecular's AbM抗体建模软件(现在的Accelrys®)获得,或者CDR的“接触定义”基于观测到抗原接触,如MacCallum et al.,1996,J.Mol.Biol.,262:732-745所述。在另一方法中,在本文称作CDR的“构象定义”,所述CDR的位置可以鉴定为热力学上(enthalpic)有助于抗原结合的残基。见例如Makabe et al.,2008,Journal of Biological Chemistry,283:1156-1166。其它CDR边界定义可以不严格根据上述方法之一,尽管根据预测或实验发现CDR可以缩短或延长,但是其与Kabat CDR的至少一部分重叠,其中所述实验为发现特定残基或残基组或者甚至全部的CDR不显著影响抗原结合。如本文所用,CDR可以是指通过本领域已知的任何方法

包括方法的组合定义的 CDR。本发明使用的方法可利用根据任何这些方法定义的 CDR。对于含有一个以上 CDR 的任何指定的实施方案，所述 CDR 可以根据任何 Kabat、Chothia、扩展、AbM、接触和 / 或构象定义加以定义。

[0059] 如本领域所已知，抗体的“恒定区”是指单独或者组合的抗体轻链的恒定区或者抗体重链的恒定区。

[0060] 如本文所用，术语“PCSK9”是指保留至少一部分 PCSK9 活性的任何形式的 PCSK9 及其变体。除非特别指出，如特别提及人 PCSK9，PCSK9 包括有天然序列 PCSK9 的所有哺乳动物物种，如人、犬、猫、马和牛。人 PCSK9 的一个例子是 Uniprot 编号 Q8NBP7(SEQ ID NO:1)。

[0061] 如本文所用，“PCSK9 拮抗抗体”是指抗 PCSK9 抗体，其能抑制 PCSK9 生物活性和 / 或由 PCSK9 信号传导介导的下游途径，包括 PCSK9 介导的 LDLR 下调，及 PCSK9 介导的 LDL 血液清除率降低。PCSK9 拮抗抗体涵盖了这样的抗体，其阻断、拮抗、抑制或降低（至任何程度，包括显著地）PCSK9 生物活性，包括由 PCSK9 信号传导介导的下游途径，如 LDLR 相互作用和 / 或对 PCSK9 的细胞应答的激发。对于本发明，应明确地理解术语“PCSK9 拮抗抗体”涵盖了所有先前确定的术语、名称和功能状态及特性，由此 PCSK9 自身、PCSK9 生物活性（包括但不限于其介导与 LDLR 相互作用、下调 LDLR 及降低血液 LDL 清除率的任何方面的能力）或者生物活性的后果被充分失效、降低或中和化至任何有意义的程度。在一些实施方案中，PCSK9 拮抗抗体与 PCSK9 结合并阻止其与 LDLR 的相互作用。在例如美国专利申请公开号 20100068199 中提供了 PCSK9 拮抗抗体的例子，所述专利以其全部内容在此并入本文。

[0062] 如本文所用，“完全拮抗剂”是在有效浓度基本完全阻断 PCSK9 的可测作用的拮抗剂。部分拮抗剂是指能部分阻断可测作用的拮抗剂，但是即使在最高浓度也不是完全拮抗剂。基本完全是指至少大约 80%、优选至少大约 90%、更优选地至少大约 95、最优选至少大约 98% 或 99% 的可测作用被阻断。在本文中描述了相关的“可测作用”，其包括在 Huh7 细胞中体外测定的通过 PCSK9 拮抗剂下调 LDLR，在体内降低血液（或者血浆）中总胆固醇水平，及在体内降低血液（或者血浆）中 LDL 水平。

[0063] 如本文所用，术语“有临床意义的”是指人体中血液 LDL- 胆固醇水平降低至少 15%，或者小鼠中血液总胆固醇水平降低至少 15%。需明确的是在血浆或血清中的测量可以替代血液中水平的测量。

[0064] 如本文所用，术语“给药方案”是指给患者施用的整个治疗过程，例如用 PCSK9 拮抗抗体治疗。

[0065] 如本文所用，在血液中 LDL 胆固醇的平均水平在特定水平范围内的时间的背景下，术语“连续”是指所述平均水平在该特定范围内的时间不被任何平均水平不在特定水平范围内的时间打断。

[0066] 如本文所用，在血液中 LDL 胆固醇的平均水平在特定水平范围内的时间的背景下，术语“不连续的”是指平均水平在特定范围内的时间被一些量的时间打断（如 15 分钟、20 分钟、30 分钟、45 分钟、1 小时、2 小时、3 小时、4, 小时、5 小时、6 小时、8 小时、10 小时、12 小时、14 小时、16 小时 18 小时、20 小时、24 小时 28 小时、32 小时、36 小时、40 小时、44 小时、48 小时、60 小时、72 小时、84 小时、90 小时，或者以任何上述特别陈述时间为上限和下限的任何范围的时间），在此期间平均水平不在特定水平范围内。

[0067] 术语“多肽”、“寡肽”、“肽”和“蛋白质”在本文可互换使用，是指任何长度的氨基

酸链，优选相对较短的链（如 10-100 个氨基酸）。氨基酸链可以是线性的或者是分支的，其可包含修饰的氨基酸，和 / 或可以由非氨基酸打断。该术语还涵盖了已经天然修饰或通过介入修饰的氨基酸链。例如二硫键形成、糖基化、脂化、乙酰化、磷酸化，或者任何其它处理或修饰，如与标记成分配合。该定义还包括例如含有一或多个氨基酸类似物（包括例如非天然氨基酸等）以及本领域已知的其它修饰的多肽。应理解多肽可以作为单链或者结合的链的形式存在。

[0068] 如本领域已知，“多核苷酸”或者“核酸”在本文互换使用，是指任何长度的核苷酸链，包括 DNA 和 RNA。核苷酸可以是脱氧核糖核苷酸、核糖核苷酸、修饰的核苷酸或碱基和 / 或其类似物，或者可以通过 DNA 或 RNA 聚合酶掺入链中的任何底物。多核苷酸可包含修饰的核苷酸，如甲基化核苷酸及其类似物。如果存在，则可以在链装配之前或之后对核苷酸结构的进行修饰。核苷酸序列可以由非核苷酸成分打断。多核苷酸在聚合后可进一步修饰，如通过与标记成分配合而修饰。其它类型的修饰包括例如“帽”，用类似物替换一或多个天然存在的核苷酸；核苷酸间修饰，如具有无电荷键（如甲基磷酸酯，磷酸三酯，氨基磷酸酯，氨基甲酸酯等）及具有带电荷键（如硫代磷酸酯，二硫代磷酸酯等）的修饰，含有悬垂成分如蛋白质（如核酸酶，毒素，抗体，信号肽，聚 -L- 赖氨酸等）的修饰，具有嵌入剂（如吖啶，补骨脂素等）的修饰，含有螯合剂（如金属，放射性金属，硼，氧化金属等）的修饰，含有烷化剂的修饰，具有修饰的键（如 α 端基异构体核酸等）以及未修饰形式的多核苷酸的修饰。进一步地，任何通常存在于糖中的羟基基团均可以被例如膦酸酯基团、磷酸酯基团置换，由标准的保护基团保护，或者激活为制备与另外的核苷酸的另外的键，或者可以与固体支持物配合。5’ 和 3’ 末端 OH 可以磷酸化的或者用胺或 1-20 个碳原子的有机加帽基团取代。其它羟基也可以衍生为标准的保护基团。多核苷酸也可以含有本领域通常已知的核糖或脱氧核糖的相似形式，包括例如 2’-O- 甲基 -、2’-O- 烯丙基、2’- 氟 - 或者 2’- 叠氮基 - 核糖，碳环状糖类似物，α - 或 β - 异头糖，差向异构体糖如阿拉伯糖、木糖或来苏糖，吡喃糖，呋喃糖，景天庚糖，无环类似物和无碱基核苷类似物如甲基核苷。一或多个磷酸二酯键可以由各种连接基团置换。所述各种连接基团包括但不限于其中磷酸酯由 P(O)S（硫代）、P(S)S（二硫代）、(O)NR_2（酰胺酯 (amidate)）、P(O)R、P(O)OR’、CO 或 CH_2（甲缩醛 (formacetal)）置换，其中 R 或 R’ 独立地是 H 或者是取代或非取代的烷基 (1-20C)，任选含有醚 (-O-) 键、芳基、烯基、环烷基、环烯基或者芳烷基。多核苷酸中并非所有键都需要是相同的。前述内容适用于本文提及的所有多核苷酸，其包括 RNA 和 DNA。

[0069] 如本文所用，当平衡解离常数等于或低于 20nM、优选低于大约 6nM、更优选低于大约 1nM、最优选低于大约 0.2nM 时，抗体与 PCSK9 “相互作用”，所述平衡解离常数是通过在美国专利申请公开 No.20100068199 的实施例 2 中揭示的方法测量的。

[0070] 与表位“优先结合”或者“特异性结合”（在本文可互换使用）的抗体是本领域熟知的术语，确定这种特异性或优先结合的方法也为本领域熟知。如果一个分子与一特定细胞或物质较与另外的细胞或物质更频繁、更迅速、更持久和 / 或更亲和地反应或结合，则称该分子呈现出“特异性结合”或者“优先结合”。如果一种抗体与靶较与其它物质以更高的亲和性、亲合力、更容易和 / 或更持久地结合，则该抗体“特异性结合”或者“优先结合”所述靶。例如，特异性或优先结合一个 PCSK9 表位的抗体是与其结合其它 PCSK9 表位或非 -PCSK9 表位相比以更高的亲和性、亲合力、更容易地和 / 或更持久地结合这个表位的抗体。通过阅读

这个定义，也应理解，如特异性或优先结合第一个靶的一种抗体（或部分或表位）可能特异性或优先结合第二个靶或者可能不特异性或优先结合第二个靶。因此，“特异性结合”或者“优先结合”不是必须要求（但是可以包括）专一结合。通常但非必须地，提及结合时是指优先结合。

[0071] 如本文所用，“基本纯的”是指材料是至少 50% 纯的（即无污染物）、更优选至少 90% 纯的、更优选至少 95% 纯的、再优选至少 98% 纯的、最优选至少 99% 纯的。

[0072] “宿主细胞”包括可以是或者已经是掺入多核苷酸插入体的载体的接受体的细胞个体或细胞培养物。宿主细胞包括单一宿主细胞的后代，以及可以是与原始亲代细胞非必需完全相同（在形态学或者在基因组 DNA 补体中）的后代，其中的不同是由于天然、偶然或有意的突变。宿主细胞包括在体内用本发明的多核苷酸转染的细胞。

[0073] 正如本领域所已知，术语“Fc 区”用于定义免疫球蛋白重链的 C- 末端区域。“Fc 区”可以是天然序列 Fc 区或者变体 Fc 区。尽管免疫球蛋白重链的 Fc 区的边界可变，但是人 IgG 重链 Fc 区通常定义为从位置 Cys226 或者从 Pro230 的氨基酸残基至其羧基末端。Fc 区中残基的编号是如 Kabat 所述 EU 索引编号。Kabat et al.，Sequences of Proteins of Immunological Interest，5th Ed. Public Health Service，National Institutes of Health，Bethesda，Md.，1991。免疫球蛋白的 Fc 区通常包含两个恒定结构域，即 CH2 和 CH3。

[0074] 如在本领域中所用，“Fc 受体”和“FcR”描述了结合抗体的 Fc 区的受体。优选的 FcR 是天然序列人 FcR。此外，优选的 FcR 是结合 IgG 抗体的 FcR（γ 受体），包括 FcγRI、FcγRII 和 FcγRIII 亚类受体，包括这些受体的等位基因变体和可变剪接形式。FcγRII 受体包括 FcγRIIA（“激活受体”）和 FcγRIIB（“抑制受体”），其具有相似的氨基酸序列，主要在其细胞质结构域中有不同。FcR 在 Ravetch and Kinet，1991、Ann. Rev. Immunol.，9:457-92、Capel et al.，1994、Immunomethods，4:25-34 及 de Haas et al.，1995，J. Lab. Clin. Med.，126:330-41 中进行了综述。“FcR”也包括新生儿的受体 FcRn，其与母亲 IgG 转移至胎儿相关 (Guyer et al.，1976 J. Immunol.，117:587 ；及 Kim et al.，1994，J. Immunol.，24:249)。

[0075] 如本文所用，关于抗体的术语“竞争”是指第一种抗体或其抗原结合部分结合表位的方式与第二种抗体或其抗原结合部分结合表位的方式足够相似，这样，与不存在第二种抗体的条件下相比，第一种抗体与其关联表位的结合的结果在存在第二种抗体的条件下明显降低。或者，可以但无须是第二种抗体与其表位结合在存在第一种抗体的条件下也明显降低的情况。即第一种抗体可抑制第二种抗体与其表位的结合，而无须第二种抗体抑制第一种抗体与其各自的表位的结合。然而，在每种抗体明显抑制另一抗体与其关联表位或配体结合的情况中，无论是相同、较高或较低程度，所述抗体均被称作彼此“交叉竞争”结合各自的表位。竞争和交叉竞争抗体均涵盖在本发明中。无论这种竞争或者交叉竞争发生的机制是什么（如位阻、构象改变，或者结合共有表位或者其一部分），技术人员基于本发明提供的教导将意识到这种竞争和 / 或交叉竞争抗体涵盖在本发明内，并可用于本发明公开的方法中。

[0076] 抗体的一个表位与另一（第二个）表位或者与和 LDLR 的 EGF 样结构域相互作用的 PCSK9 表面“重叠”，是指共享相互作用的 PCSK9 残基的空间。为了计算重叠百分比，例如要求保护的抗体的 PCSK9 表位与和 LDLR 的 EGF 样结构域相互作用的 PCSK9 表面的重叠

百分比，当与 LDLR 复合时隐藏的 PCSK9 表面积基于每个残基 (per-residue) 进行计算。还要计算在 PCSK9: 抗体复合物中这些残基的隐藏面积。为了防止可能的重叠超过 100%，在 PCSK9: 抗体复合物中比在 LDLR:PCSK9 复合物中具有更高隐藏的表面积的残基的表面积根据 LDLR:PCSK9 复合物设定数值 (100%)。表面重叠百分比是通过合计 LDLR:PCSK9 相互作用残基进行计算并且用相互作用面积加权。

[0077] “功能性 Fc 区”具有天然序列 Fc 区的至少一个效应子功能。“效应子功能”的例子包括 C1q 结合，补体依赖性细胞毒性，Fc 受体结合，抗体依赖性细胞介导的细胞毒性，吞噬作用，细胞表面受体（如 B 细胞受体）的下调等等。这种效应子功能通常需要 Fc 区与结合结构域（如抗体可变结构域）组合，且可以使用本领域已知的评估这种抗体效应子功能的各种测定评定。

[0078] “天然序列 Fc 区”包含与天然发现的 Fc 区的氨基酸序列相同的氨基酸序列。“变体 Fc 区”包含与天然序列 Fc 区由于至少一个氨基酸修饰而不同的氨基选序列，但是仍保留天然序列 Fc 区的至少一个效应子功能。优选地，变体 Fc 区与天然 Fc 区或者与亲代多肽的 Fc 区相比具有至少一个氨基酸替换，例如在天然序列 Fc 区或者亲代多肽的 Fc 区中具有大约 1-10 个氨基酸替换，优选大约 1-5 个氨基酸替换。本文变体 Fc 区优选与天然序列 Fc 区和 / 或与亲代多肽的 Fc 区具有至少大约 80% 序列相同性，最优选具有至少大约 90%、更优选至少大约 95%、至少大约 96%、至少大约 97%、至少大约 98%、至少大约 99% 序列相同性。

[0079] 如本文所用，术语“阿托伐他汀”、“西立伐他汀”、“氟伐他汀”、“洛伐他汀”、“美伐他汀”、“匹伐他汀”、“普伐他汀”、“罗苏伐他汀”和“辛伐他汀”分别包括阿托伐他汀、西立伐他汀、氟伐他汀、洛伐他汀、美伐他汀、匹伐他汀、普伐他汀、罗苏伐他汀、辛伐他汀，及其药物可接受的盐或者立体异构体。如本文所用，术语“药物可接受的盐”包括患者生理学耐受的盐。典型地，这种盐是由无机酸或碱和 / 或有机酸或碱制备的。这些酸和碱的实例为本领域技术人员熟知。

[0080] 如本文所用，“治疗”是获得有益或希望的结果的方法。对于本发明，有益或希望的临床结果包括但不限于如下一或多项 :增强 LDL 清除及降低异常胆固醇和 / 或脂蛋白水平的发病率或加以改善，所述异常胆固醇和 / 或脂蛋白水平是由代谢和 / 或饮食失调造成的，或者包括家族性高胆固醇血症、致动脉粥样硬化性血脂异常、动脉粥样硬化、ACS 及更普遍地，心血管疾病 (CVD)。

[0081] “降低发病率”是指任何降低严重度（可包括减少通常用于这种病症的（如接受）其它药物和 / 或治疗的需要和 / 或量）。如本领域技术人员所理解，个体在其对治疗的反应方面可能不同，因此，如“降低发病率的方法”表达的是基于合理预期施用 PCSK9 拮抗抗体，这种施用可能导致特定个体的这种发病率降低。

[0082] “改善”是指与未施用 PCSK9 拮抗抗体相比，一或多个症状的减轻或改善。“改善”也包括缩短或减少症状的持续时间。

[0083] 如本文所用，药物、化合物或者药物组合物的“有效剂量”或者“有效量”，是足以实现任一或多个有益或希望的结果的量。对于预防性用途，有益或希望的结果包括消除或降低疾病风险，减轻疾病严重度，或者延迟疾病发生，包括疾病的生物化学、组织学和 / 或行为症状、其并发症以及在疾病发生期间存在的中间病理学表型。对于治疗性用途，有益或希望的结果包括这样的临床结果，如减少高胆固醇血症或者减少血脂异常、动脉粥样硬化、心

血管疾病或者冠心病的一或多个症状，降低治疗该疾病所需的其它药物的剂量，增强另一药物的作用，和 / 或延迟患者疾病的进展。有效剂量可以在一或多次用药中施用。对于本发明，药物、化合物或者药物组合物的有效剂量是足以直接或间接实现预防或治疗的量。如临床上所理解，药物、化合物或者药物组合物的有效剂量可以联合或者不联合另一药物、化合物或者药物组合物而实现。因此，在施用一或多种治疗剂的情况下，“有效剂量”可以认为是这样的，如果与一或多种其它物质联合时，单一药剂可以实现或实现了希望的结果，则可以认为其是以有效剂量施用的。

[0084] “个体”或“对象”是哺乳动物，更优选是人。哺乳动物还包括但不限于家畜、比赛动物 (sport animals)、宠物、灵长类动物、马、狗、猫、小鼠和大鼠。

[0085] 如本文所用，“载体”是指一种构建体，其在宿主细胞中能输送及优选地，表达一或多个感兴趣的基因或序列。载体例子包括但不限于病毒载体、裸 DNA 或 RNA 表达载体、质粒、粘粒或噬菌体载体、与阳离子缩合剂相关的 DNA 或 RNA 表达载体、包裹在脂质体中的 DNA 或 RNA 表达载体，以及某些真核细胞如生产细胞。

[0086] 如本文所用，“表达控制序列”是指指导核酸转录的核酸序列。表达控制序列可以是启动子，如组成型或可诱导启动子，或者是增强子。表达控制序列与被转录的核酸序列可操纵地连接。

[0087] 如本文所用，“药物可接受的载体”或者“药物可接受的赋形剂”包括当与活性成分组合时使得所述成分保留生物活性及与对象的免疫系统不反应的任何材料。例如包括但不限于任何标准药物学载体，如磷酸盐缓冲盐水溶液，水，乳状液如油 / 水乳状液，以及各种类型的增湿剂。对于喷雾或肠道外施用，优选的稀释剂是磷酸盐缓冲盐水 (PBS) 或者生理盐水 (0.9%)。包含这种载体的组合物通过熟知的常规方法配制 (见例如 Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990 ; and Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing, 2000)。

[0088] 如本文所用，术语“k_{on}”是指抗体与抗原结合的速率常数。特别地，速率常数 (k_{on} 和 k_{off}) 及平衡解离常数是使用 Fab 抗体片段 (即一价) 和 PCSK9 测量的。

[0089] 如本文所用，术语“k_{off}”是指抗体从抗体 / 抗原复合物中解离的速率常数。

[0090] 如本文所用，术语“K_D”是指抗体 - 抗原相互作用的平衡解离常数。

[0091] 关于本文一个数值或参数所用术语“大约”包括 (及描述了) 针对该数值或参数的实施方案。例如，涉及“大约 X”的描述包括“X”的描述。数字范围包含在数字限定范围内。

[0092] 应理解在本文无论何处用词语“包含”描述实施方案时，也提供了“由…组成”和 / 或“基本上由…组成”描述的类似的实施方案。

[0093] 在以 Markush 组或其他并列组描述的本发明的方面或实施方案时，本发明不仅涵盖整体列出的整个组，还涵盖了所述组的所有单个成员和主要组中所有可能的亚组，以及缺少一或多个所述组成员的主要组。本发明还设想在要求保护的发明中明确排除任何所述组的成员中的一或多个。

[0094] 除非特别指出，本文使用的所有技术和学术术语具有与本发明所属领域中的技术人员通常理解的相同含义。本文描述了方法和材料的例子，尽管也可以使用与本文所述方

法和材料相似或等价的方法和材料实施或检测本发明。本文提及的所有出版物及其它参考文献均以其全部内容并入本文。在内容有冲突的情况中，以本说明书，包括定义为准。尽管本文引用了许多文献，但是这种引用不表示承认这些文献任何一篇组成本领域公知常识一部分。在本说明书和权利要求书中，单词“包含 (comprise)”或其变化形式用语如“comprises”或“comprising”，应理解为意味着包含一个所陈述的整体或一组整体，但是不排除任何其它整体或其他组整体。除非特别指出，单数术语应包括复数形式，复数术语应包括单数形式。所述材料、方法和实施例只是举例说明本发明，无限制本发明之意。

[0095] 关于抗 PCSK9 抗体的公开信息包括如下公开申请 :PCT/IB2009/053990，2010 年 3 月 18 日以 WO2010/029513 公开；及美国专利申请号 12/558312，2010 年 3 月 18 日以 US2010/0068199 公开，所述专利均以其全部内容并入本文。

[0096] 用抗 PCSK9 抗体治疗

[0097] 本发明提供了治疗特征在于血浆中 LDL 颗粒显著升高的疾病的治疗方案。所述治疗方案包括施用 PCSK9 拮抗抗体。在一些实施方案中，所述治疗方案包括将 PCSK9 拮抗抗体施用于已经在接受稳定剂量他汀类药物治疗的患者。本发明公开的治疗方案提供了有效量的 PCSK9 拮抗抗体，其拮抗循环 PCSK9，用于在个体中治疗或预防高胆固醇血症，和 / 或血脂异常、动脉粥样硬化、心血管疾病、急性冠脉综合征 (ACS) 或者冠心病的至少一个症状。

[0098] 有利地，本发明公开的治疗方案导致 LDL-C 显著而持久降低。优选地，血液胆固醇和 / 或血液 LDL 较施用前降低至少大约 10% 或 15%。更优选地，血液胆固醇和 / 或血液 LDL 较施用所述抗体之前降低至少大约 20、30、40、50、60、70 或 80%。

[0099] 给药方案

[0100] 在一些实施方案中，给药方案包括施用初始剂量为大约 2mg/kg 的 PCSK9 抗体，随后每 4 周施用一次大约 2mg/kg 的维持剂量。在另一些实施方案中，给药方案包括施用初始剂量为大约 4mg/kg 的 PCSK9 抗体，随后每 4 周施用一次大约 4mg/kg 的维持剂量。在另一些实施方案中，给药方案包括施用初始剂量为大约 4mg/kg 的 PCSK9 抗体，随后每 8 周施用一次大约 4mg/kg 的维持剂量。在另一些实施方案中，给药方案包括施用初始剂量为大约 8mg/kg 的 PCSK9 抗体，随后每 8 周施用一次大约 8mg/kg 的维持剂量。在另一些实施方案中，给药方案包括施用初始剂量为大约 12mg/kg 的 PCSK9 抗体，随后每 8 周施用一次大约 12mg/kg 的维持剂量。

[0101] 在另一些实施方案中，给药方案包括每周施用一次大约 0.25mg/kg 的 PCSK9 抗体。在另一些实施方案中，给药方案包括每周施用一次大约 0.5mg/kg 的 PCSK9 抗体。在另一些实施方案中，给药方案包括每周施用一次大约 1mg/kg 的 PCSK9 抗体。在另一些实施方案中，给药方案包括每周施用一次大约 1.5mg/kg 的 PCSK9 抗体。

[0102] 然而，根据执业医生希望达到的药动学衰退模式可以使用另一些给药方案。这种治疗的进展易于通过常规技术和试验进行监测。在优选的实施方案中，初始剂量与第一个后续剂量及再后续剂量彼此间隔时间至少 4 周。所述给药方案（包括使用的 PCSK9 拮抗剂）可以根据时间而变化。

[0103] 通常地，对于施用 PCSK9 抗体，初始候选剂量可以是大约 0.3mg/kg-18mg/kg 的 PCSK9 拮抗抗体。对于本发明，根据上述因素，典型的剂量范围可以是大约 3μg/kg 至 30μg/kg 至 300μg/kg 至 3mg/kg、至 30mg/kg、至 100mg/kg 或更高剂量。例如，可以使

用的剂量为大约 0.3mg/kg、大约 0.5mg/kg、大约 1mg/kg、大约 1.5mg/kg、大约 2mg/kg、大约 2.5mg/kg、大约 3mg/kg、大约 3.5mg/kg、大约 4mg/kg、大约 4.5mg/kg、大约 5mg/kg、大约 5.5mg/kg、大约 6mg/kg、大约 6.5mg/kg、大约 7mg/kg、大约 7.5mg/kg、大约 8mg/kg、大约 8.5mg/kg、大约 9mg/kg、大约 9.5mg/kg、大约 10mg/kg、大约 10.5mg/kg、大约 11mg/kg、大约 11.5mg/kg、大约 12mg/kg、大约 12.5mg/kg、大约 13mg/kg、大约 13.5mg/kg、大约 14mg/kg、大约 14.5mg/kg、大约 15mg/kg、大约 15.5mg/kg、大约 16mg/kg、大约 16.5mg/kg、大约 17mg/kg、大约 17.5mg/kg、大约 18mg/kg、大约 18.5mg/kg、大约 19mg/kg、大约 19.5mg/kg、大约 20mg/kg、大约 20.5mg/kg、大约 21mg/kg、大约 21.5mg/kg、大约 22mg/kg、大约 22.5mg/kg、大约 23mg/kg、大约 23.5mg/kg、大约 24mg/kg、大约 24.5mg/kg，以及大约 25mg/kg。对于在几天或更长时间重复施用，根据具体情况，持续治疗直至出现希望的症状抑制或者直至达到足够的治疗水平，例如降低血液 LDL 水平。

[0104] 给药方案的例子包括施用初始剂量为大约 0.25mg/kg、大约 0.5mg/kg、大约 1mg/kg、大约 1.5mg/kg、大约 2mg/kg、大约 2.5mg/kg、大约 3mg/kg、大约 4mg/kg、大约 5mg/kg、大约 6mg/kg、大约 7mg/kg、大约 8mg/kg、大约 9mg/kg、大约 10mg/kg、大约 11mg/kg、大约 12mg/kg、大约 13mg/kg、大约 14mg/kg、大约 15mg/kg、大约 16mg/kg、大约 17mg/kg，或者大约 18mg/kg，随后施用维持剂量为大约 0.25mg/kg、大约 0.5mg/kg、大约 1mg/kg、大约 1.5mg/kg、大约 2mg/kg、大约 2.5mg/kg、大约 3mg/kg、大约 4mg/kg、大约 5mg/kg、大约 6mg/kg、大约 7mg/kg、大约 8mg/kg、大约 9mg/kg、大约 10mg/kg、大约 11mg/kg、大约 12mg/kg、大约 13mg/kg、大约 14mg/kg、大约 15mg/kg、大约 16mg/kg、大约 17mg/kg，或者大约 18mg/kg 的 PCSK9 抗体。在一些实施方案中，维持剂量每周施用一次。在一些实施方案中，维持剂量隔周施用一次。在一些实施方案中，维持剂量每三周施用一次。在一些实施方案中，维持剂量每四周施用一次。在一些实施方案中，维持剂量大约每五周施用一次。在一些实施方案中，维持剂量大约每六周施用一次。在一些实施方案中，维持剂量大约每七周施用一次。在一些实施方案中，维持剂量大约每八周施用一次。在优选的实施方案中，初始剂量与第一次后续剂量及再后续剂量施用时间彼此间隔至少大约四周。在一些实施方案中，维持剂量每月施用一次。

[0105] 在另一些实施方案中，可以使用固定剂量。例如，可以使用的 PCSK9 拮抗抗体剂量为大约 0.25mg、大约 0.3mg、大约 0.5mg、大约 1mg、大约 1.5mg、大约 2mg、大约 2.5mg、大约 3mg、大约 4mg、大约 5mg、大约 6mg、大约 7mg、大约 8mg、大约 9mg、大约 10mg、大约 11mg、大约 12mg、大约 13mg、大约 14mg、大约 15mg、大约 16mg、大约 17mg、大约 18mg、大约 19mg、大约 20mg、大约 21mg、大约 22mg、大约 23mg、大约 24mg、大约 25mg、大约 26mg、大约 27mg、大约 28mg、大约 29mg、大约 30mg、大约 31mg、大约 32mg、大约 33mg、大约 34mg、大约 35mg、大约 36mg、大约 37mg、大约 38mg、大约 39mg、大约 40mg、大约 41mg、大约 42mg、大约 43mg、大约 44mg、大约 45mg、大约 46mg、大约 47mg、大约 48mg、大约 49mg、大约 50mg、大约 51mg、大约 52mg、大约 53mg、大约 54mg、大约 55mg、大约 56mg、大约 57mg、大约 58mg、大约 59mg、大约 60mg、大约 61mg、大约 62mg、大约 63mg、大约 64mg、大约 65mg、大约 66mg、大约 67mg、大约 68mg、大约 69mg、大约 70mg、大约 71mg、大约 72mg、大约 73mg、大约 74mg、大约 75mg、大约 76mg、大约 77mg、大约 78mg、大约 79mg、大约 80mg、大约 81mg、大约 82mg、大约 83mg、大约 84mg、大约 85mg、大约 86mg、大约 87mg、大约 88mg、大约 89mg、大约 90mg、大约 91mg、

大约 92mg、大约 93mg、大约 94mg、大约 95mg、大约 96mg、大约 99mg、大约 98mg、大约 99mg、大约 100mg,about101mg、大约 102mg、大约 103mg、大约 104mg、大约 105mg、大约 106mg、大约 107mg、大约 108mg、大约 109mg、大约 110mg、大约 111mg、大约 112mg、大约 113mg、大约 114mg、大约 115mg、大约 116mg、大约 117mg、大约 118mg、大约 119mg、大约 120mg、大约 121mg、大约 122mg、大约 123mg、大约 124mg、大约 125mg、大约 126mg、大约 127mg、大约 128mg、大约 129mg、大约 130mg、大约 131mg、大约 132mg、大约 133mg、大约 134mg、大约 135mg、大约 136mg、大约 137mg、大约 138mg、大约 139mg、大约 140mg、大约 141mg、大约 142mg、大约 143mg、大约 144mg、大约 145mg、大约 146mg、大约 147mg、大约 148mg、大约 149mg、大约 150mg、大约 151mg、大约 152mg、大约 153mg、大约 154mg、大约 155mg、大约 156mg、大约 157mg、大约 158mg、大约 159mg、大约 160mg、大约 161mg、大约 162mg、大约 163mg、大约 164mg、大约 165mg、大约 166mg、大约 167mg、大约 168mg、大约 169mg、大约 170mg、大约 171mg、大约 172mg、大约 173mg、大约 174mg、大约 175mg、大约 176mg、大约 177mg、大约 178mg、大约 179mg、大约 180mg,about181mg、大约 182mg、大约 183mg、大约 184mg、大约 185mg、大约 186mg、大约 187mg、大约 188mg、大约 189mg、大约 190mg、大约 191mg、大约 192mg、大约 193mg、大约 194mg、大约 195mg、大约 196mg、大约 199mg、大约 198mg、大约 199mg、大约 200mg、大约 250、大约 300、大约 350、大约 400、大约 450 或者大约 500mg。在一些实施方案中,所述固定剂量是经皮下或静脉内施用的。

[0106] 可以根据本发明公开的一或多个给药方案将 PCSK9 拮抗抗体施用于接受稳定剂量他汀类药物的个体。所述稳定剂量可以是,例如但不限于日剂量或者隔日剂量他汀类药物。本领域技术人员已知许多他汀类药物,包括,例如但不限于阿托伐他汀、辛伐他汀、洛伐他汀、普伐他汀、罗苏伐他汀、氟伐他汀、西立伐他汀、美伐他汀、匹伐他汀,以及他汀类药物组合治疗。他汀类药物组合治疗的非限制性实例包括阿托伐他汀加氨氯地平 (CADUET™),辛伐他汀加依泽替米贝 (VYTORIN™),洛伐他汀加烟酸 (ADVICOR™),以及辛伐他汀加烟酸 (SIMCOR™)。

[0107] 在一些实施方案中,在施用初始剂量的 PCSK9 拮抗抗体之前,个体已经接受稳定剂量的他汀类药物至少 1、2、3、4、5 或 6 周时间。优选地,在施用初始剂量的 PCSK9 拮抗抗体之前,接受稳定剂量的他汀类药物的个体的空腹 LDL-C 水平高于或等于大约 70mg/dL。在一些实施方案中,在施用初始剂量的 PCSK9 拮抗抗体之前,接受稳定剂量的他汀类药物的个体的空腹 LDL-C 水平高于或等于大约 80、90、100、110、120、130、140、150、160、170、180、190 或 200mg/dL。

[0108] 对于本发明,根据上述因素,典型的他汀类药物剂量范围可以是大约 1mg-80mg。例如,可以使用的他汀类药物的剂量为大约 0.3mg、大约 0.5mg、大约 1mg、大约 2.5mg、大约 3mg、大约 4mg、大约 5mg、大约 6mg、大约 7mg、大约 8mg、大约 9mg、大约 10mg、大约 11mg、大约 12mg、大约 13mg、大约 14mg、大约 15mg、大约 16mg、大约 17mg、大约 18mg、大约 19mg、大约 20mg、大约 21mg、大约 22mg、大约 23mg、大约 24mg、大约 25mg、大约 26mg、大约 27mg、大约 28mg、大约 29mg、大约 30mg、大约 31mg、大约 32mg、大约 33mg、大约 34mg、大约 35mg、大约 36mg、大约 37mg、大约 38mg、大约 39mg、大约 40mg、大约 41mg、大约 42mg、大约 43mg、大约 44mg、大约 45mg、大约 46mg、大约 47mg、大约 48mg、大约 49mg、大约 50mg、大约 51mg、大约 52mg、大约 53mg、大约 54mg、大约 55mg、大约 56mg、大约 57mg、大约 58mg、大约 59mg、大

约 60mg、大约 61mg、大约 62mg、大约 63mg、大约 64mg、大约 65mg、大约 66mg、大约 67mg、大约 68mg、大约 69mg、大约 70mg、大约 71mg、大约 72mg、大约 73mg、大约 74mg、大约 75mg、大约 76mg、大约 77mg、大约 78mg、大约 79mg，或者大约 80mg。

[0109] 在优选的实施方案中，使用剂量为 40mg 或 80mg 的阿托伐他汀。在另一些实施方案中，使用剂量为 20mg 或 40mg 的罗苏伐他汀。在另一些实施方案中，使用剂量为 40mg 或 80mg 的辛伐他汀。

[0110] 在一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 2mg/kg 的 PCSK9 抗体，随后大约每四周施用一次大约 2mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 3mg/kg 的 PCSK9 抗体，随后大约每四周施用一次大约 3mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 4mg/kg 的 PCSK9 抗体，随后大约每四周施用一次大约 4mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 5mg/kg 的 PCSK9 抗体，随后大约每四周施用一次大约 5mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 4mg/kg 的 PCSK9 抗体，随后大约每八周施用一次大约 4mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 6mg/kg 的 PCSK9 抗体，随后大约每四周施用一次大约 6mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 8mg/kg 的 PCSK9 抗体，随后大约每八周施用一次大约 8mg/kg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 12mg/kg 的 PCSK9 抗体，随后大约每八周施用一次大约 12mg/kg 的维持剂量 .

[0111] 在另一些实施方案中，给药方案包括经皮下注射对接受稳定剂量他汀类药物的对象施用初始剂量为大约 200mg 的 PCSK9 抗体，随后大约每四周施用一次大约 200mg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 300mg 的 PCSK9 抗体，随后大约每四周施用一次大约 300mg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 50mg 的 PCSK9 抗体，随后大约每两周施用一次大约 50mg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 100mg 的 PCSK9 抗体，随后大约每两周施用一次大约 100mg 的维持剂量。在另一些实施方案中，给药方案包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 150mg 的 PCSK9 抗体，随后大约每两周施用一次大约 150mg 的维持剂量。

[0112] 另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 0.25mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 0.25mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 0.5mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 0.5mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 1mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 1mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象

施用初始剂量为大约 1.5mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 1.5mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 2mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 2mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 3mg/kg 的 PCSK9 拮抗抗体。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 4mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 4mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 5mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 5mg/kg 的 PCSK9 拮抗抗体的维持剂量。另一给药方案的例子包括对接受稳定剂量他汀类药物的对象施用初始剂量为大约 6mg/kg 的 PCSK9 拮抗抗体。在一些实施方案中，给药方案进一步包括每月施用一次大约的 6mg/kg 的 PCSK9 拮抗抗体的维持剂量。

[0113] 然而，根据执业医生希望达到的药动学衰退模式，也可以使用其它给药方案。这个治疗的进展易于通过常规的技术和试验进行监测。在优选的实施方案中，初始剂量与第一次后续剂量及再后续剂量的施用时间彼此间隔至少四周。所述给药方案（包括使用的 PCSK9 拮抗抗体）随着时间可以变化。

[0114] PCSK9 拮抗抗体

[0115] 如下描述是产生本发明中使用的抗体的技术的例子。用于产生抗体的 PCSK9 抗原可以是例如全长人 PCSK9、全长小鼠 PCSK9 以及 PCSK9 的各种肽片段。可用于产生抗体的其它形式 PCSK9 对本领域技术人员是显而易见的。

[0116] 通过用重组全长 PCSK9 蛋白免疫 PCSK9 基因敲除小鼠产生单克隆抗体。这种抗体制备方式产生的拮抗抗体显示，其完全阻断 PCSK9 结合 LDLR，完全阻断 PCSK9- 介导的降低 Huh7 细胞中 LDLR 水平，及降低体内包括在小鼠内 LDL 胆固醇水平至相当于在 PCSK9-/- 小鼠中的水平，如美国专利申请号 12/558312 的实施例 7 所示。

[0117] 正如所理解的，用于本发明中的抗体可以衍生自杂交瘤，但是也可以在杂交瘤之外的细胞系中表达。编码特定抗体的 cDNA 或基因组克隆的序列可用于转化合适的哺乳动物或非哺乳动物宿主细胞。可用作表达宿主的哺乳动物细胞系为本领域熟知，包括可得自美国典型培养物保藏中心 (ATCC) 的许多永生化细胞系，包括但不限于中国仓鼠卵巢 (CHO) 细胞、NSO、HeLa 细胞、幼仓鼠肾 (BHK) 细胞、猴肾细胞 (COS) 及人肝细胞癌细胞（如 HepG6）。也可以使用非哺乳动物细胞，包括细菌、酵母、昆虫和植物细胞。可优选定点突变抗体 CH6 结构域以消除糖基化，从而防止由非人糖基化引起的免疫原性、药动学和 / 或效应子功能的变化。谷氨酰胺合酶表达系统在欧洲专利 616 846,656 055 和 363 997 及欧洲专利申请 89303964.4 中进行了全部或部分讨论。此外，二氢叶酸还原酶 (DHFR) 表达系统，包括本领域已知的那些，可用于产生所述抗体。

[0118] 在一些实施方案中，本发明使用 PCSK9 拮抗抗体 L1L3 实施。在一些实施方案中，本发明使用识别 PCSK9 表位的抗体实施，该表位与由 L1L3 抗体识别的表位相同。

[0119] 在一些实施方案中，本发明使用包含来自具有 SEQ ID NO:11 所示氨基酸序列的重链可变区的三个 CDR 及来自具有 SEQ ID NO:12 所示氨基酸序列的轻链可变区的三个 CDR

的抗体实施。

[0120] 在一些实施方案中，本发明使用特异性结合 PCSK9 的抗体实施，所述抗体包含：VH 互补决定区 1(CDR1)，其具有 SEQ ID NO:2(SYYMH)、SEQ ID NO:13(GYTFTSY) 或者 SEQ ID NO:14(GYTFTSYYMH) 所示氨基酸序列；VH CDR2，其具有 SEQ ID NO:3(EISPFGGRTNYNEKFKS) 或者 SEQ ID NO:15(ISPFGGR) 所示氨基酸序列；和 / 或 VH CDR3，其具有 SEQ ID NO:4(ERPLYASDL) 所示氨基酸序列，或者在所述 CDR1、CDR2 和 / 或 CDR3 序列中具有一或多个保守氨基酸替换的其变体，其中所述变体基本保留与所述序列定义的 CDR 相同的结合特异性。优选地，所述变体包含上至大约 10 个氨基酸替换，及更优选地包含上至大约 4 个氨基酸替换。

[0121] 在一些实施方案中，本发明使用这样的抗体实施，所述抗体包含：具有 SEQ ID NO:5(RASQGISSALA) 所示氨基酸序列的 VL CDR1，具有 SEQ ID NO:6(SASYRYT) 所示氨基酸序列的 CDR2，和 / 或具有 SEQ ID NO:7(QQRYSLWRT) 所示氨基酸序列的 CDR3，或者具有在所述 CDR1、CDR2 和 / 或 CDR3 序列中具有一或多个保守氨基酸替换的其变体，其中所述变体基本上保留与所述序列限定的 CDR1 相同的结合特异性。优选地，所述变体包含直至大约 10 个氨基酸替换，更优选直至大约 4 个氨基酸替换。

[0122] 在一些实施方案中，本发明使用具有包含或由 SEQ ID NO:8 或 10 组成的重链序列和包含或由 SEQ ID NO:9 组成的轻链序列的抗体实施。

[0123] 在一些实施方案中，本发明使用具有包含或由 SEQ ID NO:11 组成的重链可变区和包含或由 SEQ ID NO:12 组成的轻链可变区的抗体实施。

[0124] 在一些实施方案中，本发明使用识别人 PCSK9 上的表位的抗体实施，所述表位包含 SEQ ID NO:1 所示 PCSK9 氨基酸序列的 153-155、194、195、197、237-239、367、369、374-379 和 381 位氨基酸残基。优选地，人 PCSK9 上的抗体表位不包含 SEQ ID NO:1 所示 PCSK9 氨基酸序列的 71、72、150-152、187-192、198-202、212、214-217、220-226、243、255-258、317、318、347-351、372、373、380、382 和 383 位中的一或多个氨基酸残基。

[0125] 在一些实施方案中，本发明使用这样的抗体实施，所述抗体识别 PCSK9 的第一个表位，其与由以下单克隆抗体识别的第二个表位相同或重叠，所述单克隆抗体选自：5A10，其由在美国典型培养物保藏中心 (ATCC) 以编号 PTA-8986 保藏的杂交瘤细胞系产生；4A5，其由在美国典型培养物保藏中心以编号 PTA-8985 保藏的杂交瘤细胞系产生；6F6，其由在美国典型培养物保藏中心以编号 PTA-8984 保藏的杂交瘤细胞系产生；以及 7D4，其由在美国典型培养物保藏中心以编号 PTA-8983 保藏的杂交瘤细胞系产生。在优选的实施方案中，本发明使用 PCSK9 拮抗抗体 L1L3 实施（见 PCT/IB2009/053990，2010 年 3 月 18 日以 WO2010/029513 公开，及美国专利申请 No.12/558312，2010 年 3 月 18 日以 US2010/0068199 公开）。

[0126] 优选地，变体包含上至大约 20 个氨基酸替换，更优选地，上至大约 8 个氨基酸替换。优选地，所述抗体进一步包含免疫学惰性恒定区，和 / 或所述抗体具有选自如下的同种型：IgG_2、IgG_4、$IgG_{2\Delta a}$、$IgG_{4\Delta b}$、$IgG_{4\Delta c}$、IgG_4S228P、$IgG_{4\Delta b}$ S228P 和 $IgG_{4\Delta c}$ S228P。在另一优选的实施方案中，所述恒定区是非糖基化 (aglycosylated) 的 Fc 区。

[0127] 可用于本发明中的抗体可涵盖单克隆抗体、多克隆抗体、抗体片段（如 Fab、Fab'、F(ab')2、Fv、Fc 等）、嵌合抗体、双特异性抗体、异源配合物 (heteroconjugate) 抗体、单链

(ScFv)、其突变体、包含抗体部分的融合蛋白（如结构域抗体）、人抗体、人源化抗体，以及免疫球蛋白分子的任何另一些修饰的构型，其包含所需要的特异性的抗原识别位点，包括抗体的糖基化变体、抗体的氨基酸序列变体，以及共价修饰的抗体。抗体可以是鼠类、大鼠、人或者任何另一些来源的抗体（包括嵌合或人源化抗体）。

[0128] 在一些实施方案中，所述 PCSK9 拮抗抗体是单克隆抗体。所述 PCSK9 拮抗抗体也可以是人源化抗体。在另一些实施方案中，抗体是人抗体。

[0129] 在一些实施方案中，所述抗体包含修饰的恒定区，如免疫学惰性的恒定区，具有降低的引发免疫应答的潜力。在一些实施方案中，所述恒定区如 Eur. J. Immunol., 1999, 29:2613-2624、PCT 公开号 WO99/58572 和 / 或英国专利申请号 9809951.8 所述修饰。Fc 可以是人 IgG_2 或者人 IgG_4。Fc 可以是含有 A330P331 突变为 S330S331 的人 IgG_2（$IgG_{2\Delta a}$），其中氨基酸残基参考野生型 IgG2 序列进行编号。Eur. J. Immunol., 1999, 29:2613-2624。在一些实施方案中，抗体包含一个 IgG_4 恒定区，其包含如下突变（Armour et al., 2003, Molecular Immunology 40585-593）：E233F234L235 突变为 P233V234A235（$IgG_{4\Delta c}$），其中编号参考野生型 IgG4。在另一个实施方案中，Fc 是人 IgG_4E233F234L235 突变为 P233V234A235，具有 G236 删除（$IgG_{4\Delta b}$）。在另一个实施方案中，Fc 是任何人 IgG_4Fc（IgG_4、$IgG_{4\Delta b}$ 或 $IgG_{4\Delta c}$），其含有铰链稳定的突变，S228 突变为 P228（Aalberse et al., 2002, Immunology 105, 9-19）。在另一个实施方案中，Fc 可以是非糖基化（aglycosylated）的 Fc。

[0130] 在一些实施方案中，所述恒定区是通过突变寡糖附着残基（如 Asn297）和 / 或是恒定区中糖基化识别序列一部分的侧翼残基而非糖基化的。在一些实施方案中，所述恒定区是针对 N- 连接的糖基化用酶进行非糖基化的。所述恒定区可以针对 N- 连接的糖基化用酶进行或者通过在糖基化缺陷的宿主细胞中表达进行非糖基化。

[0131] 在一些实施方案中，可以存在一种以上的拮抗抗体。可以存在至少一种、至少两种、至少三种、至少四种、至少五种不同的或更多的拮抗抗体和 / 或肽。通常地，这些 PCSK9 拮抗抗体或肽可具有互补活性，其彼此无不利影响。PCSK9 拮抗抗体也可以与另一些 PCSK9 拮抗剂或 PCSK9 受体拮抗剂联合使用。例如，可以使用如下一或多种 PCSK9 拮抗剂：针对 PCSK9 的反义分子（包括针对编码 PCSK9 的核酸的反义分子），PCSK9 抑制化合物，及 PCSK9 结构类似物。PCSK9 拮抗抗体也可以与增强和 / 或互补所述药剂效果的另一些药剂联合使用。

[0132] 关于本发明所述的所有方法，PCSK9 拮抗抗体还包括包含一或多种其它药剂的组合物。这些组合物可进一步包含合适的赋形剂，如药物可接受的赋形剂，包括缓冲液，这些为本领域熟知。本发明可以单独使用或者与其它常规治疗方法组合使用。

[0133] PCSK9 拮抗抗体可以通过任何合适途径施用于个体。本发明描述的实施例只是例证可用技术而不是限制本发明，这对本领域技术人员是显而易见的。因此，在一些实施方案中，所述 PCSK9 拮抗抗体是根据已知方法施用于个体的，例如静脉内施用，如在一段时间内推注或持续输注，肌内、腹膜内、脑脊髓内、经皮、皮下、动脉内、舌下、滑膜内、通过吹入、鞘内、口服、吸入或者局部的途径。施用可以是全身性施用，如静脉内注射，或者局部施用。可以使用可商购的液体配制物雾化器，包括喷雾式雾化器和超声雾化器进行施用。液体配制物可以直接雾化，冻干粉末可以在冲调后雾化。或者，PCSK9 拮抗抗体可以使用碳氟化合物

配制物和计量吸入器气雾化，或者作为冻干和研磨粉末吸入。

[0134] 在一个实施方案中，PCSK9 拮抗抗体是通过部位特异性或者靶向局部输送技术施用的。部位特异性或靶向局部输送技术的例子包括 PCSK9 拮抗抗体的各种可植入持久药性 (depot) 源或者局部输送导管，如输注管、留置管或者针管，合成的移植物，外膜敷料 (adventitial wraps)，分流装置 (shunts) 和支架 (stents) 或者其它可植入装置，位点特异性载体，直接注射，或者直接施用。见例如 PCT 公开号 WO00/53211 和美国专利号 5,981,568。

[0135] 可以使用 PCSK9 拮抗抗体的各种配制物进行施用。在一些实施方案中，PCSK9 拮抗抗体可以单独 (neat)) 施用。在一些实施方案中，PCSK9 拮抗抗体与药物可接受的赋形剂可存在于多种配制物中。药物可接受的赋形剂为本领域已知，是相对惰性的物质，其辅助药理学有效物质的施用。例如，赋形剂可以给予形状或稠度，或作为稀释剂。合适的赋形剂包括但不限于稳定剂、增湿和乳化剂、改变渗透性的盐、包裹剂、缓冲液和皮肤渗透增强剂。赋形剂以及用于肠胃外和非肠胃外药物输送的配制物如 Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing (2000) 所示。

[0136] 这些药剂可以与药物学可接受的载体如盐水、Ringer's 溶液、葡萄糖溶液等组合。特定的给药方案，即剂量、时间和重复，根据特定个体及个体的病史决定。

[0137] 可接受的运载体、赋形剂或稳定剂在使用的剂量和浓度上对于受者是无毒的，并可包含缓冲液如磷酸盐、柠檬酸盐和其它有机酸缓冲液；盐如氯化钠；抗氧化剂，包括抗坏血酸和甲硫氨酸；防腐剂（例如氯化十八烷基二甲基苄基铵；氯化六甲铵；氯化苯甲烷铵、氯化苯乙铵；苯酚、丁醇或苯甲醇；烷基对羟基苯甲酸酯，如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯；儿茶酚；间苯二酚；环己醇；3- 戊醇；和 m- 甲酚）；低分子量（少于约 10 个残基）多肽；蛋白质，如血清白蛋白、明胶或免疫球蛋白；亲水多聚体如聚乙烯吡咯烷酮；氨基酸如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸；单糖、二糖和其它碳水化合物，包括葡萄糖、甘露糖或糊精；螯合剂如 EDTA；糖如蔗糖、甘露醇、海藻糖或山梨糖醇；成盐的反荷离子如钠；金属络合物（例如 Zn- 蛋白质络合物）；和 / 或非离子型表面活性剂，如 TWEEN™、PLURONICS™ 或者聚乙二醇 (PEG)。

[0138] 通过本领域已知方法制备含有 PCSK9 拮抗抗体的脂质体，如在 Epstein, et al., Pro″Natl. Acad. Sci. USA82:3688 (1985)；Hwang, et al., Proc. Natl Acad. Sci. USA77:4030 (1980) 和美国专利号 4,485,045 和 4,544,545 中描述。在美国专利号 5,013,556 中公开了循环时间提高的脂质体。通过反相蒸发法用脂质组合物可以产生特别有用的脂质体，所述脂质组合物含有磷脂酰胆碱、胆固醇和 PEG 衍生的磷脂酰乙醇胺 (PEG-PE)。通过指定孔径的滤器滤过脂质体以产生具有期望直径的脂质体。

[0139] 活性成分也可以被包埋在通过凝聚技术或界面聚合法制备的微胶囊（例如，分别是羟甲基纤维素或明胶微胶囊和聚异丁烯酸甲酯微胶囊），胶体药物运输（例如脂质体、白蛋白微球、微乳剂、纳米颗粒和纳米胶囊）或大乳剂中。在 Remington, The Science and Practice of Pharmacy, 20th Ed., Mack Publishing (2000) 中公开了这类技术。

[0140] 可制备缓释制剂。合适的缓释制剂的例子包括含有抗体的固体疏水多聚体半渗透性基质，所述基质是有形状物体形式，例如薄膜或微胶囊。缓释基质的例子包括聚酯、水凝胶（例如聚 (2- 羟乙基 - 异丁烯酸酯）或聚（乙烯醇））、聚乳酸（美国专利号 3,773,919）、

L-谷氨酸与7乙基-L-谷氨酸的共聚物、不可降解的乙烯-醋酸乙烯酯、可降解的乳酸-羟基乙酸共聚物如LUPRON DEPOT™(由乳酸-羟基乙酸共聚物和醋酸亮丙瑞林组成的可注射微球)、乙酸异丁酸蔗糖酯和聚-D-(-)-3-羟基丁酸。

[0141] 用于体内施用的配制物必须是无菌的。这可以通过例如无菌滤膜过滤轻易实现。治疗性PCSK9拮抗抗体组合物通常被置于具有无菌接入口的容器中,例如带有可被皮下注射针穿透的塞子的静脉注射溶液袋或瓶。

[0142] 合适的乳剂可以使用可商购的脂肪乳剂来制备,所述脂肪乳剂例如Intralipid™、Liposyn™、Infonutrol™、Lipofondin™和Lipiphysan™。活性成分可被溶于预先混合的乳剂组合物中,或者其可溶于油(例如大豆油、红花油、棉籽油、芝麻油、玉米油或杏仁油)和与磷脂(例如卵磷脂、大豆磷脂或大豆卵磷脂)及水混合形成的乳剂中。应意识到可添加其它成分,例如甘油或葡萄糖,以调节乳剂的弹性(tonicity)。典型地,合适的乳剂含有多至20%的油,例如5%和20%之间。脂肪乳剂包含0.1-1.0μm之间、尤其是0.1-0.5μm之间的脂肪微滴,并具有5.5-8.0范围内的pH。

[0143] 乳剂组合物可以通过将PCSK9拮抗抗体与Intralipid™或其组分(大豆油、卵磷脂、甘油和水)混合而制备。

[0144] 用于吸入或吹入的组合物包括在药物可接受的、水性或有机溶剂或其混合物中的溶液和悬浮液,以及粉末。液体或固体组合物可含有上述的合适的药物可接受的赋形剂。在一些实施方案中,通过口或鼻呼吸途径施用组合物用于局部或全身作用。优选地在无菌药物可接受的溶剂中的组合物可以通过使用气体进行雾化。被雾化的溶液可以从雾化装置中直接被呼吸,或者雾化设备可以附着于面罩、帷罩(tent)或间歇性正压呼吸机。溶液、悬浮液或粉末组合物可以从以适当方式输送所述配制物的装置中施用,优选经口或鼻。

[0145] 编码抗体L1L3的重链和轻链可变区的多核苷酸于2009年8月25日保藏在美国典型培养物保藏中心(ATCC),10801University Boulevard,Manassas,VA90110,U.S.A.。L1L3重链可变区多核苷酸保藏物编号为ATCC Accession No.PTA-10302,L1L3轻链可变区多核苷酸保藏物编号为ATCC Accession No.PTA-10303。所述保藏物按照国际承认用于专利程序的微生物保存布达佩斯条约(Budapest Treaty)及其规章的规定进行保藏。这确保保藏的存活培养物从保藏日起被维持30年。根据布达佩斯条约的规定,保藏物可以从ATCC获得,并且遵循Pfizer,Inc.与ATCC之间的协议,所述协议确保:相关美国专利公布或任何美国或外国专利申请公开后(先公开为准),保藏的培养物后代永久和无限制地能够被公众获得,并根据35USC第122节及其委员规则(包括37CFR1.14节,尤其是指886OG638)确保美国专利和商标委员会授权的人能够获得所述后代。

[0146] 本专利申请的受让人已经同意,如果保藏的材料的培养物在合适的条件下培养时死亡或丢失或损坏,将在通知后迅速用另一相同材料替换。保藏材料的可获得性不应被解释为可以违背任何政府当局根据其专利法授予的权利来实施本发明的许可。

实施例

[0147] 如下实施例只是阐明本发明的方法和材料。本领域技术人员在本发明的精神和范围内可以对与本领域通常有冲突的条件和参数加以适当修改和调整。

[0148] 实施例1:用人源化PCSK9拮抗抗体L1L3的治疗有效降低血清胆固醇和LDL胆固

醇水平

[0149] 本实施例举例说明了人源化 PCSK9 拮抗抗体在动物模型中降低血清胆固醇和 LDL 胆固醇水平中的效力。

[0150] L1L3 是人源化（小鼠残基 <5%）单克隆抗体，其结合分泌性 PCSK9，有效阻止其下调 LDLR，使得血清中 LDL 清除改善及 LDL-C 降低。

[0151] 当将 10mg/kg 的 L1L3 以腹膜内 (IP) 单剂量施用正常饮食饲养的 C57BL/6 小鼠 (n=10) 时，在处理后 48 小时小鼠血清胆固醇水平降低至 47mg/dL（降低 37%），相比之下盐水处理的对照小鼠血清胆固醇水平为 75mg/dL，在处理后 4 天为 44mg/dL（降低 47%），相比之下对照动物的水平为 83mg/dL。在处理后 7 天，血清胆固醇水平恢复至 69mg/dL。

[0152] 在正常饮食饲养的 Sprague-Dawley 大鼠中进行剂量应答实验，施用单 IP 剂量 0、0.1、1、10 和 80mg/kg(n=6/ 组）的 L1L3。血清胆固醇水平呈现剂量依赖性降低，最大效应为在施用 10 和 80mg/kg 剂量后 48 小时可见降低 50%。胆固醇水平下降的持续时间从 1-21 天也是剂量依赖性的。L1L3 的降低胆固醇作用的量级和持续时间均与药物处理相关联。非空腹血清甘油三酯水平也是剂量依赖性升高，在 80mg/kg 剂量出现最大升高大约三倍，时程与药物暴露相关。由于在其它物种如小鼠和非人灵长类动物中未观测到 L1L3 对血清甘油三酯水平的相似作用（见下文），并且血液甘油三酯水平改变在携带 PCSK9 突变的人体中未报道 (Abifadel et al., 2003, Nat. Genet., 34:154-156 ; Cohen et al., 2005, Nat. Genet. 37:161-165 ; Zhao et al., 2006, Am. J. Hum. Genet. 79:514-523)，因此由 L1L3 处理所致血清甘油三酯水平增加看起来是在大鼠中物种特异性现象。

[0153] 在正常饮食饲养的食蟹猕猴中，经 IV 施用单一剂量 0.1、1、3 和 10mg/kg(n=4/ 组）的 L1L3。施用 0.1mg/kg L1L3 导致在第 2 天 LDL-C 水平短暂降低 50%，在第 5 天迅速恢复。施用 1mg/kg 剂量在第 5 天达到最大效应，LDL-C 水平降低 71%，之后立即开始恢复，在第 14 天达到给药前水平。施用 3mg/kg 剂量在第 7 天达到最大效应，LDL-C 水平降低 72%，在第 13 天开始恢复，在第 22 天恢复至基线水平。施用 10mg/kg 剂量在施用后直至 21 天仍保持 LDL-C 水平降低 70%，在第 31 天动物完全恢复。L1L3 的降低 LDL-C 作用的量级和持续时间均与药物处理相关联。在所有剂量组中，HDL-C 水平均不受 L1L3 处理的影响。

[0154] 在研究的第 42 天和 56 天（间隔 2 周），将 3mg/kg 剂量组 (n=4) 的食蟹猕猴再施用两次额外的 3mg/kg L1L3。这两次额外的剂量再次降低 LDL-C 及保持 LDL-C 水平低于 50% 持续 4 周时间。LDL-C 水平在两周后恢复正常。血清 HDL-C 水平保持不变。

[0155] 在食蟹猕猴中通过单次静脉内推注施用 0.1、1.0、3.0、10.0 和 100.0mg/kg 剂量 L1L3 进行 PK 研究，测量总抗体浓度。据估计在 0.1mg/kg 单剂量 L1L3 的 β- 相半衰期为 0.67 天，在 1.0、3.0、10.0 和 100.0mg/kg 剂量分别延长为 1.91、2.33、3.49 和 5.25 天。因此，在食蟹猕猴中，证实 L1L3 的半衰期的剂量依赖性和非线性缩短作用，这与抗原介导的降解及用具有膜结合的抗原的抗体治疗可见的结果一致。

[0156] 概括而言，在动物模型中，L1L3 结合并拮抗血清 PCSK9 功能，导致血清胆固醇和 LDL 胆固醇水平快速及显著降低。

[0157] 实施例 2 :在单次、逐步增加剂量的经静脉内施用 PCSK9 拮抗抗体 L1L3 之后的药动学和药效学结果

[0158] 这个实施例举例说明了评估其它方面健康的人对象中施用单次、逐步增加剂量的

静脉内人源化PCSK9拮抗抗体L1L3给药之后的药动学和药效学的临床试验研究，所述人对象是降低胆固醇疗法的候选者。在所有评估的剂量组中，施用L1L3均使得LDL-C水平降低。

[0159] 该研究限定是随机的、安慰剂对照的、增加的、单剂量的L1L3研究。所述对象、研究人员和现场工作人员（除了与准备药物相关的现场人员之外）对于治疗任务均是不知情的，是CRO指定人员；而赞助商临床研究组是知情的。这项研究是在每组8个对象共6组进行工作，以寻求最大耐受剂量或MTD（总共大约48个对象）。在每组中，向对象随机施用L1L3或安慰剂（3:1分配比率）。在前一夜空腹之后施用药剂，经静脉内输注60分钟。每个方案通过输注装置小心控制输注速度。单次输注60分钟。

[0160] 剂量如下表1所示。

[0161] 表1

[0162]

组	L1L3剂量	施用的对象数
1	0.3 mg/kg 安慰剂	6 2
2	1.0 mg/kg 安慰剂	6 2
3	3.0 mg/kg 安慰剂	6 2
4	6.0 mg/kg 安慰剂	6 2
5	12 mg/kg 安慰剂	6 2
6	18 mg/kg 安慰剂	6 2

[0163] 调整给药方案以允许施用较低、中等或较高剂量，以获得最大耐受剂量及无作用剂量。无论其组别分配，登记入该研究中的每个对象，在其研究参与期间仅接受一次研究药物。为了安全性，对所有患者在其研究完成之前要再观测21天（共28天）。

[0164] 该项研究的初级PK终点是L1L3的$AUC_{(0-t[last])}$、T_{max}和C_{max}。二级PK终点包括L1L3的终末消除半衰期（$T_{1/2}$）、清除率（CL）、稳态体积（Vss）及$AUC_{(0-\infty)}$。评定血清脂质（总胆固醇，LDL，HDL，甘油三酯，非-HDL-C和Apoprotein B）的改变。

[0165] 对每个对象在给药28天内进行筛查。对象在第0天接受单剂量L1L3，在限制期间（研究第-1、0和1天）以及在第4、7、14、21、28天，每天一次进行PK和安全性评定，第28天后根据初始PK结果评定。

[0166] 这项研究的纳入标准如下：健康，非卧床、年龄在18-70岁之间的男性和/或女性（女性应为非备孕女性），兼具以上；基础总胆固醇水平≥200mg/dl，基础LDL≥130mg/dl；体重指数（BMI）为18.5-35kg/m^2BMI18.5-35，体重≤150kg，兼具以上；个人签名和注明日期的知情同意书表明该对象（或者法律可接受的代理人）已经被告知所有实验相关方面内容；并且自愿及能遵从计划的探访、治疗计划、实验室检查及其它实验程序。

[0167] 这项研究的排除标准如下：临床显著的血液、肾脏、内分泌、肺、胃肠道、心血管、肝、精神、神经或者过敏性疾病（包括药物过敏，但是排除在给药时未治疗的无临床症状的季节性过敏反应）的迹象或病史；继发高血脂症；对象在本发明处理之前至少1周不应服

用其它处方药。如果患者已经接受降脂药治疗，这些药物应终止足够时间以使得血清脂质恢复至治疗前水平；在给药前5天内有发热病史；中风或短暂脑缺血发作病史；在过去的一年内有心肌梗塞病史；尿液药物筛查阳性；在筛选的6个月内有定期饮酒对女性超过7杯/周）或者对男性超过14杯/周（1杯=5盎司（150mL）葡萄酒或者12盎司（360mL）啤酒或者1.5盎司（45mL）烈性酒；在第一次施用实验药物之前30天内或者5个半衰期（选择更长的）内用研究性药物治疗；在筛选时12-导联ECG证实QTc>450msec；怀孕或哺乳女性；备孕女性；在给药前56天内献血大约1品脱（500mL）；肝素或肝素诱导的血小板减少症敏感性病史（如果肝素用于冲洗静脉内注射导管）；其它急性或慢性医学或精神病或实验室检查异常，其可增加与参与研究或施用学术研究产物相关的危险，或者可干扰研究结果的解释，以及研究人员的判断，使得对象不适合纳入这项研究中。

[0168] 将对象随机进行研究，只要满足所有的对象选择标准即可。使用计算机产生的随机方案指定对象治疗顺序。

[0169] 对于增加剂量，由赞助商和研究人员在回顾来自在施用前一次剂量水平之后至少7天的所有组的对象的可用的安全性和耐受能力数据之后决定施用更高剂量的L1L3。

[0170] L1L3药物制品（100mg）以无菌液体形式提供，浓度为10mg/mL，在玻璃小瓶中以供静脉内（IV）施用，具有一个橡皮塞和铝封。每个小瓶含有10mL（可取体积）的L1L3，浓度为10mg/mL，pH为5.5。L1L3和安慰剂根据药学手册中的剂量和施用说明（Dosage and Administration Instructions in the Pharmacy Manual）要求在现场制备。药物由合格的非盲现场人员制备，并以不知情形式分配给患者和直属研究人员。根据位于药学手册和研究参考指南（Pharmacy Manual and Study Reference Guide）的施用剂量说明（Dosage Administration Instructions，DAI），用大约60分钟通过控速静脉内输注施用L1L3。

[0171] 研究方案

[0172] 第-1天：在开始第0天活动之前至少12小时随机指定对象编号并录入临床研究单元（Clinical Research Unit），需要保留在临床研究单元（CRU）内直至第1天的程序完成。对象在第0天进行计划血脂检测（Lipid Panel）之前晚上开始禁食至少10小时。完成如下程序：复查自筛选以来的病史中的改变；复查自筛选以来伴随的药物治疗中的改变；复查自筛选以来的药物、乙醇和烟草使用史；通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状；身体检查，包括体重；尿液药物筛查；获得仰卧位生命体征；获得大约间隔2-4分钟的三次重复12导联ECG。

[0173] 第0天：在给药之前，完成如下程序：收集在空腹至少10小时后的空腹脂质谱（总胆固醇，LDL，HDL，非-HDL胆固醇，Apo B和甘油三酯）；收集进行常规和额外实验室检查的样品：血液学、化学、凝集淀粉酶、尿液分析；收集进行给药前（pre-dose）PK的样品；收集PCSK9水平/PD感兴趣的标志物的样品；收集抗-L1L3抗体的样品；复查自筛选以来伴随药物治疗中的改变；通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状；获得仰卧位生命体征；药学手册说明（Pharmacy Manual Instructions）施用研究药物输注（Study Drug Infusion）。

[0174] 在给药后，完成如下程序：获得间隔大约2-4分钟的三次重复12导联ECG，自输注结束（EOI）10分钟内开始；获得在EOI的仰卧位生命体征；收集在EOI及在输注后如下时间点（即EOI+如下时间点）的血液样品进行PK分析：60分钟，120分钟及360分钟。

[0175] 第 1 天 :完成如下程序 :收集在给药后 1440 分钟 (24 小时)+/-30 分钟的血液样品进行 PK 分析 ;进行简短的身体检查 ;收集在空腹至少 10 小时后的空腹脂质谱 (总胆固醇, LDLHDL 非 -HDL 胆固醇,Apo B 和甘油三酯) ;收集感兴趣的 PCSK9 水平 /PD 标志物的样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来的伴随药物治疗中的改变 ;获得仰卧位生命体征 ;从 CRU 中撤出。

[0176] 第 4 天 :完成如下程序 :收集进行常规实验室检查的样品 :血液学、化学和尿液检查 ;收集空腹至少 10 小时后的空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单一血液样品进行 PK 分析 ;收集 PCSK9 水平 / 感兴趣的 PD 标志物的样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来伴随药物治疗中的改变 ;获得仰卧位生命体征。

[0177] 第 7 天 :完成如下程序 :进行简短身体检查 ;收集进行常规和额外的实验室检查的样品 :血液学、化学、凝集、淀粉酶、尿液分析检查 ;收集空腹至少 10 小时的空腹脂质概况 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单一血液样品进行 PK 分析 ;收集 PCSK9 水平 / 感兴趣的 PD 标志物样品 ;收集抗 -L1L3 抗体的样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来的伴随药物治疗中的改变 ;复查自筛选以来药物、乙醇和烟草使用史 ;获得仰卧位生命体征 ;获得间隔大约 2-4 分钟的三次重复 12 导联 ECG。

[0178] 第 14 天 :完成如下程序 :进行简短身体检查 ;收集进行常规和额外实验室检查的样品 :血液学、化学、凝集、淀粉酶、尿液分析检查 ;收集空腹至少 10 小时的空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单一血液样品进行 PK 分析 ;收集 PCSK9 水平 / 感兴趣的 PD 标志物样品 ;收集抗 -L1L3 抗体样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来的伴随药物治疗中的改变 ;复查自筛选以来的药物、乙醇和烟草的使用史 ;获得仰卧位生命体征。

[0179] 第 21 天 :完成如下程序 :进行简短身体检查 ;收集进行常规和额外实验室检查的样品 :血液学、化学、凝集、淀粉酶、尿液分析检查 ;收集空腹至少 10 小时的空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单一血液样品进行 PK 分析 ;收集 PCSK9 水平 / 感兴趣的 PD 标志物样品 ;收集抗 -L1L3 抗体样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来的伴随药物治疗中的改变 ;复查自筛选以来的药物、乙醇和烟草的使用史 ;获得仰卧位生命体征。

[0180] 第 28 天 :完成如下程序 :进行全面身体检查 ;获得对象体重 ;收集进行常规和额外实验室检查的样品 :血液学、化学、凝集、淀粉酶、尿液分析检查 ;收集空腹至少 10 小时的空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单一血液样品进行 PK 分析 ;收集 PCSK9 水平 / 感兴趣的 PD 标志物样品 ;收集抗 -L1L3 抗体样品 ;通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状 ;复查自筛选以来的伴随药物治疗中的改变 ;复查自筛选以来的药物、乙醇和烟草的使用史 ;获得仰卧位生命体征 ;获得间隔大约 2-4 分钟三次重复的 12 导联 ECG。

[0181] 对延长的 PK 的额外随访 :当适当时完成如下程序 :进行简短身体检查 ;收集进行常规和额外实验室检查的样品 :血液学、化学、凝集、淀粉酶、尿液分析检查 ;收集空腹至少 10 小时的空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯) ;收集单

一血液样品进行 PK 分析；收集 PCSK9 水平 / 感兴趣的 PD 标志物样品；收集抗 -L1L3 抗体样品；通过自主报告不利事件及通过询问对象回答非引导性问题如“你感觉如何”等评定症状；复查自筛选以来的伴随药物治疗中的改变；复查自筛选以来的药物、乙醇和烟草的使用史；获得仰卧位生命体征；获得间隔大约 2-4 分钟三次重复的 12 导联 ECG。

[0182] 各个患者的总血液取样体积为大约 183-210mL。在第 0 天给药之前、输注结束时、在输注结束后 60、120、360 和 1440 分钟 (24- 小时) 收集血浆样品以分析 L1L3 水平。此外，在第 4、7、14、21、28 天及额外的 PK 随访 (适当时) 时获得单一 PK 样品。在每个时间点抽取一份样品。

[0183] 在第 0 天给药之前和在第 1、4、7、14、21、28 天及适当时额外的随访时获得血液样品，以评定 PCSK9 水平及其它感兴趣的实验性药效学标志物。

[0184] 收集在空腹至少 10 小时后空腹脂质谱 (总胆固醇、LDL、HDL、非 -HDL 胆固醇、Apo B 和甘油三酯)。

[0185] 研究结果

[0186] L1L3PK NCA 结果：以 0.3mg/kg 剂量施用 L1L3 的半衰期中值是 2.71 天。以 1mg/kg 剂量施用 L1L3 的半衰期中值是 4.77 天。以 3mg/kg 剂量施用 L1L3 的半衰期中值是 8.1 天。以 6mg/kg 剂量施用 L1L3 的半衰期中值是 7.75 天。以 12mg/kg 剂量施用 L1L3 的半衰期中值是 12.24 天。以 18mg/kg 剂量施用 L1L3 的半衰期中值是 11.76 天。L1L3PK 浓度 - 时间图是多相的，与靶 - 介导的药物分配一致。然而，在人对象中 L1L3 的半衰期意外地及显著长于 L1L3 在食蟹猕猴中的半衰期 (即在食蟹猕猴中施用 1.0、3.0、10.0 和 100.0mg/kg 剂量的半衰期分别为 1.91、2.33、3.49 和 5.25 天 (见实施例 1))。施用 0.3、1、3、6、12 和 18mg/kg 剂量的 L1L3 的平均药物清除率 (Cl) 分别为 8.70、6.58、4.54、4.33、3.28 和 3.85mL/ 天 /kg。得自这项研究的 PK NCA 结果在下表 2 中概括示出。在该表的 2-7 列，上方数值表示平均值，下方数值是中值。

[0187] 表 2：PK NCA 结果

[0188]

剂量 (mg/kg)	Cmax (ng/mL)	Tmax (天)	半衰期 (天)	Cl (mL/天/kg)	Vss (mL/kg)	$AUC_{(0-\infty)}$ (天*ng/mL)
0.3	10319.67 10537.50	0.083 0.06	2.74 2.71	8.70 8.92	31.77 30.74	34997.88 33748.15
1	29251.83 28231.50	0.063 0.06	4.80 4.77	6.58 6.00	41.59 42.34	156399.94 166736.58
3	96711.50 100620.5	0.049 0.04	8.74 8.1	4.54 4.12	49.06 48.94	709485.10 728278.54

[0189]

剂量 (mg/kg)	Cmax (ng/mL)	Tmax (天)	半衰期 (天)	Cl (mL/天/kg)	Vss (mL/kg)	$AUC_{(0-\infty)}$ (天*ng/mL)
6	175854.33 177485	0.056 0.04	8.36 7.75	4.33 4.65	60.45 61.33	1446945.71 1289916.44
12	353960.17 357671.00	0.090 0.08	20.53 12.24	3.28 3.36	72.25 57.52	3768691.17 3599992.39
18	532449.17 560463.50	0.090 0.08	12.97 11.76	3.85 3.71	65.46 60.83	4812012.99 4857618.28

[0190] 用 L1L3 处理导致明显的及持久的剂量依赖性空腹 LDL- 胆固醇 (LDL-C) 水平降低。LDL-C vs. 时间谱在图 1 中示出。空腹 LDL-C 基线水平为大约 145mg/dL。在给药后第 7 天，用单一剂量 0.3、1、3、6、12 或 18mg/kg 的 L1L3 处理的对象中 LDL-C 水平在 50-100mg/dL 之间。相反，施用安慰剂的对象中 LDL-C 水平通常保持在大约基线水平。在给药后第 14 天，用 1、3、6、12 或 18mg/kg L1L3 处理的对象中 LDL-C 水平为大约 70mg/dL 或更低。在给药后第 14 天，用 6mg/kg 或 12mg/kg L1L3 处理的对象中 LDL-C 水平为大约 55mg/dL，用 18mg/kg L1L3 处理的对象的 LDL-C 水平为大约 20mg/dL。用单一 12mg/kg 剂量的 L1L3 处理的对象中 LDL-C 水平在直至给药后至少大约 57 天（研究结束）保持在或低于大约 60mg/dL。用单一 18mg/kg 剂量的 L1L3 处理的对象中 LDL-C 水平在直至给药后至少大约 57 天保持在或低于大约 50mg/dL。用单一 6mg/kg 剂量的 L1L3 处理的对象中 LDL-C 水平在给药后大约 42 天保持低于 50mg/dL，在直至给药后至少大约 57 天低于 100mg/dL。用单一 3mg/kg 剂量 L1L3 处理的对象中 LDL-C 水平在给药后第 14 天为大约 70mg/dL，在给药后第 21 天为大约 60mg/dL，在直至给药后大约 36 天保持低于 100mg/dL。用单一 1mg/kg 剂量 L1L3 处理的对象中 LDL-C 水平在给药后第 14 天为大约 65mg/dL，在直至给药后第 21 天保持低于 100mg/dL。用单一 0.3mg/kg 剂量 L1L3 处理的对象中 LDL-C 水平在给药后第 7 天为大约 85mg/dL，在直至给药后大约 10 天保持低于 100mg/dL。

[0191] 血液中空腹 LDL-C 水平相对基线的变化的百分比在图 2 中示出（数据以平均值 +/-SE 示出）并在下表 3 中概括示出。在该表中，“N”表示对象数，“平均值”表示空腹 LDL-C 水平相对基线的变化的平均百分比，“PBO”是安慰剂。

[0192] 表 3

[0193]

随访天数	PBO		L1L3											
			0.3 mg/kg		1 mg/kg		3 mg/kg		6 mg/kg		12 mg/kg		18 mg/kg	
	N	平均值	N	平均值	N	平均值	N	平均值	N	平均值	N	平均值	N	平均值
1	12	0.000	6	0.000	6	0.000	6	0.000	6	0.000	6	0.000	6	0.000
2	12	3.26	6	-15.8	6	-0.33	6	-1.97	6	-1.44	6	-8.26	4	-11.52
3	12	-0.5	6	-14.13	6	-9.79	6	-13.20	6	-9.23	6	-14.05	6	-22.43
4	12	2.25	6	-30.14	6	-19.14	6	-19.10	6	-18.80	5	-23.23	6	-34.36
8	11	8.32	6	-42.86	6	-33.33	6	-39.78	6	-43.77	5	-37.96	5	-43.70
15	11	-3.24	6	-23.68	6	-50.50	6	-57.93	6	-61.52	5	-66.25	5	-82.89
22	11	6.26	6	-11.36	6	-22.40	6	-65.09	6	-68.92	5	-59.79	6	-72.97
29	11	11.87	6	-9.12	6	-3.36	6	-64.77	6	-64.19	6	-74.67	6	-67.40
36	5	17.38							6	-67.70	5	-65.23	4	-61.47
43	5	12.14					3	-27.56	6	-64.18	4	-69.31	3	-80.21
50	4	3.67							6	-49.17	4	-56.08		
57	4	12.08							6	-36.12	3	-63.10		

[0194] 接受安慰剂的对象中 LDL-C 水平通常保持在基线或之上，在图 2 中以“0”表示。如上所示，空腹 LDL-C 基线为大约 145mg/dL。施用 18mg/kg L1L3 导致相对基线的变化百分比升高至大约 83%（图 2）。单一 18mg/kg 剂量的 L1L3 使 LDL-C 水平在给药后至少直至 57 天保持低于基线大约 65%。单一 6mg/kg 或 12mg/kg 剂量的 L1L3 使 LDL-C 水平在给药后直至 43 天保持低于基线大约 60%。单一 3mg/kg 剂量的 L1L3 使 LDL-C 水平在给药后第 29 天保持低于基线大约 60%，在给药后直至 50 天保持低于基线 20%。

[0195] 用 L1L3 处理导致空腹总胆固醇 (TC) 明显和持久的剂量依赖性降低。血液中空腹 TC 水平相对基线的变化百分比在图 3 中示出（数据以平均值 +/-2SE 表示）。空腹 TC 基线为大约 230mg/dL；基线在图 3 中以“0”表示。在给药后大约第 9 天，施用单一 12 或 18mg/kg 剂量的 L1L3 的对象中 TC 水平降低至低于基线大约 30% 或者更低；TC 降低作用持续至少至给药后第 57 天（研究结束）。施用单一 6mg/kg 剂量 L1L3 的对象中 TC 水平在给药后第 9 天降低至低于基线大约 30% 或更低直至给药后大约 52 天。施用单一 3mg/kg 剂量 L1L3 的对象中 TC 水平在给药后第 9 天降低至低于基线大约 30%，在给药后大约第 22 天低于基线大约 40%。施用单一 3mg/kg 剂量 L1L3 的对象中 TC 水平在给药后大约第 22 天降低至低于基线大约 40%。施用单一 1mg/kg 剂量 L1L3 的对象中 TC 水平在给药后第 15 天降低至低于基线大约 36%。施用单一 0.3mg/kg 剂量 L1L3 的对象中 TC 水平在给药后大约第 9 天降低至低于基线大约 25%。在施用单一 12 或 18mg/kg 剂量 L1L3 之后，在给药后第 15 天，大多对象的 TC 水平低于基线 50%。在施用单一 6mg/kg 剂量 L1L3 之后，在给药后第 30 天，许多对象的 TC 水平低于基线 50%。在研究期间，在施用安慰剂的对象中 TC 水平保持在基线或在基线下 2% 之上。

[0196] 用处理导致空腹阿朴脂蛋白 B(apo B) 水平明显和持久地剂量依赖性降低。血液中空腹 apo B 水平相对基线的变化百分比在图 4 中示出数据以平均值 +/-2SE 示出。空腹 apo B 的基线为大约 119mg/dL；基线在图 4 中以“0”表示。施用安慰剂的对象中 Apo B 水平在研究期间保持在大约基线。施用 12 或 18mg/kg 剂量 L1L3 的对象中 Apo B 水平在第

14 天降低至低于基线大约 50%，在剩余的研究期间保持在低于基线大约 50% 或更低。施用 6mg/kg 剂量 L1L3 的对象中 Apo B 水平在第 14 天降低至低于基线大约 40%，在第 21 天低于基线大约 50%，在剩余的研究期间一般低于基线大约 30%。施用 3mg/kg 剂量 L1L3 的对象中 Apo B 水平在第 14 天降低至低于基线大约 40%，在第 28 天低于基线大约 50%。施用 1mg/kg 剂量 L1L3 的对象中 Apo B 水平在第 14 天降低至低于基线大约 40%。施用 0.3mg/kg 剂量 L1L3 的对象中 Apo B 水平在第 7 天降低至低于基线大约 25%。

[0197] 如图 5 所示，高密度脂蛋白胆固醇 (HDL-C) 水平在用 L1L3 处理后不显著改变。在图 5 中示出的数据是平均值 +/-2SE。空腹 HDL-C 的基线是大约 49mg/dL ；基线在图 5 中以“0”表示。施用安慰剂的对象中 HDL-C 水平在研究期间保持大约基线。空腹甘油三酯 (TGs) 水平在研究期间保持不变。血液中空腹 TG 水平相对基线的变化百分比在图 6 中示出。数据以平均值 +/-2SE 示出。空腹 TG 基线为 173mg/dL ；基线在图 6 中以“0”表示。

[0198] 在这项研究中，未发生严重不利事件，也无对象由于处理出现的不利事件 (TEAEs) 而终止。大多数 TEAEs 强度是轻度的，无一是严重程度。

[0199] 概括而言，施用 L1L3 导致在所有评估的剂量组中 LDL-C 水平均降低。通常地，在第 15 天或第 22 天测量出现 LDL-C 水平最大百分比降低。降低作用在早如第 3 天即可见。LDL-C 水平降低的程度和持续时间是剂量依赖性的。结果表明 L1L3 具有长期持续作用，即施用 0.3mg/kg 和 1.0mg/kg 剂量 L1L3 抗体的最大作用分别在第 7 天和第 14 天，在 3.0mg/kg 剂量最大作用直至 4 周，在 6mg/kg、12mg/kg 和 18mg/kg 剂量最大作用超过 6 周。这些持续作用基于 L1L3 的 $T^1/_2$ 数据是未曾预料的。

[0200] 实施例 3 ：单剂量 PCSK9 拮抗抗体 L1L3 组合他汀类药物的药动学和药效学

[0201] 这个实施例举例说明了临床实验研究以评估在施用稳定剂量阿托伐他汀的人对象中施用单剂量 PCSK9 拮抗抗体 (L1L3) 的药动学和药效学。

[0202] 在这项研究中，对接受稳定剂量阿托伐他汀的人对象施用单剂量 L1L3 抗体，剂量为 0.5mg/kg 或者 4mg/kg 的 PCSK9 拮抗抗体。在大约 60 分钟单次输注施用 L1L3。每个方案通过输注装置小心控制输注速度。在本研究方案中如下文所述施用阿托伐他汀 (每日 40mg)。在这项研究中，在其参与期间 (不包括在临床留观期间第 1 天至第 7 天之内) 自行服用阿托伐他汀的对象，在留观期间由专业的现场人员施用相同剂量。

[0203] 以无菌溶液呈现以经静脉内 (IV) 注射施用 L1L3 注射液，10mg/mL。每个含有 100mg L1L3 的 10mL 水相缓冲液的小瓶用镀膜的塞子和铝封条密封。阿托伐他汀 (40mg) 是一种白色药片，一面编码为“PD157”，另一面为“40”。

[0204] 在对每个对象施用药剂的 28 天内进行筛选。在筛选之前施用对象稳定剂量的阿托伐他汀至少 45 天。对象在第 4 天接受单剂量 L1L3，在留观其间进行多次 PK 和安全性评定 (研究第 -1，1-7 天)。将对象送回临床研究单元随访。

[0205] 对于对象而言的关键纳入标准是 ：在第 1 天之前施用稳定剂量阿托伐他汀 (每日 40mg)45 天，体重指数 (BMI) 为 18.5-40kg/m^2，兼具以上，体重等于或低于 150kg。对于对象而言的关键排除标准是 ：在过去一年期间的心血管事件病史 (如心肌梗塞 (MI)) ；控制不良的 1 型或 2 型糖尿病 (定义 ：不受控制的糖尿病定义为 HBIAc>9%) ；及控制不良的高血压 (不受控制的高血压定义为即使施用治疗时收缩压高于 140mm Hg 或者舒张压高于 90mm Hg)。可包括患有高血压及可以稳定剂量抗高血压药物控制的对象。所述研究包括两种性

别，最小年龄限于 18，最大年龄限于 80。

[0206] 在存在阿托伐他汀和阿托伐他汀条件下施用单剂量 0.5 或 4mg/kg L1L3 抗体之后评估 L1L3 抗体的药动学参数。测量在施用 L1L3 抗体之后空腹 LDL 胆固醇 (LDL-C) 相对基线的变化的绝对值和百分比。在这项研究中，测量对象经历毒性或不耐受剂量标准的发生率。也测量因治疗出现的不利事件 (TEAEs) 的发生率，以严重度和与研究药物的因果关系分类。测量每个上述结果的时间范围是 2 个月。

[0207] 研究方案

[0208] 第 -1 天 ：安排对象进入临床研究单元 (CRU)，并完成如下程序 ：复查和更新纳入和排除标准 ；复查和更新病史 ；复查和更新在施用计划的第一次剂量之前 28 天内所有处方药和非处方药及饮食补充剂的服用史 ；简单身体检查 ；仰卧位和直立体位生命体征测量 (血压、脉搏、体温 ）；10 小时空腹后收集血液和尿液样品进行安全性实验室检查 (血清化学、血液学、尿液分析、凝集、脂肪酶、淀粉酶、CRP 检查 ）；尿液药物和乙醇筛查 ；尿妊娠试验 (备孕女性 ）；收集血液样品进行免疫原性分析 (抗 -L1L3 抗体 ）；收集血液样品进行药效学分析 (PCSK9 和脂质颗粒 ）；收集血液样品进行药物基因组学分析 (任选，对象同意 ）；三次重复仰卧位 ECG ；评定乙醇、咖啡因和烟草使用情况 ；评定基线症状 / 不利事件 ；及随机化对象。

[0209] 第 1 天 ：在给药之前，完成如下程序 ：三次重复仰卧位 ECG(适当时在插入 IV 导管之前 ）；仰卧位和直立体位生命体征测量 (血压、脉搏、体温 ）；收集血液样品 (第 1 天，0 小时 ）进行 PK(阿托伐他汀 ）；对象服用赞助商提供的阿托伐他汀 (40mg) ；在给药后，在如下时间点收集血液样品进行 PK(阿托伐他汀 ）：第 1 天，0.25、0.5、1、2、3、4、6、8 和 12 小时。完成如下程序 ：评定基线症状 / 不利事件 ；复查伴随药物治疗。对象在第 2 天进行血液样品脂质检查之前空腹至少 10 小时。

[0210] 第 2 天 ：在给药之前，完成如下程序 ：仰卧位和直立体位生命体征测量 (血压、脉搏、体温 ）；收集血液样品 (第 2 天，0 小时 ）进行 PK(阿托伐他汀 ）；空腹 10 小时后收集脂质检查 ；对象服用赞助商提供的阿托伐他汀 (40mg)。完成如下程序 ：评定基线症状 / 不利事件及复查伴随药物治疗。

[0211] 第 3 天 ：在给药之前，完成如下程序 ：收集血液样品 (第 3 天，0 小时 ）进行 PK(阿托伐他汀 ）；仰卧位和直立体位生命体征测量 (血压，脉搏，体温 ）；对象服用赞助商提供的阿托伐他汀 (40mg)。完成如下程序 ：评定基线症状 / 不利事件 ；复查伴随药物治疗。对象空腹至少 10 小时之后，在第 4 天进行血液样品脂质检查。

[0212] 第 4 天 ：在施用阿托伐他汀和 L1L3 之前，完成如下程序 ：三次重复仰卧位 ECG ；仰卧位和直立体位生命体征测量 (血压、脉搏、体温 ）；收集血液样品 (第 4 天，0 小时 ）进行阿托伐他汀 PK ；收集血液样品 (第 4 天，0 小时 ）进行 L1L3PK ；在空腹 10 小时后收集血液和尿液样品进行安全性实验室检查 (血清化学、血液学、尿液分析、脂肪酶、淀粉酶、CRP 检查 ）；称重 ；空腹 10 小时后收集脂质检查 ；收集血液样品进行药效学分析 (PCSK9 和脂质颗粒 ）；收集血液样品进行免疫原性分析 (抗 -L1L3 抗体)。给药 ：对象服用赞助商提供的阿托伐他汀 (40mg)。L1L3 通过控制速度的静脉内输注在大约 60 分钟内施用。给药后，完成如下程序 ：在第 4 天施用阿托伐他汀后 0.25、0.5、1、2、3、4、6、8 和 12 小时收集血液样品进行 PK(阿托伐他汀 ）；在第 4 天从输注开始 1、4、8 和 12 小时收集血液样品进行 PK(L1L3) ；

给药后 1 小时三次重复仰卧位 ECGt ;在 L1L3 输注开始第 1 和 4 小时仰卧位和直立体位生命体征测量（血压，脉搏，体温）;评定基线症状 / 不利事件 ;复查伴随药物治疗。在第 5 和第 6 天进行血液样品脂质检查之前，对象空腹至少 10 小时。

[0213] 第 5 和 6 天 :在给药之前，完成如下程序 :仰卧位和直立体位生命体征测量（血压，脉搏，体温）;收集血液样品（第 5 天，0 小时）进行 PK（阿托伐他汀）;收集（第 5 天）血液样品进行 PK(L1L3) ;空腹 10 小时后收集脂质检查。仅第 5 天 :收集血液样品进行药效学分析 (PCSK9 和脂质颗粒）。对象服用赞助商提供的阿托伐他汀 (40mg)。完成如下程序 :评定基线症状 / 不利事件 ;复查伴随药物治疗。在第 7 天收集脂质检查血液样品之前，对象空腹至少 10 小时。

[0214] 第 7 天 :在给药之前，完成如下程序 :三次重复仰卧位 ECG ;仰卧位和直立体位生命体征测量（血压、脉搏、体温）;收集（第 7 天）血液样品进行 PK（阿托伐他汀）;收集（第 7 天）血液样品进行 (L1L3) ;在空腹 10 小时后收集脂质检查 ;收集血液样品进行药效学分析 (PCSK9 和脂质颗粒）;空腹 10 小时后收集血液和尿液样品进行安全性实验室检查（血清化学、血液学、尿液分析、凝集、脂肪酶、淀粉酶、CRP 检查）。对象服用最后一次赞助商提供的阿托伐他汀 (40mg)。在从所述单元撤出之前，完成如下程序 :简单身体检查 ;评定基线症状 / 不利事件 ;复查伴随药物治疗。提醒对象回到临床门诊，在第 15 天收集脂质检查血液样品之前空腹至少 10 小时。在剩余的研究期间对象持续服用其处方药阿托伐他汀。

[0215] 第 15 天（±1 天）:完成如下程序 :简单身体检查 ;阿托伐他汀顺应性检查 ;标准仰卧位 ECG ;仰卧位和直立体位生命体征测量（血压，脉搏，体温）;收集（第 15 天）血液样品进行 PK(L1L3) ;空腹 10 小时后收集脂质检查 ;收集血液样品进行免疫原性分析（抗 -L1L3 抗体）;收集血液样品进行药效学分析 (PCSK9 和脂质颗粒）;在空腹 10 小时后收集血液和尿液样品进行安全性实验室检查（血清化学，血液学，尿液分析，CRP) ;评定基线症状 / 不利事件 ;复查伴随药物治疗。提醒对象回到临床门诊，在第 22 天收集脂质检查血液样品之前空腹至少 10 小时。

[0216] 第 22 天（±1 天）:完成如下程序 :简单身体检查 ;检查对阿托伐他汀的依从性 ;仰卧位和直立体位生命体征测量（血压、脉搏、体温）;收集（第 22 天）血液样品进行 PK(L1L3) ;空腹 10 小时后收集脂质检查 ;在空腹 10 小时后收集血液和尿液样品进行安全性实验室检查（血清化学，血液学，尿液分析，CRP) ;评定基线症状 / 不利事件 ;复查伴随药物治疗。提醒对象回到临床门诊，在第 29 天收集脂质检查血液样品之前空腹至少 10 小时。

[0217] 第 29 天（±1 天）:完成如下程序 :全面身体检查 ;阿托伐他汀顺应性检查 ;仰卧位和直立体位生命体征测量（血压、脉搏、体温）;收集（第 29 天）血液样品进行 PK(L1L3) ;收集血液样品进行药效学分析 (PCSK9 和脂质颗粒）;收集血液样品进行免疫原性分析（抗 -L1L3 抗体）;在空腹 10 小时后收集脂质检查 ;三次重复仰卧位 ECG ;在空腹 10 小时后收集血液和尿液样品进行安全性实验室检查（血清化学、血液学、尿液分析、CRP)，尿液药物和乙醇筛查 ;血清妊娠试验（备孕女性）;评定基线症状 / 不利事件 ;复查伴随药物治疗。提醒对象回到临床门诊，在第 36 天收集脂质检查血液样品之前空腹至少 10 小时。

[0218] 第 36、43、50、57 和 64 天（终止随访）:完成如下程序 :简单身体检查 ;阿托伐他汀顺应性检查 ;标准仰卧位 ECG ;仰卧位和直立体位生命体征测量（血压、脉搏、体温）;收集血液样品进行 PK(L1L3) ;收集血液样品进行免疫原性分析（抗 -L1L3 抗体）;在空腹 10 小

时后收集脂质检查；在空腹 10 小时后收集血液和尿液样品进行安全性实验室检查（血清化学，血液学，尿液分析，CRP），评定基线症状 / 不利事件；复查伴随药物治疗。仅第 64 天：尿液妊娠试验（备孕女性）；凝集试验；称重；收集血液样品进行药效学分析 (PCSK9 和脂质颗粒）。

[0219] 第 78 和 92 天：在一些情况中，在等待自第 57 天的药动学结果期间，增加两次随访，第 78 天和第 92 天。在这种情况中，第 57 天的程序后接续第 78 天程序，第 64 天程序随后续接第 92 天程序。第 92 天成为终止随访。

[0220] 结果

[0221] 在这项研究中没有中止的对象。没有严重的不利事件 (SAE)，即偏头痛恶化，这不是药物相关的。TEAE 通常是非特异性的，强度不严重。此外，TEAE 是短暂的，丙氨酸转氨酶 (ALT) 和 / 或天冬氨酸转氨酶 (AST) 高于 3x ULN，无临床迹象 / 症状，所有均在一周内解决。

[0222] 表 4 概括示出这项研究的 L1L3PK 参数。

[0223]

表 4：L1L3 PK 参数：几何平均数(CV%)

参数	4 mg/kg L1L3+ 阿托伐他汀	0.5 mg/kg L1L3+ 阿托伐他汀
N, n	12,12	7,7
AUC_{inf}(ng.天/mL)	777167(13)	46338(28)
AUC_t(ng.天/mL)	726337(17)	38310(35)
C_{max}(ng/mL)	105048(16)	13827(9)
T_{max}(天)	0.1(0.04 – 0.50)	0.17(0.04 – 0.33)
$t_{1/2}$(天)	7.3(33)	2.6(34)
CL(mL/天/kg)	5.2(15)	10.8(29)
Vss(mL/kg)	52.3(16)	40.2(14)

[0224] 表 5 概括示出这项临床实验研究结果，评估在接受稳定剂量阿托伐他汀的人对象中单次施用 L1L3 的药动学和药效学结果。表中提供了在施用 L1L3 抗体后（表 4）空腹 LDL-C 水平相对基线的平均变化百分比值。

[0225]

表 5. 平均(SD) LDL-C vs 时间数据				
天数	0.5 mg/kg L1L3 (n=12)		4 mg/kg L1L3 (n=12)	
	平均值	SD	平均值	SD
0	0.0	0.0	0.0	0.0
1	-28.8	20.2	-20.9	18.5
2	-48.5	26.3	-38.4	13.0
3	-66.7	28.2	-43.3	18.1
11	-34.4	27.0	-64.6	26.0
18	9.0	39.7	-73.2	21.2
25	23.3	43.5	-70.8	20.4
32	14.8	37.0	-69.9	14.8
39	21.2	36.7	-45.1	16.9
46	17.0	37.2	-19.2	16.4
53	27.9	42.7	-3.6	25.4
60	30.1	39.3	7.1	25.8

[0226] 在存在阿托伐他汀（剂量 =40mg）条件下用 L1L3 处理导致空腹 LDL-C 水平明显及持久地剂量依赖性降低。空腹 LDL-C 基线为大约 72.5mg/dL。图 7A 示出在施用 L1L3 抗体之后的绝对空腹 LDL-C 水平。图 7B 示出在施用 L1L3 抗体之后空腹 LDL-C 水平相对基线的变化百分比。基线在图 7B 中以“0”表示。使用 0.5mg/kg 剂量 L1L3，在施用 L1L3 之后第 3 天观测到最大 LDL-C 降低作用。使用 4mg/kg 剂量 L1L3，在施用 L1L3 之后直至 32 天均观测到最大 LDL-C 降低作用。LDL-C 降低的剂量依赖性反应在图 8 中示出。如图 8 示出，每个 L1L3 的施用剂量均使接受稳定剂量他汀类药物的患者的 LDL-C 水平降低。此外，接受稳定剂量他汀类药物的患者中 LDL-C 降低作用高于仅施用 L1L3 的患者中的作用（图 8）。

[0227] 实施例 4 ：PK-PD 建模及模拟时间谱

[0228] 基于上述研究中提供的数据，产生模拟血清 L1L3- 时间谱和 LDL-C- 时间谱。图 9A-F 示出在施用指定剂量 L1L3 或者安慰剂之后 L1L3（上方）和 LDL-C（下方）的模拟时间谱。该模拟图是施用 2mg/kg L1L3（左侧）或 6mg/kg L1L3（中间）相比施用安慰剂（右侧）而产生的。在第 0 天和第 29 天施用 L1L3 或安慰剂，即间隔四周施用两次。图 10 示出在施用如下剂量的 L1L3 之后的模拟 LDL-C- 时间谱 ：0.25mg/kg、0.5mg/kg、1mg/kg、2mg/kg、4mg/kg 和 6mg/kg，每次在第 0、29 和 56 天施用（图 10）。模拟 L1L3- 时间谱和 LDL-C- 时间谱证实每四周施用一次低剂量的 L1L3 产生持久的 LDL-C 降低。

[0229] 实施例 5 ：多次 L1L3 给药之后的药动学和药效学

[0230] 这个实施例举例说明了一项临床实验研究，评估在人对象中多次 PCSK9 拮抗抗体 (L1L3) 静脉内给药之后的药动学和药效学。

[0231] 这项研究是随机的、多中心的、双盲的、安慰剂对照的平行设计的实验，其具有 28 天筛查期，4 周治疗期和 8 周随访期（图 11）。在这项研究中，对日本人对象施用 L1L3 抗体，剂量为 0.25mg/kg、0.5mg/kg、1.0mg/kg 或者 1.5mg/kg 的 PCSK9 拮抗抗体。对于每个对象，本研究由三个时期组成 ：审查期，治疗期和随访期。治疗期持续直至大约 28 天，在第 1、8、15 和 22 天施用 4 次单一 I.V. L1L3 或安慰剂给药。随访期持续大约 8 周，从大约第 29 天开

始至最后随访（第 78 天）。对象定期在临床门诊进行安全性评定及收集进行常规实验室检查、脂质谱、PK、PD 和免疫原性检查的血液样品。

[0232] 每周一次施用所有检测剂量的 L1L3 处理，导致空腹水平持久、明显及长期的剂量依赖性降低。空腹 LDL-C 基线为大约 155mg/dL。图 12 示出施用 L1L3 抗体之后的绝对空腹 LDL-C 水平。图 13 示出在施用 L1L3 抗体之后的空腹 LDL-C 水平相对基线的变化百分比。基线在图 13 中以“0”表示。

[0233] 图 14 中的表概括示出这个临床实验研究结果，评估在接受稳定剂量阿托伐他汀的人对象中多次 L1L3 给药之后的药动学和药效学结果。图中提供了在施用 L1L3 抗体之后空腹 LDL-C 水平相对基线的变化平均百分比（平均值）（图 14）。

[0234] 实施例 6：组合他汀类药物多次 L1L3 给药之后的药动学和药效学

[0235] 这个实施例举例说明了一项临床实验研究，评估在服用阿托伐他汀、辛伐他汀或罗苏伐他汀的人对象中多次 PCSK9 拮抗抗体 (L1L3) 给药之后的药动学和药效学结果。

[0236] 这项研究是随机的、多中心的、双盲的、安慰剂对照的平行设计的实验，3 周筛查期，12 周治疗期和 8 周随访期。

[0237] 纳入这项研究中的对象符合所有如下标准：男性和女性对象年龄超过或等于 18 岁；体重指数为 18.5-40kg/m^2；总体重高于 50kg(110lbs) 及低于 150kg(330lbs)；稳定日剂量的他汀类药物，规定为阿托伐他汀 40 或 80mg，罗苏伐他汀 20 或 40mg，或者辛伐他汀 40 或 80mg，在第 1 天之前最少服用 45 天；在两次资格审查 (qualifying visits) 时（筛查和第 -7 天）脂质水平符合如下标准：空腹 LDL-C 水平高于或等于 100mg/dL。

[0238] 对象定期在临床门诊进行安全性评定及收集血液提供安全性实验、脂质谱、PK、PD 和免疫原性样品。在筛查期间及在第 3 天每次随访之前进行电话联系，以提醒对象需要空腹 10 小时，评定不利事件并在对象源文件中记录该联系。对象在第 1、29 和 57 天接受一次 1mg/kg L1L3、3mg/kg L1L3、6mg/kg L1L3 或安慰剂输注，在治疗期和随访期进行多次效力、安全性和 PK 评定。每个方案通过输注装置小心控制输注速度。在大约 60 分钟内施用单一输注。

[0239] 结果

[0240] 3mg/kg 的给药方案和 6mg/kg 的给药方案均达到 LDL-C 相对基线变化 30%，这是统计学显著并且超出目标值的。观测到 L1L3 对甘油三酯无作用。可见 HDL 水平略微升高，最高升高 9%。处理组和记录在表 6 中示出。

[0241] 表 6

[0242]

	安慰剂 (N=19)	L1L3 0.25 mg/kg (N=19)	L1L3 1 mg/kg (N=18)	L1L3 3 mg/kg (N=18)	L1L3 6 mg/kg (N=18)
	n (%)	n (%)	n (%)	n (%)	n (%)
对象编号					
阿托伐他汀	6 (31.6)	6 (31.6)	6 (33.3)	6 (33.3)	6 (33.3)
罗苏伐他汀	6 (31.6)	5 (26.3)	6 (33.3)	5 (27.8)	5 (27.8)
辛伐他汀	7 (36.8)	6 (31.6)	6 (33.3)	7 (38.9)	6 (33.3)

[0243] 预定的主要效力终点是在第85天LDL-C相对基线的变化百分比，这是使用ANCOVA模型分析的。最终的ANCOVA模型含有关于基线LDL-C和处理的期限(term)。为了防止主要终点分析在0.05水平的整体I型误差率，采用Haybittle-Peto，以0.001α损耗(alpha spent)为界限。

[0244] 在3和6mg/kg治疗组中，通过由剂量漏服(missing-ness of doses)驱动的LDL-C变化观测到明确的剂量应答的强治疗作用(图15和16)。随后使用混合模型重复测量分析LDL-C数据，以预估这两种治疗的时间和根据经验的剂量应答谱。

[0245] 当加入他汀类药物时另外降低30%LDL-C的预定目标值是成功的概念证明标准。当加入他汀类药物治疗时，在每4周施用3和6mg/kg剂量组明确实现了这个LDL-C降低30%或更多的目标水平(图15和16)。图15中的图表示出研究和治疗期相对基线的变化百分比，图16中的图表示出除了错过给药的对象之外的研究和治疗期的相对基线的变化百分比。在接受稳定日剂量他汀类药物的患者中，3mg/kg L1L3的给药方案在第29天实现LDL-C水平低于基线大约50%(图15)。在接受稳定日剂量他汀类药物的患者中，6mg/kg L1L3的给药方案在第29天实现LDL-C水平低于基线大约65%(图15)。施用3mg/kg和6mg/kg给药方案均使LDL-C水平降低30%以上持续28天(图16)。在表7中提供了在第85天由安慰剂调节的治疗效力的统计学概述。在表7中，脂质的基线定义为在第-7天和第1天观测数值的平均值。

[0246] 表7：在第85天LDL-C数据相对基线的变化百分比的统计学分析(MMRM)总结

[0247]

对比 (检测 vs. 参照)	LS平均值差异 (检测-参照)	标准误差	95% CI	*P-值
L1L3 0.25 mg/kg vs. 安慰剂 第85天	2.67	10.252	(-17.87, 23.20)	0.7958
L1L3 1 mg/kg vs. 安慰剂 第85天	0.83	10.013	(-19.23, 20.89)	0.9340
L1L3 3 mg/kg vs. 安慰剂 第85天	-38.92	9.721	(-58.39, -19.46)	0.0002
L1L3 6 mg/kg vs. 安慰剂 第85天	-50.14	10.266	(-70.70, -29.57)	<0.0001

[0248] L1L3Cmax和低谷浓度在表8中示出。

[0249] 表8:L1L3药动学

[0250]

剂量 (mg/kg)	第一次给药后		第3次给药后	
	C_{max} (μg/mL)	C_{trough} (μg/mL)	C_{max} (μg/mL)	C_{trough} (μg/mL)
0.25	10.9 ± 13.0	0.109 ± 0.406	6.95 ± 1.55	0.122 ± 0.226

[0251]

	(n=17)	(n=14)	(n=13)	(n=8)
1	28.3 ± 6.6 (n=17)	0.256 ± 0.310 (n=14)	37.3 ± 26.4 (n=11)	0 ± 0 (n=9)
3	92.2 ± 22.6 (n=18)	3.16 ± 2.30 (n=13)	86.5 ± 15.0 (n=12)	3.04 ± 4.42 (n=7)
6	182 ± 64 (n=17)	17.4 ± 11.6 (n=14)	179 ± 66 (n=8)	15.6 ± 15.3 (n=7)

[0252] 在接受稳定日剂量他汀类药物的患者中每月一次用 3 和 6mg/kg 剂量的 L1L3 处理导致血液中 LDL-C 水平相对基线降低 30% 以上。观测到 HDL 水平的升高较低（至多 9%），L1L3 对甘油三酯的作用较小。L1L3 通常是安全的并具有良好的耐受性。LFTs、CK、ECG 和 BP 中的改变是短暂的、适度的，在大多数情况中被认为与治疗不相关。没有对象存在阳性 ADA。

[0253] 实施例 7 :组合他汀类药物多次 L1L3 给药之后的药动学和药效学

[0254] 这个实施例举例说明了一项临床实验研究，评估在人对象中组合他汀类药物在多次经皮下 PCSK9 拮抗抗体 (L1L3) 给药之后的 LDL-C 水平。

[0255] 这项研究是随机的、多中心的、双盲的、安慰剂对照的、平行设计组的、剂量变化研究设计的实验，在接受他汀类药物的高胆固醇血症患者中每月经皮下施用一次及每月经皮下施用两次，共施用 L1L3 六个月，以评定其效力、安全性和可耐受性。计划在两个给药方案 (Q28d 或 Q14d) 中共分 7 个剂量组，每组 50 个对象。方案设计在表 9 中示出。

[0256] 表 9

[0257]

分支	指定的干预
实验: Q28d 剂量分支 Q28d 剂量组接受每月一次皮下施用 L1L3 抗体或安慰剂	组 1: 安慰剂, Q28d 组 2: L1L3 200 mg, Q28d 组 3: L1L3 300 mg, Q28d
实验: Q14d 剂量分支 Q14d 剂量组接受每两周一次皮下施用 L1L3 抗体或安慰剂	组 4: 安慰剂, Q14d 组 5: L1L3 50mg, Q14d 组 6: L1L3 100 mg, Q14d 组 7: L1L3 150 mg, Q14d

[0258] 资格 :18 岁或更大年龄。

[0259] 纳入标准 :对象应接受稳定剂量的（至少 6 周）任何他汀类药物并在这个实验期间继续接受相同剂量他汀类药物。在筛查及在第 1 天随机化之前至少 7 天的 2 次筛查时脂质水平应符合以下治疗背景水平 :空腹 LDL-C 高于或等于 80mg/dL(2.31mmol/L) ;空腹 TG 低于或等于 400mg/dL(4.52mmol/L) ;对象空腹 LDL-C 水平必须高于或等于 80mg/dL（在最初筛查时 2.31mmol/L，在随机化 7 天内第二次筛查时该数值必须不低于初始值的 20% 以符合这个实验的资格）。

[0260] 初级的结果测量是在随机化后 12 周结束时从 LDL-C 基线变化的绝对值。次级结果测量包括如下内容 :评定在随机化后 12 周结束时 LDL-C 的变化及相对基线的变化百分比 ;血浆稳态 L1L3 药动学参数 ;LDL-C 低于指定限值 (<100mg/dL,<70mg/dL,<40mg/dL,<25mg/dL) 的对象的比例 ;评定在随机化后 12 周结束时总胆固醇的变化及相对基线的变化百分

比；评定在随机化后 12 周结束时 ApoB 的变化及相对基线的变化百分比；评定在随机化后 12 周结束时 ApoA1 的变化及相对基线的变化百分比；评定在随机化后 12 周结束时脂蛋白 (a) 的变化及相对基线的变化百分比；评定在随机化后 12 周结束时 HDL- 胆固醇的变化及相对基线的变化百分比；评定在随机化后 12 周结束时极低密度脂蛋白的变化及相对基线的变化百分比；评定在随机化后 12 周结束时甘油三酯的变化及相对基线的变化百分比；及评定在随机化后 12 周结束时非 -HDL- 胆固醇的变化及相对基线的变化百分比。

[0261] 尽管参照各种申请、方法和组合物，已经描述了本发明所公开的教导，但是应意识到在不偏离本发明教导和下文权利要求书的精神下可以对本发明进行各种改变和修改。提供前文的实施例是为了更好地阐明本发明，这些实施例无限制本文呈现的教导的范围之意。虽然在这些实施方案的例子中描述了本发明的教导，但是技术人员容易理解，不用过多的实验可以对这些实施方案的例子进行各种改变和修改。所有这种改变和修改均在本发明范围内。

[0262] 本文引用的所有参考文献，包括专利、专利申请、文章、教科书等以及其中引用的参考文献，均以其全部内容并入本文作参考。在一或多个并入的文献和相似的材料与本发明申请包括但不限于术语定义、术语应用、描述的技术等不同或有冲突时，以此申请书为准。

[0263] 前文的描述和实施例详细描述了本发明的特定实施方案并描述了由本发明人设想的最佳模式。应意识到，无论前文如何详细描述，本发明可以多种形式实施，且本发明应根据所附权利要求书及其任何等价物加以解释。

[0001]

序列表

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<120> 使用抗 PCSK9 抗体的治疗

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<151> 2011-07-14

<150> 61/614,312
<151> 2012-03-22

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<151> 2012-05-04

<160> 15

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<212> PRT
<213> Homo sapiens

<400> 1

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            20                  25                  30

Asp Glu Asp Gly Asp Tyr Glu Glu Leu Val Leu Ala Leu Arg Ser Glu
        35                  40                  45

Glu Asp Gly Leu Ala Glu Ala Pro Glu His Gly Thr Thr Ala Thr Phe
    50                  55                  60

His Arg Cys Ala Lys Asp Pro Trp Arg Leu Pro Gly Thr Tyr Val Val
65                  70                  75                  80

Val Leu Lys Glu Glu Thr His Leu Ser Gln Ser Glu Arg Thr Ala Arg
                85                  90                  95

Arg Leu Gln Ala Gln Ala Ala Arg Arg Gly Tyr Leu Thr Lys Ile Leu
            100                 105                 110

His Val Phe His Gly Leu Leu Pro Gly Phe Leu Val Lys Met Ser Gly
        115                 120                 125
```

[0002]

Asp Leu Leu Glu Leu Ala Leu Lys Leu Pro His Val Asp Tyr Ile Glu
130 135 140

Glu Asp Ser Ser Val Phe Ala Gln Ser Ile Pro Trp Asn Leu Glu Arg
145 150 155 160

Ile Thr Pro Pro Arg Tyr Arg Ala Asp Glu Tyr Gln Pro Pro Asp Gly
165 170 175

Gly Ser Leu Val Glu Val Tyr Leu Leu Asp Thr Ser Ile Gln Ser Asp
180 185 190

His Arg Glu Ile Glu Gly Arg Val Met Val Thr Asp Phe Glu Asn Val
195 200 205

Pro Glu Glu Asp Gly Thr Arg Phe His Arg Gln Ala Ser Lys Cys Asp
210 215 220

Ser His Gly Thr His Leu Ala Gly Val Val Ser Gly Arg Asp Ala Gly
225 230 235 240

Val Ala Lys Gly Ala Ser Met Arg Ser Leu Arg Val Leu Asn Cys Gln
245 250 255

Gly Lys Gly Thr Val Ser Gly Thr Leu Ile Gly Leu Glu Phe Ile Arg
260 265 270

Lys Ser Gln Leu Val Gln Pro Val Gly Pro Leu Val Val Leu Leu Pro
275 280 285

Leu Ala Gly Gly Tyr Ser Arg Val Leu Asn Ala Ala Cys Gln Arg Leu
290 295 300

Ala Arg Ala Gly Val Val Leu Val Thr Ala Ala Gly Asn Phe Arg Asp
305 310 315 320

Asp Ala Cys Leu Tyr Ser Pro Ala Ser Ala Pro Glu Val Ile Thr Val
325 330 335

Gly Ala Thr Asn Ala Gln Asp Gln Pro Val Thr Leu Gly Thr Leu Gly
340 345 350

[0003]

Thr Asn Phe Gly Arg Cys Val Asp Leu Phe Ala Pro Gly Glu Asp Ile
355 360 365

Ile Gly Ala Ser Ser Asp Cys Ser Thr Cys Phe Val Ser Gln Ser Gly
370 375 380

Thr Ser Gln Ala Ala Ala His Val Ala Gly Ile Ala Ala Met Met Leu
385 390 395 400

Ser Ala Glu Pro Glu Leu Thr Leu Ala Glu Leu Arg Gln Arg Leu Ile
405 410 415

His Phe Ser Ala Lys Asp Val Ile Asn Glu Ala Trp Phe Pro Glu Asp
420 425 430

Gln Arg Val Leu Thr Pro Asn Leu Val Ala Ala Leu Pro Pro Ser Thr
435 440 445

His Gly Ala Gly Trp Gln Leu Phe Cys Arg Thr Val Trp Ser Ala His
450 455 460

Ser Gly Pro Thr Arg Met Ala Thr Ala Val Ala Arg Cys Ala Pro Asp
465 470 475 480

Glu Glu Leu Leu Ser Cys Ser Ser Phe Ser Arg Ser Gly Lys Arg Arg
485 490 495

Gly Glu Arg Met Glu Ala Gln Gly Gly Lys Leu Val Cys Arg Ala His
500 505 510

Asn Ala Phe Gly Gly Glu Gly Val Tyr Ala Ile Ala Arg Cys Cys Leu
515 520 525

Leu Pro Gln Ala Asn Cys Ser Val His Thr Ala Pro Pro Ala Glu Ala
530 535 540

Ser Met Gly Thr Arg Val His Cys His Gln Gln Gly His Val Leu Thr
545 550 555 560

Gly Cys Ser Ser His Trp Glu Val Glu Asp Leu Gly Thr His Lys Pro
565 570 575

Pro Val Leu Arg Pro Arg Gly Gln Pro Asn Gln Cys Val Gly His Arg
580 585 590

[0004]

Glu Ala Ser Ile His Ala Ser Cys Cys His Ala Pro Gly Leu Glu Cys
595 600 605

Lys Val Lys Glu His Gly Ile Pro Ala Pro Gln Glu Gln Val Thr Val
610 615 620

Ala Cys Glu Glu Gly Trp Thr Leu Thr Gly Cys Ser Ala Leu Pro Gly
625 630 635 640

Thr Ser His Val Leu Gly Ala Tyr Ala Val Asp Asn Thr Cys Val Val
645 650 655

Arg Ser Arg Asp Val Ser Thr Thr Gly Ser Thr Ser Glu Gly Ala Val
660 665 670

Thr Ala Val Ala Ile Cys Cys Arg Ser Arg His Leu Ala Gln Ala Ser
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Gln Glu Leu Gln
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1 5

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<211> 17
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<213> Artificial Sequence

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<223> CDR

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1 5 10 15

[0005]

Ser

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<220>
<223> CDR

<400> 4

Glu Arg Pro Leu Tyr Ala Ser Asp Leu
1 5

<210> 5
<211> 11
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<213> Artificial Sequence

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 6
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<220>
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<400> 6

Ser Ala Ser Tyr Arg Tyr Thr
1 5

<210> 7
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Gln Gln Arg Tyr Ser Leu Trp Arg Thr
1 5

[0006]

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1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Glu Ile Ser Pro Phe Gly Gly Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Arg Pro Leu Tyr Ala Ser Asp Leu Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190
```

[0007]

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Thr
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
385 390 395 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405 410 415

[0008]

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 9
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> full length light chain

<400> 9

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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Tyr Ser Leu Trp Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

[0009]

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
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Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
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Phe Asn Arg Gly Glu Cys
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<210> 10
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<212> PRT
<213> Artificial Sequence

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<223> full length heavy chain without C-terminal lysine

<400> 10

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Glu Ile Ser Pro Phe Gly Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Arg Pro Leu Tyr Ala Ser Asp Leu Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

[0010]

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Thr
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

[0011]

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
385 390 395 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
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<213> Artificial Sequence

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<223> heavy chain variable region

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Glu Ile Ser Pro Phe Gly Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Arg Pro Leu Tyr Ala Ser Asp Leu Trp Gly Gln Gly Thr
100 105 110

[0012]

Thr Val Thr Val Ser Ser
115

<210> 12
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<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region

<400> 12

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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Tyr Ser Leu Trp Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 13
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<213> Artificial Sequence

<220>
<223> CDR

<400> 13

Gly Tyr Thr Phe Thr Ser Tyr
1 5

[0013]

<210> 14
<211> 10
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<220>
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<400> 14

Gly Tyr Thr Phe Thr Ser Tyr Tyr Met His
1 5 10

<210> 15
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<213> Artificial Sequence

<220>
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<400> 15

Ile Ser Pro Phe Gly Gly Arg
1 5

[0001]

关于微生物保藏的说明

申请人或代理人档案号 PC71736A	国际申请号 PCT/IB2012/053534

关于微生物保藏的说明

（专利合作条约实施细则 13 之 2）

微生物保藏的说明	
A.对说明书第 27 页，第 25-29 行 所述的已保藏的微生物或其他生物材料的说明	
B. 保藏事项	更多的保藏在附加页说明 ☒
保藏单位名称ATCC 美国典型培养物保藏中心	
保藏单位地址 （包括邮政编码和国名） 10801 University Blvd., Manassas, Virginia 20110-2209, United States of America	
保藏日期 2008-02-28	保藏号 ATCC PTA-8983, PTA-8984, PTA-8985, PTA-8986
C.补充说明（必要时）	更多信息在附加页中 ☐
无	
D.本说明是为下列指定国作的（如果说明不是为所有指定国而作的）	
所有指定国	
E.补充说明（必要时）	
下列说明将随后向国际局提供（写出说明的类别，例如："保藏的编号"） 无	

由受理局填写	由国际局填写
☐ 本页已经和国际申请一起收到	☐ 国际局收到本页日期
授权官员	授权官员

[0002]

微生物保藏(2)	
A.对说明书第 32 页，第 I-6 行 所述的已保藏的微生物或其他生物材料的说明	
B. 保藏事项	更多的保藏在附加页说明 ☐
保藏单位名称ATCC 美国典型培养物保藏中心	
保藏单位地址 （包括邮政编码和国名） 10801 University Blvd., Manassas, Virginia 20110-2209, United States of America	
保藏日期 2009-08-25	保藏号 ATCC PTA-10302, PTA-10303
C.补充说明（必要时）	更多信息在附加页中 ☐
无	
D.本说明是为下列指定国作的（如果说明不是为所有指定国而作的）	
所有指定国	
E.补充说明（必要时）	
下列说明将随后向国际局提供（写出说明的类别，例如："保藏的编号"） 无	

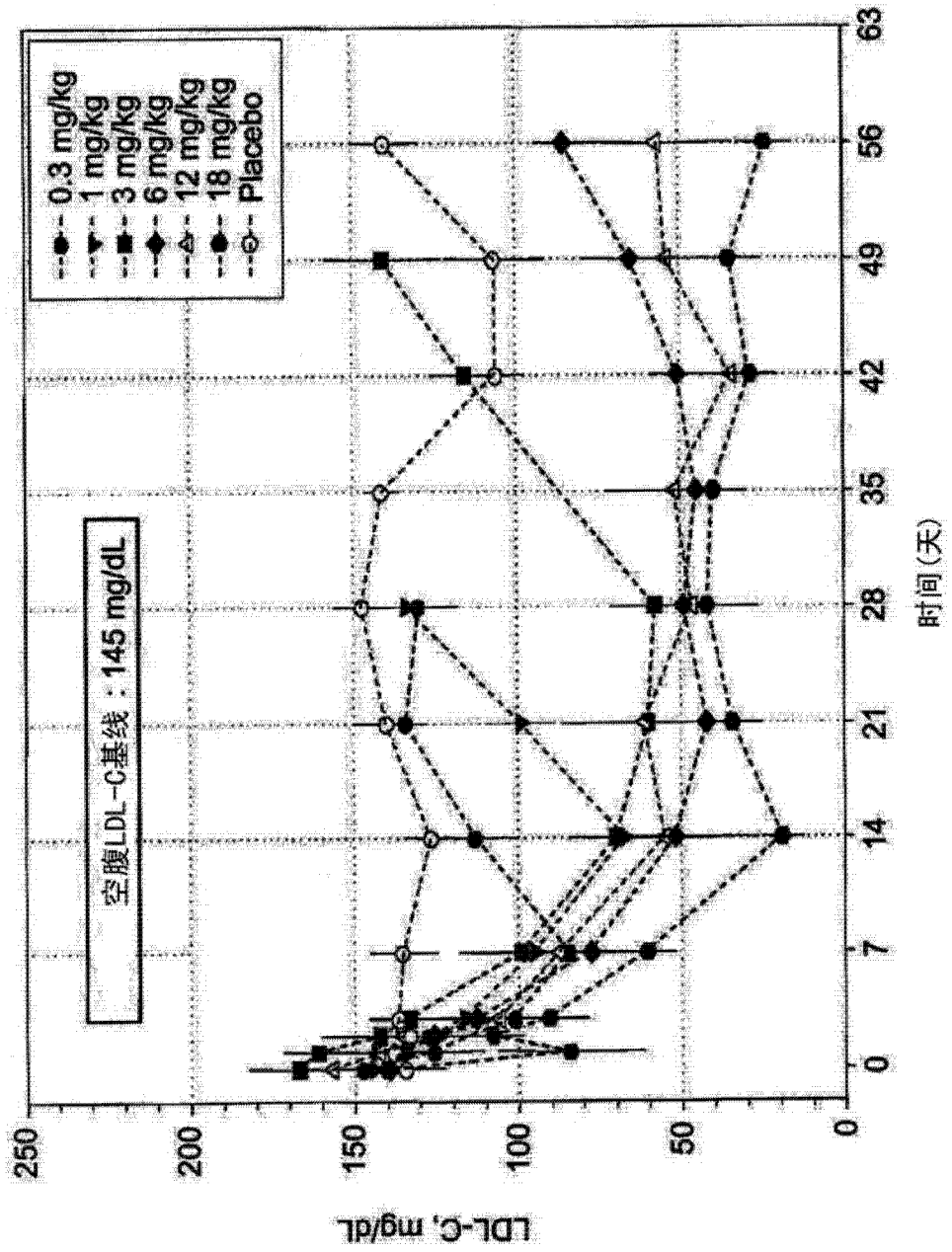
0.3 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
12 mg/kg
18 mg/kg
Placebo
空腹LDL-C基线：145 mg/dL
LDL-C, mg/dL
250
200
150
100
50
0
0
7
14
21
28
35
42
49
56
63
时间(天)

图 1

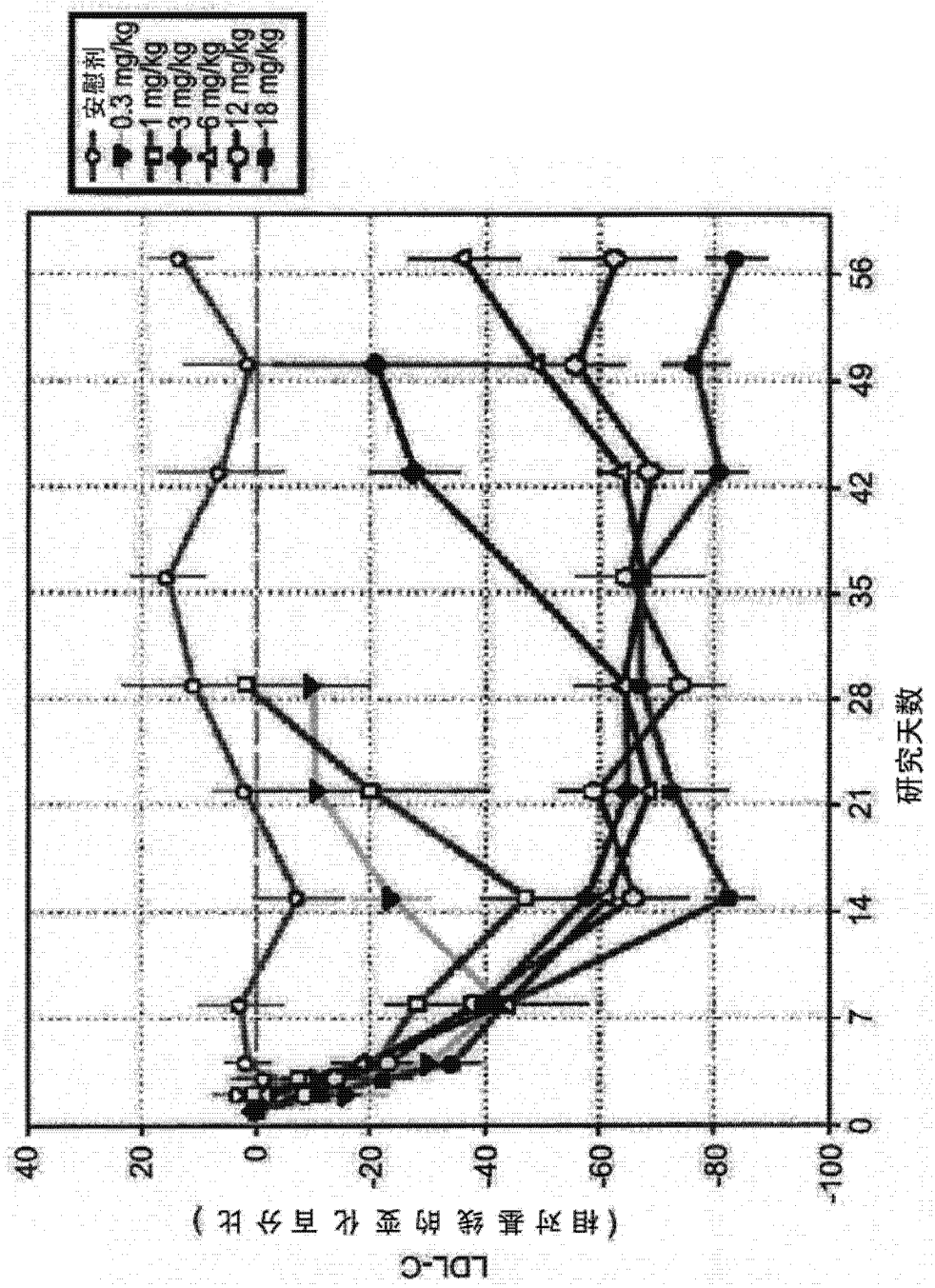
安慰剂
0.3 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
12 mg/kg
18 mg/kg
LDL-C
（相对基线的变化百分比）
40
20
0
-20
-40
-60
-80
-100
0
7
14
21
28
35
42
49
56
研究天数

图 2

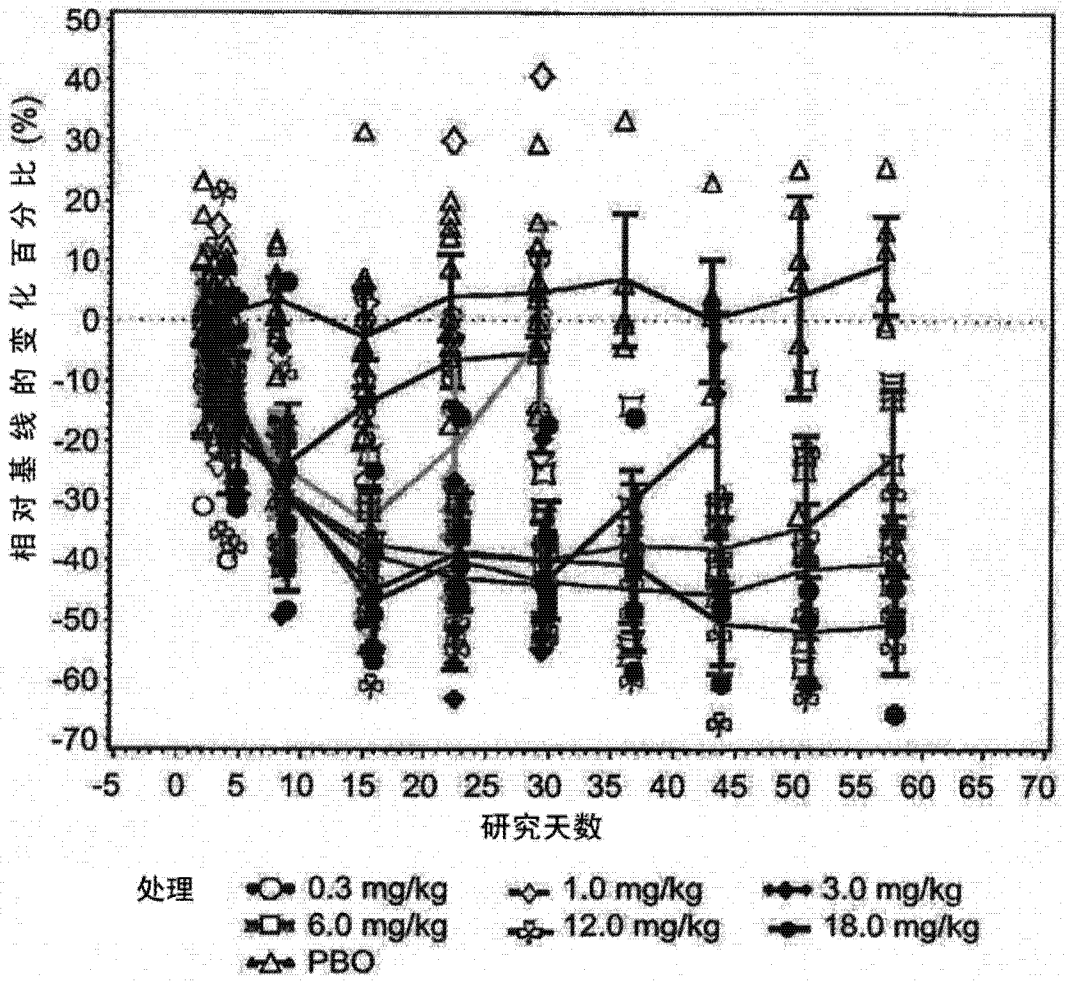
相对基线的变化百分比(%)
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
研究天数
处理
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

图 3

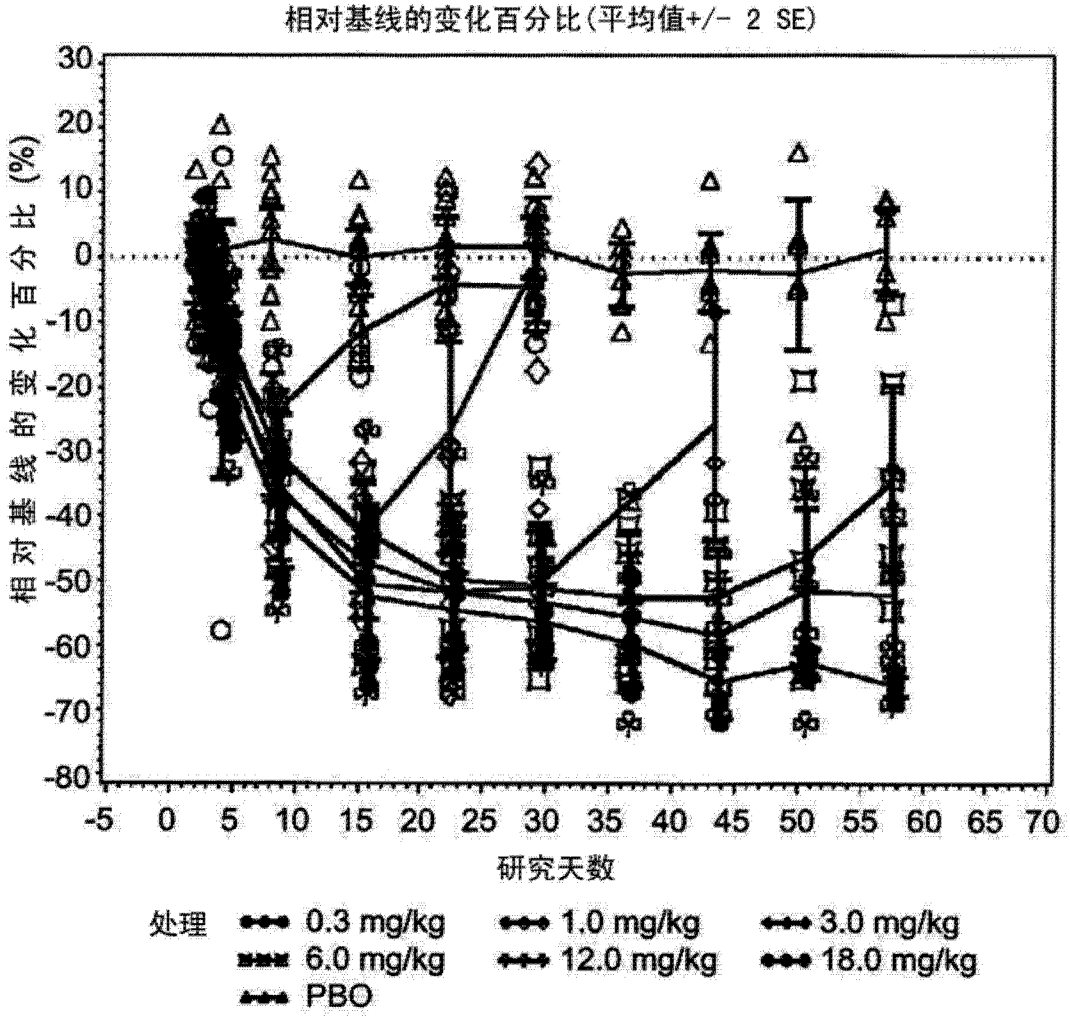
相对基线的变化百分比(平均值+/- 2 SE)
相对基线的变化百分比(%)
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
研究天数
处理
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

图 4

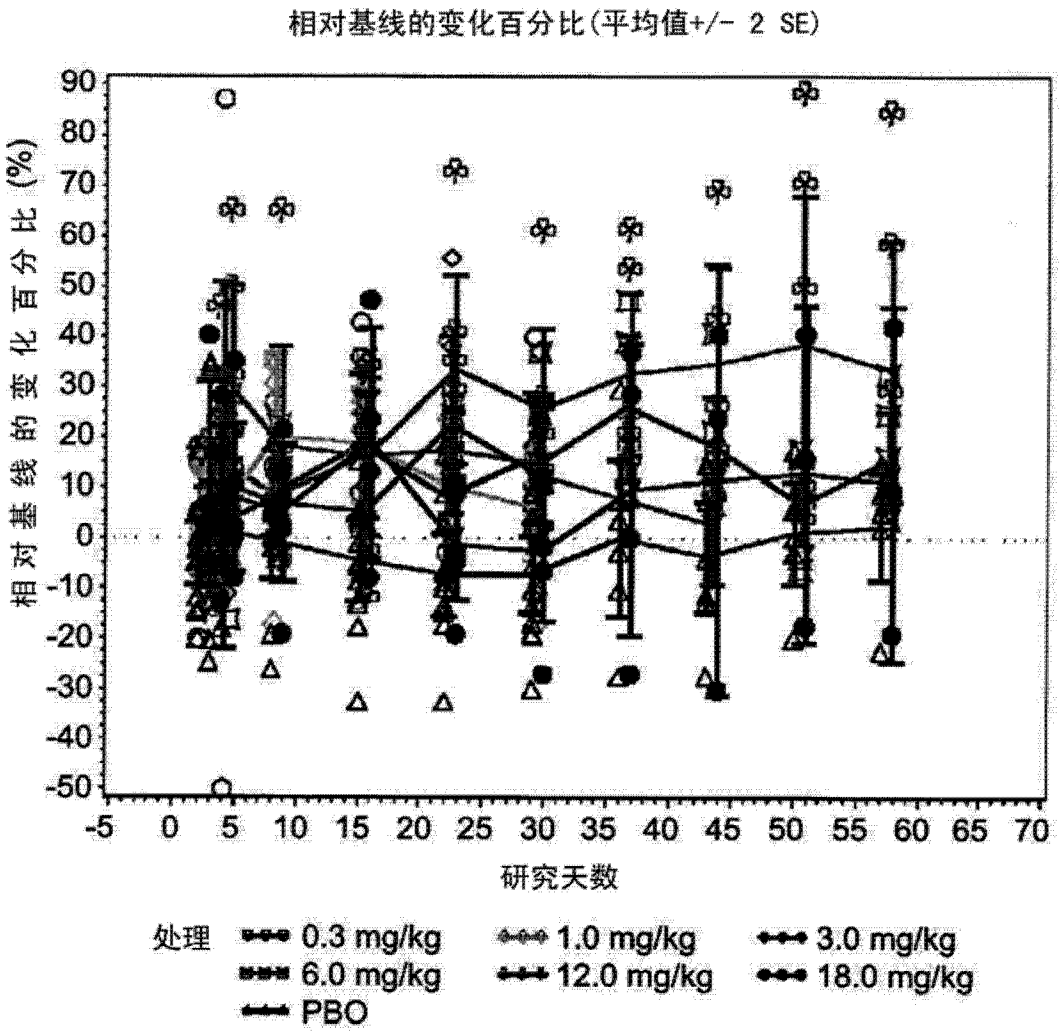
相对基线的变化百分比(平均值+/- 2 SE)
相对基线的变化百分比(%)
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
研究天数
处理
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

图 5

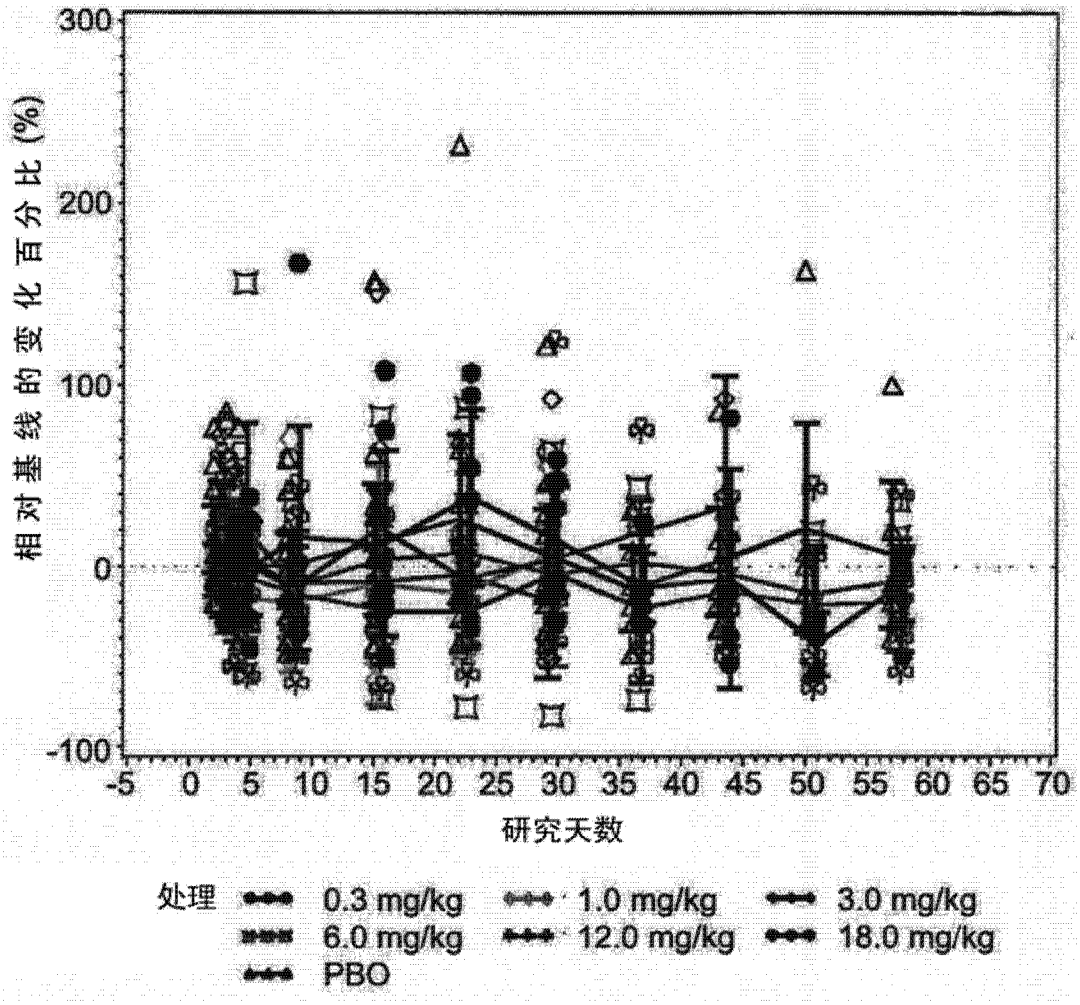
300
200
100
0
-100
相对基线的变化百分比(%)
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
研究天数
处理
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg
6.0 mg/kg
12.0 mg/kg
18.0 mg/kg
PBO

图 6

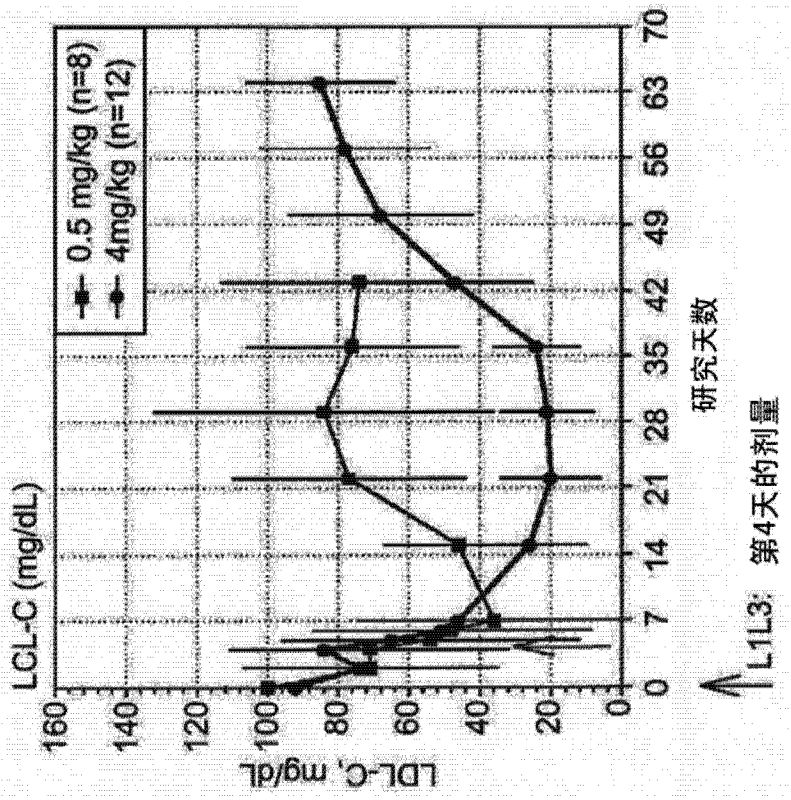
LCL-C (mg/dL)
0.5 mg/kg (n=8)
4mg/kg (n=12)
LDL-C, mg/dL
160
140
120
100
80
60
40
20
0
0
7
14
21
28
35
42
49
56
63
70
研究天数
L1L3: 第4天的剂量

图 7A

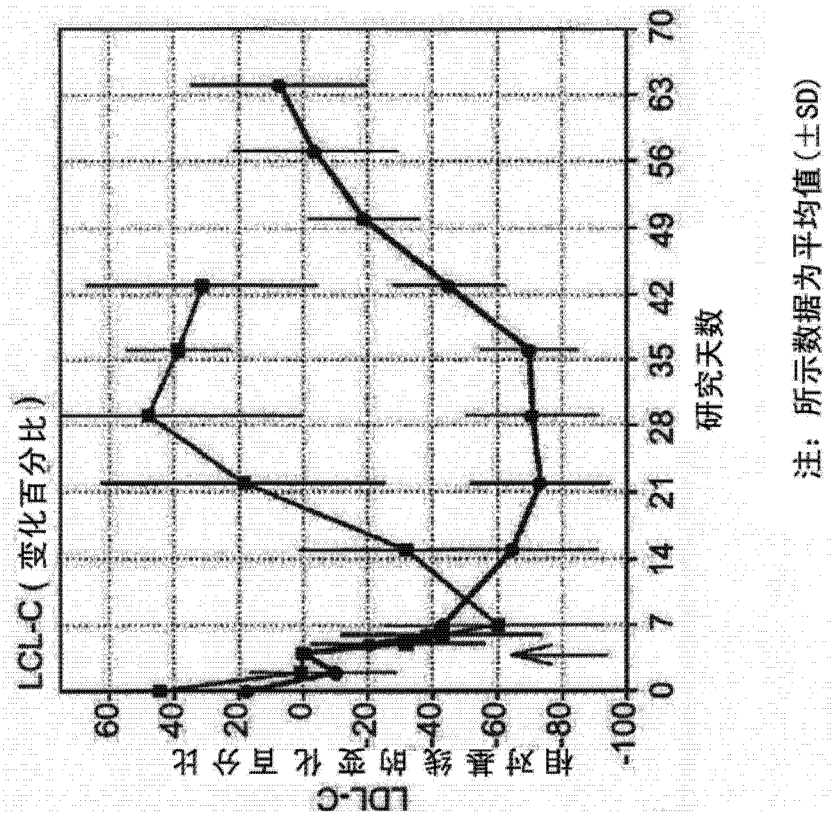
LCL-C（变化百分比）
LDL-C
相对基线的变化百分比
60
40
20
0
-20
-40
-60
-80
-100
0
7
14
21
28
35
42
49
56
63
70
研究天数
注：所示数据为平均值（±SD）

图 7B

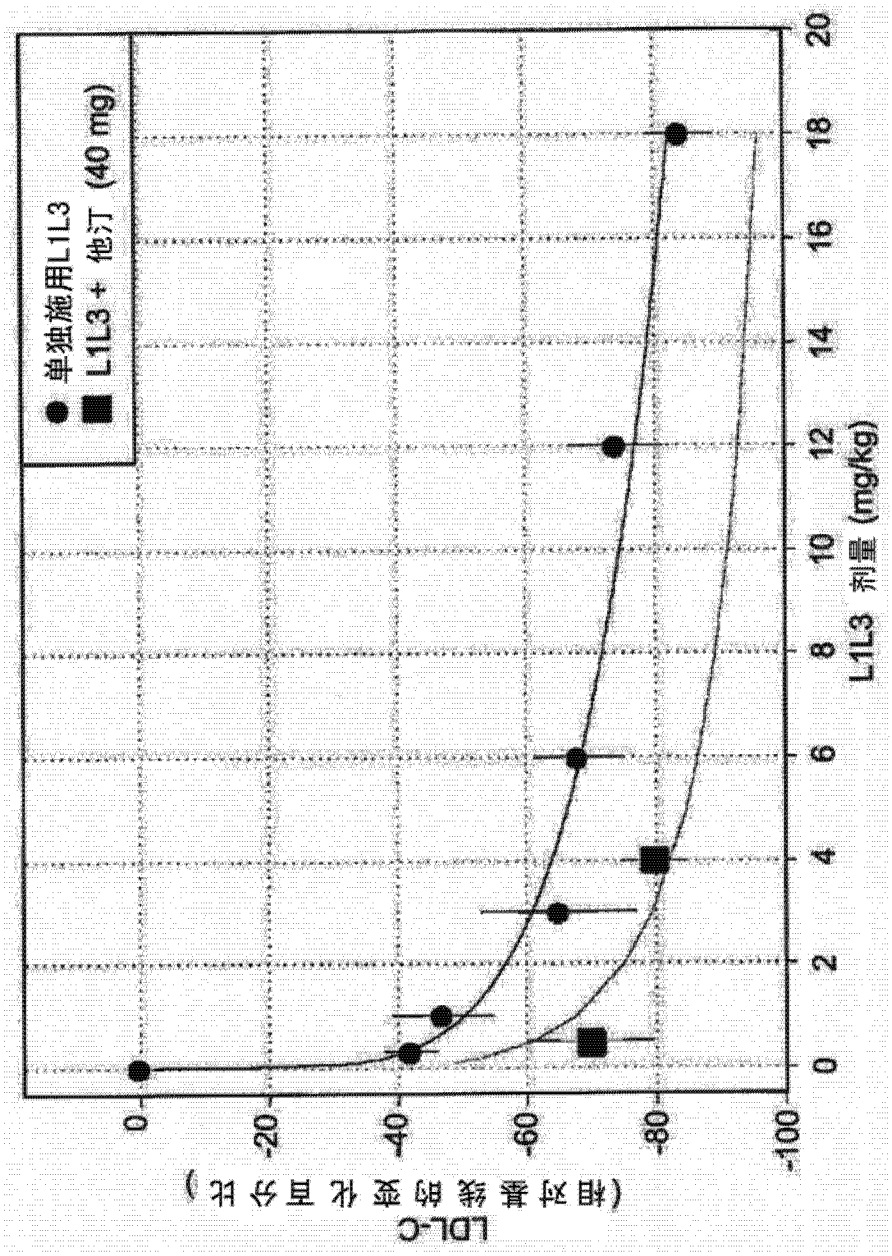
单独施用L1L3
L1L3 + 他汀 (40 mg)
LDL-C
(相对基线的变化百分比)
0
-20
-40
-60
-80
-100
0
2
4
6
8
10
12
14
16
18
20
L1L3 剂量 (mg/kg)

图 8

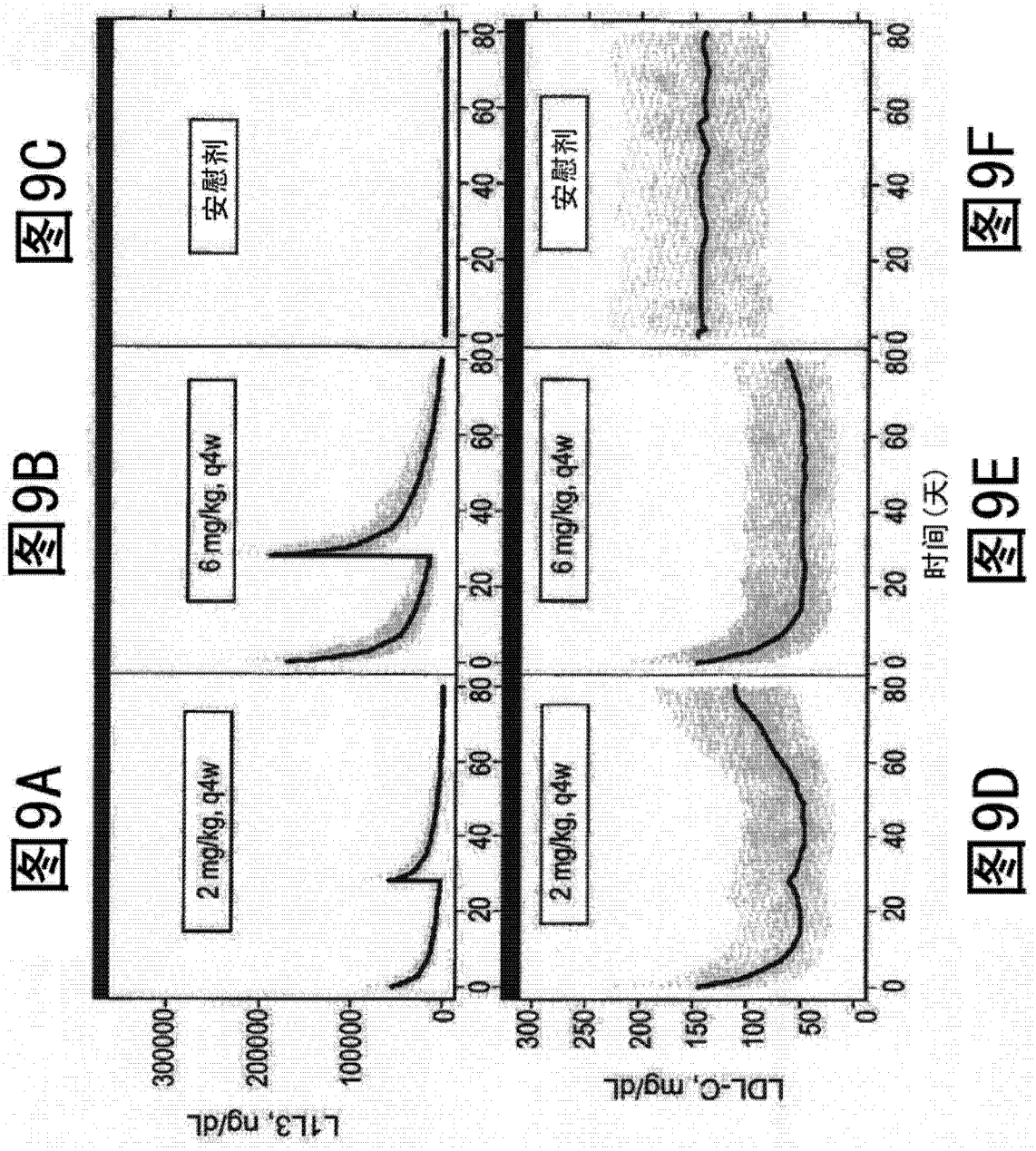
图9A
图9B
图9C
图9D
图9E
图9F
2 mg/kg, q4w
6 mg/kg, q4w
安慰剂
IL13, ng/dL
LDL-C, mg/dL
时间(天)

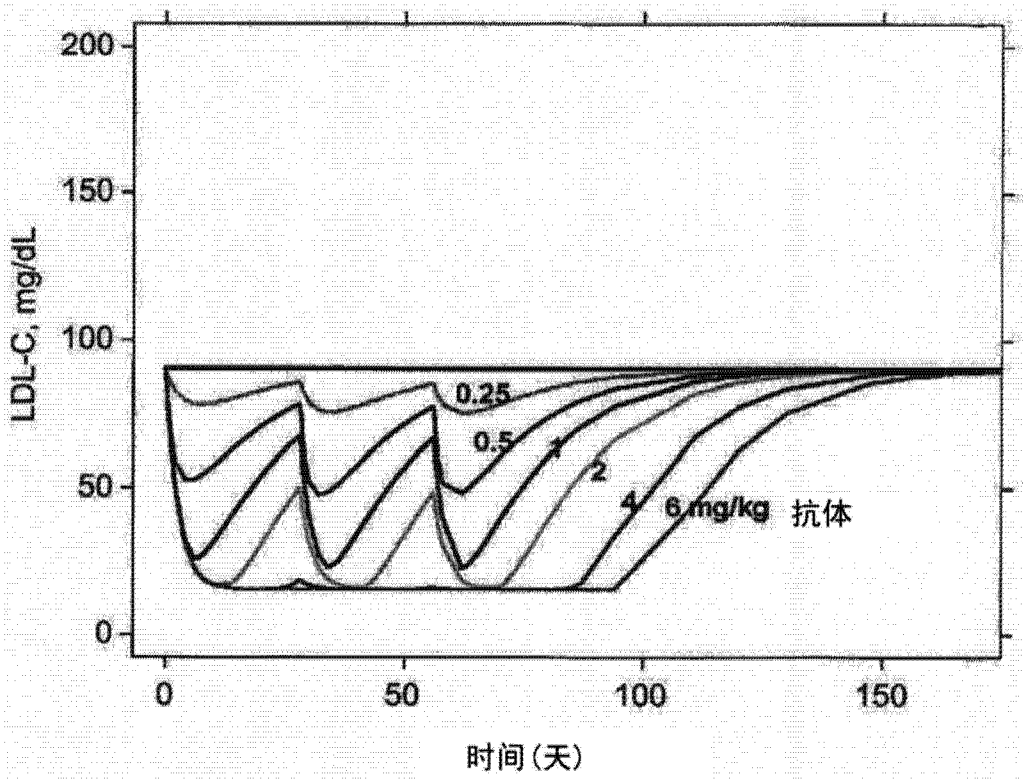

图 10

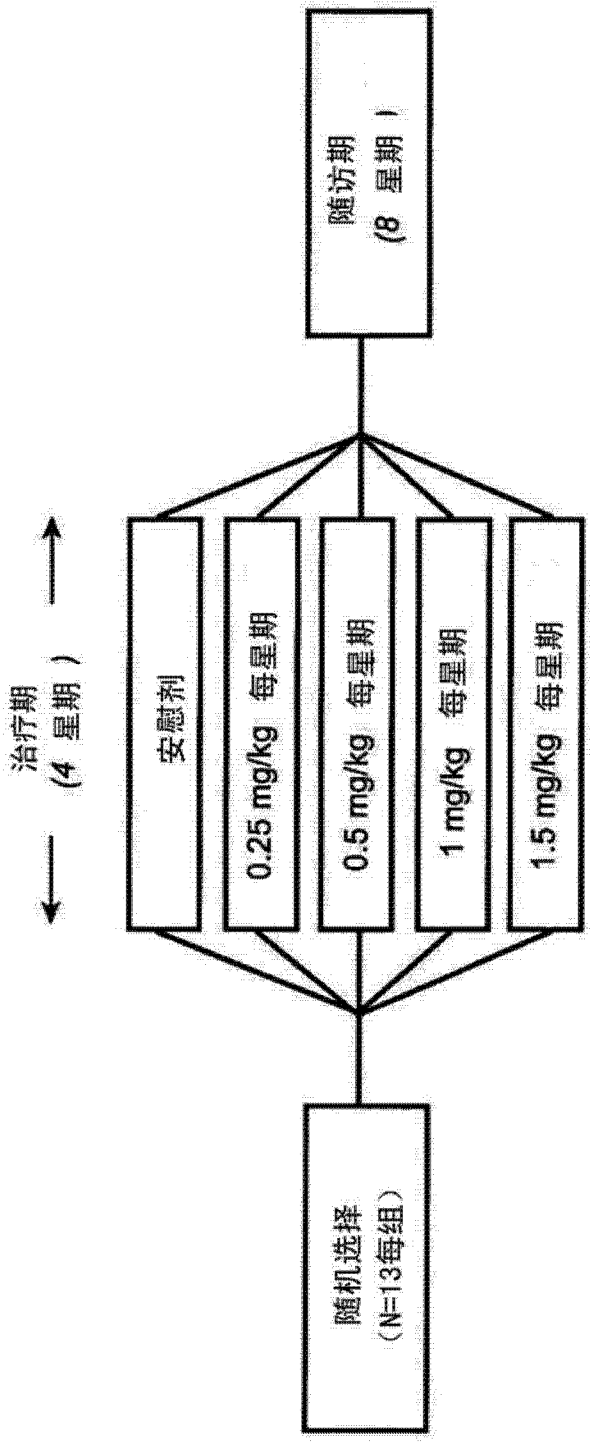
随机选择
（N=13每组）
安慰剂
0.25 mg/kg 每星期
0.5 mg/kg 每星期
1 mg/kg 每星期
1.5 mg/kg 每星期
治疗期
（4 星期）
随访期
（8 星期）

图 11

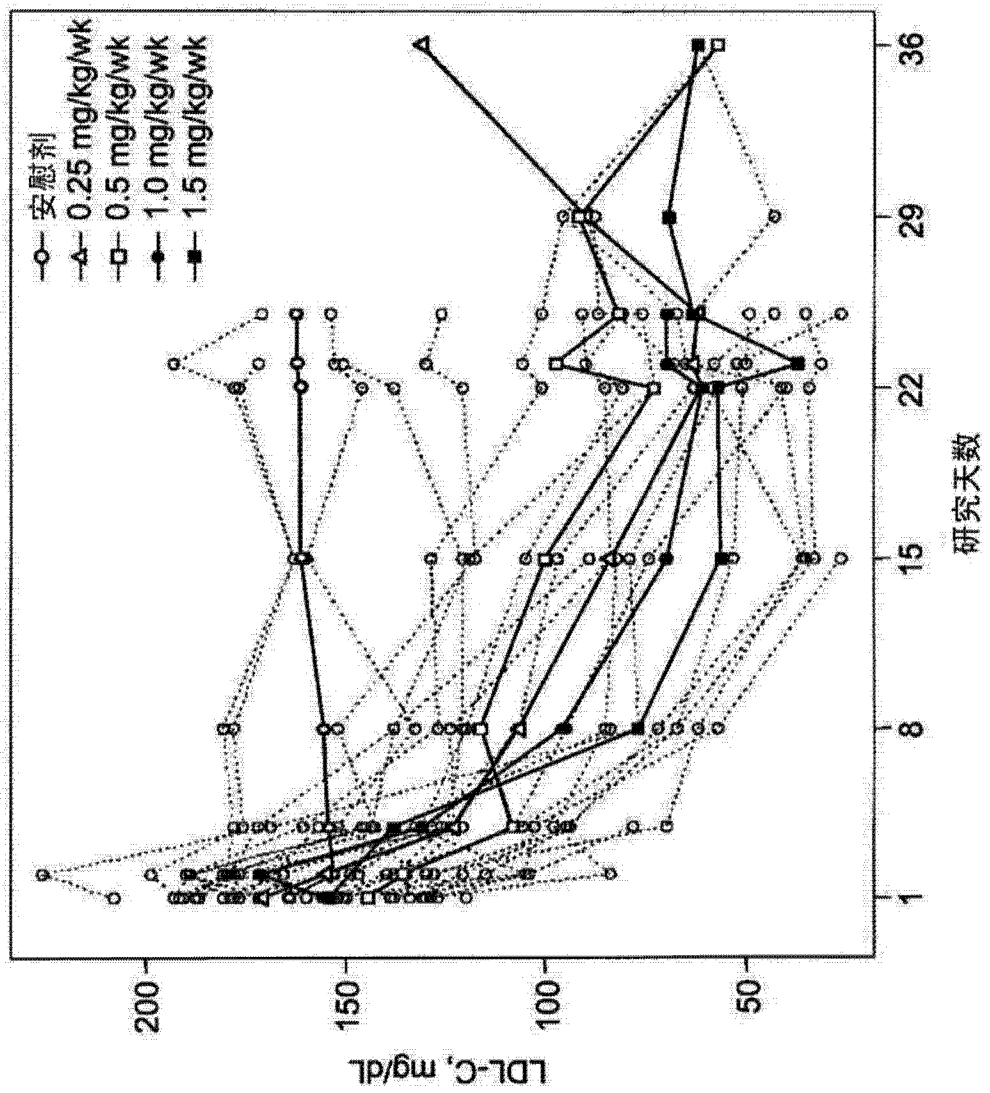
安慰剂
0.25 mg/kg/wk
0.5 mg/kg/wk
1.0 mg/kg/wk
1.5 mg/kg/wk
LDL-C, mg/dL
200
150
100
50
研究天数
1
8
15
22
29
36

图 12

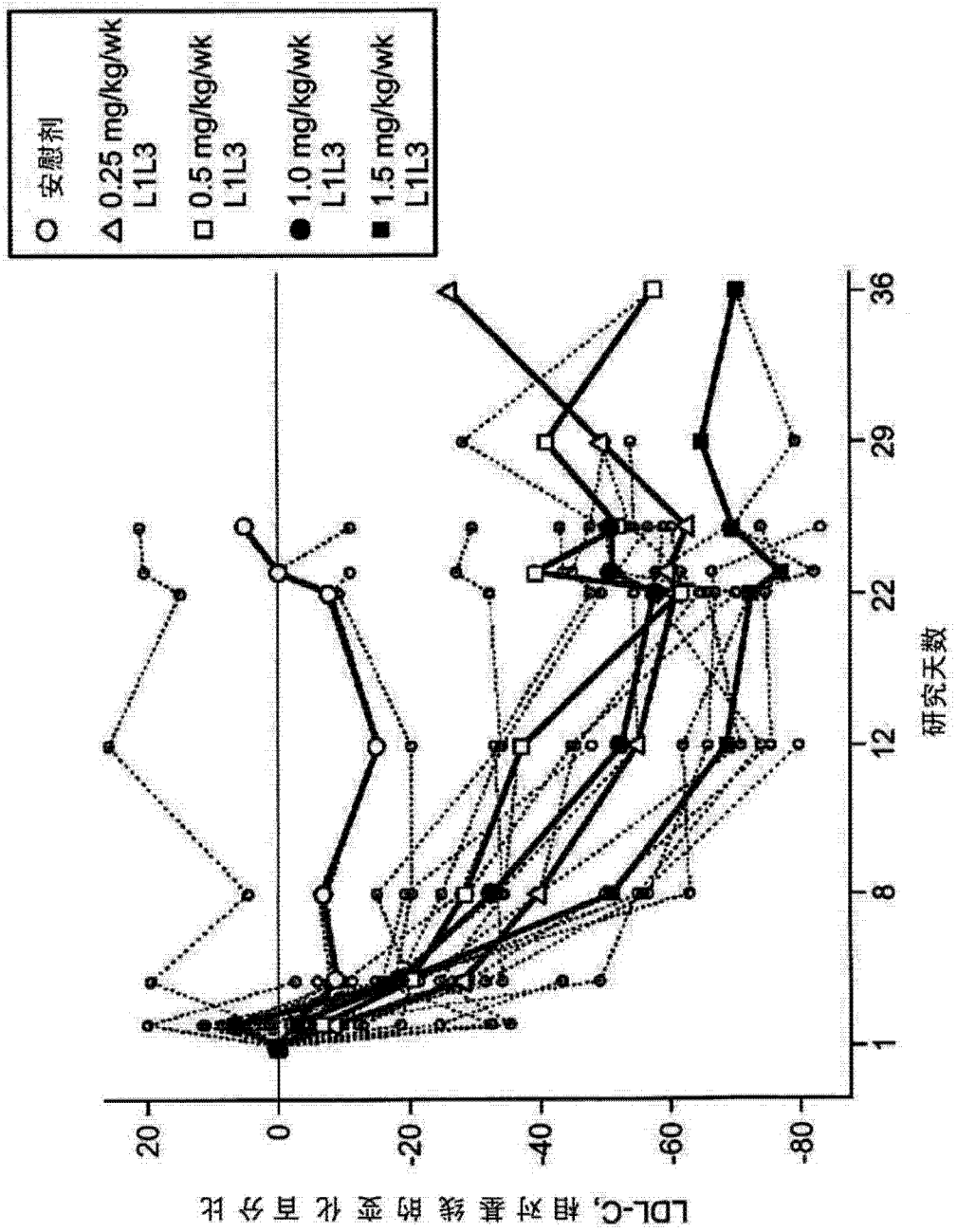
安慰剂
0.25 mg/kg/wk L1L3
0.5 mg/kg/wk L1L3
1.0 mg/kg/wk L1L3
1.5 mg/kg/wk L1L3
LDL-C, 相对基线的变化百分比
20
0
-20
-40
-60
-80
1
8
12
22
29
36
研究天数

图 13

LDL-C平均值(SD) vs 时间的数据

天数	安慰剂			0.25 mg/kg/星期			0.5 mg/kg/星期			1 mg/kg/星期			1.5 mg/kg/星期		
	n	平均值	SD	n	平均值	SD	n	平均值	SD	n	平均值	SD	n	平均值	SD
0	12	0.0	0.0	14	0.0	0.0	14	0.0	0.0	13	0.0	0.0	14	0.0	0.0
1	12	4.3	11.4	14	-1.3	7.1	14	-0.5	6.9	12	1.2	10.0	14	-2.9	15.7
2	12	3.4	11.8	14	-6.8	10.3	14	-5.8	12.5	12	-1.7	13.3	13	-2.3	18.8
4	12	0.0	10.3	14	-27.9	11.5	14	-23.1	12.8	12	-21.2	10.5	13	-20.9	14.7
8	11	-2.5	9.1	14	-38.9	12.2	13	-32.3	10.1	13	-44.3	17.2	13	-40.5	16.8
15	12	-2.7	10.5	14	-51.0	14.3	13	-46.0	16.9	12	-59.5	19.5	14	-57.8	18.1
22	12	-3.4	10.0	13	-54.8	9.3	13	-58.6	13.7	12	-64.7	13.7	14	-62.5	18.9
23	12	-0.7	10.3	13	-53.7	10.0	13	-55.2	13.7	12	-63.5	13.9	14	-62.1	21.1
25	10	-6.6	23.2	12	-60.1	11.7	13	-55.9	13.7	12	-60.1	22.3	14	-63.3	18.3
29	12	-6.8	12.2	13	-53.2	11.3	12	-54.3	14.3	12	-65.8	16.8	13	-63.3	17.0
36	11	-6.6	13.4	13	-23.6	13.7	12	-55.6	11.9	12	-65.7	17.3	12	-62.3	18.7
43	8	-11.9	22.8	8	-12.7	8.1	9	-36.3	8.7	8	-51.5	27.5	9	-69.5	10.1
50	6	13.3	17.4	7	-0.6	12.7	6	-25.4	13.2	6	-34.2	10.6	7	-67.6	9.7
57	6	5.1	17.7	7	-4.8	18.4	5	-18.4	8.9	6	-14.8	28.8	7	-66.6	8.5
64	4	2.3	7.2	5	1.8	8.0	4	-9.7	17.0	2	-17.6	27.0	4	-69.8	10.6
71	4	-5.7	3.2	4	-6.3	9.0	3	-12.2	4.5	2	-18.5	5.7	3	-59.9	18.9
78	2	-1.4	3.7	4	-19.6	23.6	2	-8.0	19.6	3	-23.6	44.6	3	-58.2	33.8

图 14

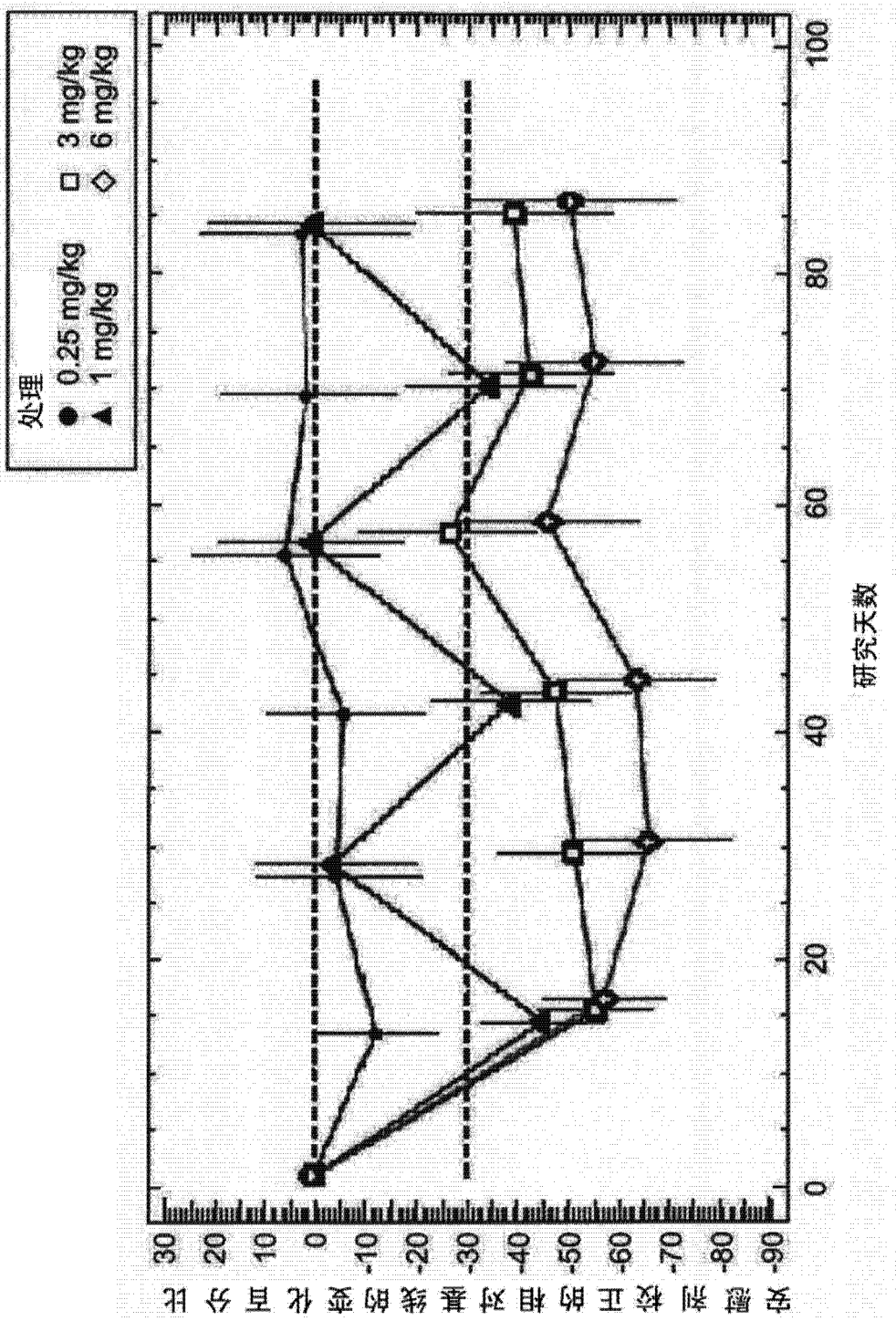
处理
0.25 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
安慰剂校正的相对基线的变化百分比
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
0
20
40
60
80
100
研究天数

图 15

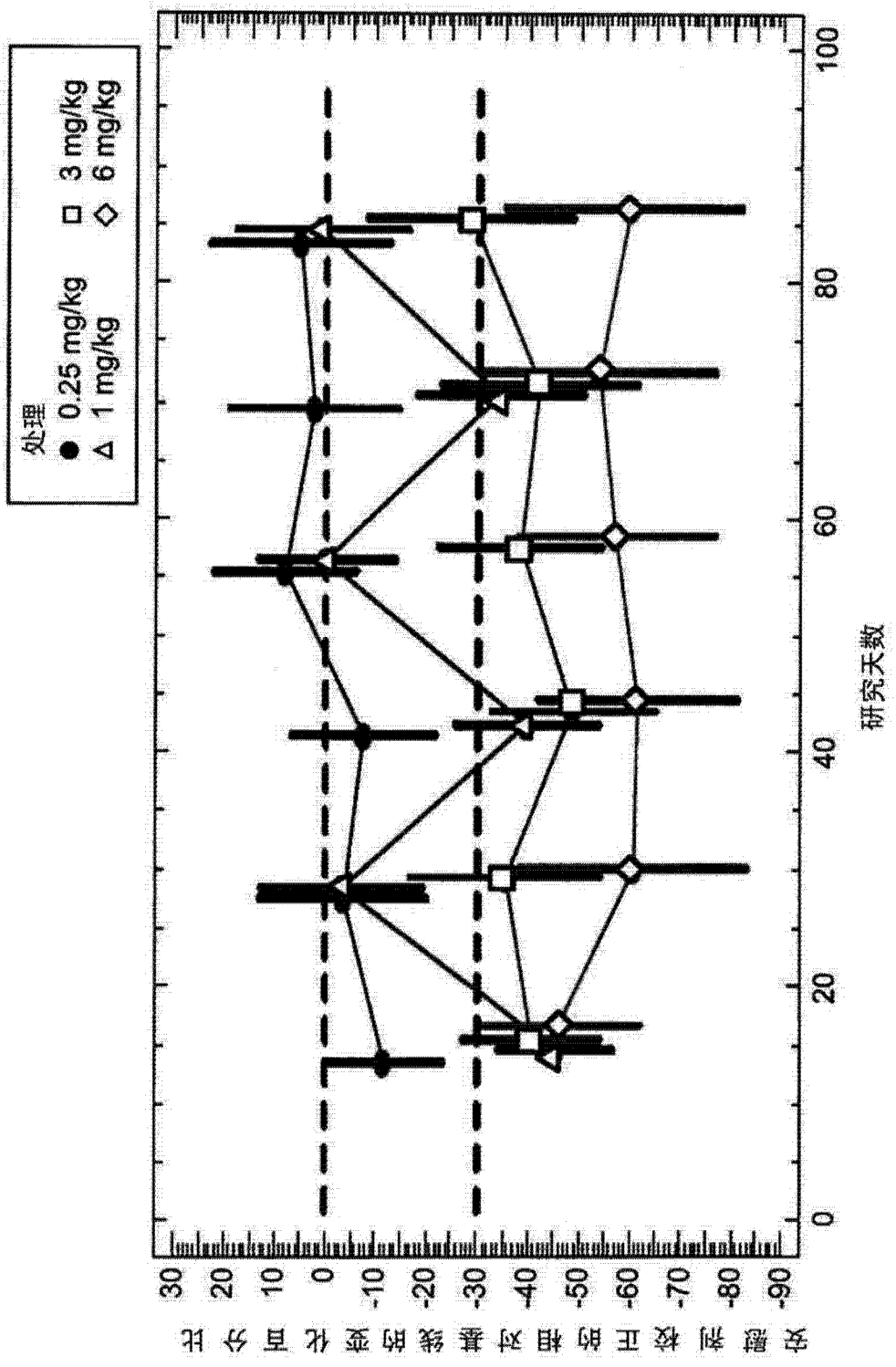
处理
0.25 mg/kg
1 mg/kg
3 mg/kg
6 mg/kg
安慰剂校正的相对基线的变化百分比
研究天数
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
0
20
40
60
80
100

图 16

Abstract

The present invention concerns dosages for the treatment of human patients susceptible to or diagnosed with a disorder characterized by marked elevations of low density lipoprotein particles in the plasma with a PCSK9 antagonist antibody alone or in combination with a statin.