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(54) Title: ANTI-PCSK9 ANTIBODIES, FORMULATIONS, DOSING, AND METHODS OF USE

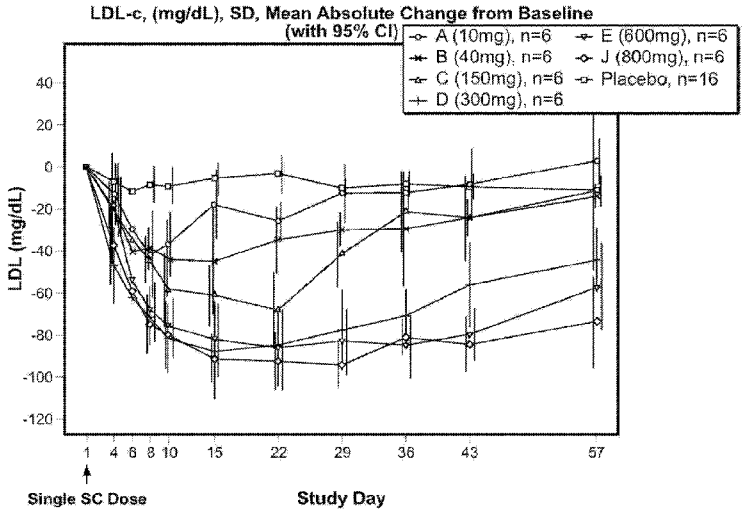

FIGURE 16

(57) Abstract: The invention provides anti-PCSK9 antibodies, formulations, dosing regimens, and methods of using the same.

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ANTI-PCSK9 ANTIBODIES, FORMULATIONS, DOSING, AND METHODS OF USE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application serial nos. 61/660,605, filed June 15, 2012, and 61/786,280, filed March 14, 2013, which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to anti-PCSK9 antibodies, antibody formulations, dosing regimens, and methods of using the same.

BACKGROUND OF THE INVENTION

[0003] Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a member of the mammalian subtilisin family of proprotein convertases. PCSK9 plays a critical role in cholesterol metabolism by controlling the levels of low density lipoprotein (LDL) particles that circulate in the bloodstream. Elevated levels of PCSK9 have been shown to reduce LDL-receptor levels in the liver, resulting in high levels of LDL-cholesterol in the plasma and increased susceptibility to coronary artery disease. (Peterson *et al.*, *J Lipid Res*. 49(7):1595-9 (2008)). Therefore, it would be highly advantageous to produce a therapeutic-based antagonist of PCSK9 that inhibits or antagonizes the activity of PCSK9 and the corresponding role PCSK9 plays in various therapeutic conditions.

SUMMARY OF THE INVENTION

[0004] The invention is in part based on a variety of antibodies to PCSK9. PCSK9 presents as an important and advantageous therapeutic target, and the invention provides antibodies as therapeutic and diagnostic agents for use in targeting pathological conditions associated with expression and/or activity of PCSK9. Accordingly, the invention provides methods, compositions, kits and articles of manufacture related to PCSK9.

[0005] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain

comprising at least one, two, three, four, five or six hypervariable region (HVR) sequences selected from the group consisting of:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IH (SEQ ID NO: 28), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSX$_1$AVA (SEQ ID NO:29), wherein X$_1$ is S or T;

(v) HVR-L2 comprising SASX$_1$LYS (SEQ ID NO:30), wherein X$_1$ is F or S; and

(vi) HVR-L3 comprising QQSYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:31) or QQAYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:37), wherein X$_1$ is P, R or T; X$_2$ is A, I, S or T; X$_3$ is L, P or Q; X$_4$ is A, H, P or S.

[0006] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain comprising the following six HVR sequences:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IH (SEQ ID NO:28), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSX$_1$AVA (SEQ ID NO:29), wherein X$_1$ is S or T;

(v) HVR-L2 comprising SASX$_1$LYS (SEQ ID NO:30), wherein X$_1$ is F or S; and

(vi) HVR-L3 comprising QQSYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:31) or QQAYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:37), wherein X$_1$ is P, R or T; X$_2$ is A, I, S or T; X$_3$ is L, P or Q; X$_4$ is A, H, P or S.

[0007] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain comprising at least one, two, three, four, five or six hypervariable region (HVR) sequences selected from the group consisting of:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$ (SEQ ID NO: 45), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T; X$_5$ is H or N;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSX$_1$AVA (SEQ ID NO:29), wherein X$_1$ is S or T;

(v) HVR-L2 comprising SASX$_1$LYS (SEQ ID NO:30), wherein X$_1$ is F or S; and

(vi) HVR-L3 comprising QQSYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:31) or QQAYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:37), wherein X$_1$ is P, R or T; X$_2$ is A, I, S or T; X$_3$ is L, P or Q; X$_4$ is A, H, P or S.

[0008] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain comprising the following six HVR sequences:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$ (SEQ ID NO: 45), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T; X$_5$ is H or N;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSX$_1$AVA (SEQ ID NO:29), wherein X$_1$ is S or T;

(v) HVR-L2 comprising SASX$_1$LYS (SEQ ID NO:30), wherein X$_1$ is F or S; and

(vi) HVR-L3 comprising QQSYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:31) or QQAYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:37), wherein X$_1$ is P, R or T; X$_2$ is A, I, S or T; X$_3$ is L, P or Q; X$_4$ is A, H, P or S.

[0009] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain comprising at least one, two, three, four, five or six hypervariable region (HVR) sequences selected from the group consisting of:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$ (SEQ ID NO: 45), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T; X$_5$ is H or N;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSTAVA (SEQ ID NO:7);

(v) HVR-L2 comprising SASFLYS (SEQ ID NO:8); and

(vi) HVR-L3 comprising $QQSYX_1X_2X_3X_4T$ (SEQ ID NO:31) or $QQAYX_1X_2X_3X_4T$ (SEQ ID NO:37), wherein X_1 is P, R or T; X_2 is A, I, S or T; X_3 is L, P or Q; X_4 is A, H, P or S.

[0010] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a variable domain comprising the following six HVR sequences:

(i) HVR-H1 comprising $GFTFX_1X_2X_3X_4IX_5$ (SEQ ID NO: 45), wherein X_1 is S or T; X_2 is G, R or S; X_3 is H, T or Y; X_4 is A or T; X_5 is H or N;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSTAVA (SEQ ID NO:7);

(v) HVR-L2 comprising SASFLYS (SEQ ID NO:8); and

(vi) HVR-L3 comprising $QQSYX_1X_2X_3X_4T$ (SEQ ID NO:31) or $QQAYX_1X_2X_3X_4T$ (SEQ ID NO:37), wherein X_1 is P, R or T; X_2 is A, I, S or T; X_3 is L, P or Q; X_4 is A, H, P or S.

[0011] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5. In certain embodiments, the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0012] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33. In certain embodiments, the antibody further comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42, (b) HVR-H2 comprising the amino acid

sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

[0013] In one embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:6;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:26; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:9.

[0014] In one embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:9.

[0015] In one embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:10.

[0016] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:11.

[0017] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:12.

[0018] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:42;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:12.

[0019] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:3;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

[0020] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:3;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:33.

[0021] In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

[0022] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, or SEQ ID NO:43; or (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44.

[0023] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, or SEQ ID NO:43. In certain embodiments, the antibody further comprises a VL sequence of SEQ ID NO: 18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44.

[0024] In one embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:18. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:44. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:19. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:19.

In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:20. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:16 and a VL sequence of SEQ ID NO:21. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:43 and a VL sequence of SEQ ID NO:21. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:22. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:23. In another embodiment, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody comprises a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:34.

[0025] In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody binds to an epitope within a fragment of PCSK9. In certain embodiments, an antibody or an antibody fragment that binds to PCSK9 or a fragment thereof is provided, wherein the antibody binds to an epitope within a fragment of PCSK9 comprising amino acids 376 to 379 of human PCSK9 amino acid sequence of SEQ ID NO:24. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue D238 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue A239 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues D238 and A239 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue E366 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue D367 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues E366 and D367 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue H391 of human PCSK9. In

certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues E366, D367 and H391 of human PCSK9. According to another embodiment, the functional and/or structural epitope of an antibody according to this invention includes residues A239 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of includes one or more of residues A239, A341, E366, D367 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of includes one or more of residues near A239, A341, E366, D367 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention comprises (i) at least one residue selected from the group consisting of R194 and E195, (ii) at least one residue selected from the group consisting of D238 and A239, (iii) at least one residue selected from the group consisting of A341 and Q342, and (iv) at least one residue selected from the group consisting of E366, D367, I369, S376, T377, C378, F379, S381 and H391, of human PCSK9. In certain embodiments, the functional and/or structural epitope comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or all of the following residues: R194, E195, D238, A239, A341, Q342, E366, D367, I369, S376, T377, C378, F379, S381 and H391 of human PCSK9.

[0026] In certain embodiments, the anti-PCSK9 antibody is a monoclonal antibody. In certain embodiments, the anti-PCSK9 antibody is humanized. In certain embodiments, the anti-PCSK9 antibody is a human antibody. In certain embodiments, at least a portion of the framework sequence of the anti-PCSK9 antibody is a human consensus framework sequence. In one embodiment, the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

[0027] In one aspect, a nucleic acid encoding any of the above anti-PCSK9 antibodies is provided. In one embodiment, a vector comprising the nucleic acid is provided. In one embodiment, the vector is an expression vector. In one embodiment, a host cell comprising the vector is provided. In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is mammalian. In yet another embodiment, the host cell is prokaryotic. In one embodiment, a method of making an anti-PCSK9 antibody is provided, wherein the method comprises culturing the host cell under conditions suitable for expression of the nucleic acid encoding the antibody, and isolating the antibody. In certain embodiment, the method further comprises recovering the anti-PCSK9 antibody from the host cell. In

certain embodiments, a composition comprising any of the anti-PCSK9 antibodies described herein is provided. In one embodiment, the composition further comprises a pharmaceutically acceptable carrier.

[0028] In one aspect, provided herein is a pharmaceutical composition comprising an anti-PCSK9 antibody at about 100 to about 225 mg/mL, arginine succinate at about 180 to about 220 mM, polysorbate at about 0.01% to about 0.03%, and pH at about 5.2 to about 6.2. In certain embodiments, the anti-PCSK9 antibody or antibody fragment in the composition is at about 150 mg/mL, arginine succinate in the composition is at about 200 mM, and polysorbate 20 in the composition is about 0.02%, and pH at about 5.5. In certain embodiments, the composition is suitable for subcutaneous administration. In certain embodiments, the viscosity of the composition is less than about 10 cP at 25°C. Any anti-PCSK9 antibodies known in the art or described herein may be formulated into the composition.

[0029] In one aspect, provided herein is a pharmaceutical composition comprising an anti-PCSK9 antibody at about 150 to about 225 mg/mL, histidine acetate at about 10 to about 30 mM, arginine acetate at about 150 to about 170 mM, polysorbate at about 0.01% to about 0.03%, and pH at about 5.8 to about 6.2. In certain embodiments, the anti-PCSK9 antibody or antibody fragment in the composition is at about 200 mg/mL, histidine acetate in the composition is at about 20 mM, arginine acetate in the composition is at about 160 mM, and polysorbate 20 in the composition is about 0.02%, and pH at about 6.0. In certain embodiments, the composition is suitable for subcutaneous administration. In certain embodiments, the viscosity of the composition is less than about 10 cP at 25°C. Any anti-PCSK9 antibodies known in the art or described herein may be formulated into the composition.

[0030] In one aspect, provided herein is a subcutaneous administration device containing an anti-PCSK9 antibody or a composition comprising an anti-PCSK9 antibody described herein. In certain embodiments, the device is for delivering to an individual a flat dose in the range of about 200 to about 1200 mg of the antibody. In certain embodiments, the device is a pre-filled syringe (e.g., 0.5-mL, 1-mL, 1.25-mL, 1.5-mL, 1.75-mL, 2-mL, 2.25-mL, or 2.5-mL syringe). In certain embodiments, the device is a 1-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 1.5-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2-mL pre-filled syringe and the

antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2.25-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2.5-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL.

[0031] In one aspect, the invention concerns methods of inhibiting binding of PCSK9 to LDL-receptor (LDLR) in a subject, said method comprising administering to the subject an effective amount of any of the anti-PCSK9 antibodies described herein. In another aspect, the invention concerns methods of reducing a level of cholesterol in a subject, said method comprising administering to the subject an effective amount of any of the anti-PCSK9 antibodies described herein. In one embodiment, the cholesterol is LDL-cholesterol. In another aspect, the invention concerns methods of reducing a level of LDL-cholesterol in a subject, said method comprising administering to the subject an effective amount of any of the anti-PCSK9 antibodies described herein. In certain embodiments, the invention concerns methods of lowering serum LDL-cholesterol level in a subject, said method comprising administering to the subject an effective amount of any one of the anti-PCSK9 antibodies described herein. In another aspect, the invention concerns methods of treating a condition associated with elevated level of LDL-cholesterol in a subject, said method comprising administering to the subject an effective amount of any one of the anti-PCSK9 antibodies described herein.

[0032] In one aspect, the invention concerns methods of treating a cholesterol related disorder. An exemplary and non-limiting list of cholesterol related disorders contemplated is provided herein under "Compositions and Methods." In certain embodiments, the cholesterol related disorder is hypercholesterolemia. In certain embodiments, the invention concerns methods of treating hypercholesterolemia comprising administering to the subject an effective amount of any one of the anti-PCSK9 antibodies described herein. In certain embodiments, the invention concerns methods of preventing and/or treating atherosclerosis and/or cardiovascular diseases. In certain embodiments, the invention concerns methods of reducing the risk of recurrent cardiovascular events in an individual comprising administering to the individual an amount effective of any one of the anti-PCSK9 antibodies described herein.

[0033] In one aspect, the invention concerns methods for treating any disease or condition which can be improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of PCSK9 activity. In certain embodiments, diseases or disorders that are either

treatable or preventable through the use of statins can also be treated using any one of the anti-PCSK9 antibodies described herein. In certain embodiments, disorders or disease that can benefit from the prevention of cholesterol synthesis or increased LDLR expression can also be treated using any one of the anti-PCSK9 antibodies described herein.

[0034] In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every two weeks, every month, every two months, or every three months. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every two weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every month. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every two months. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every three months.

[0035] In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every two weeks, every month, every two months, or every three months. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every two weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800

mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every month. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every two months. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every three months.

[0036] In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 2 weeks, every 4 weeks, every 6 weeks, every 8 weeks, every 10 weeks, or every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 2 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 4 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 6 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 10 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 220 mg, 380 mg, 400 mg, 600 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg per dose every 12 weeks.

[0037] In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 2 weeks, every 4 weeks, every 6 weeks, every 8 weeks, every 10 weeks, or every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 2 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 4 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 6 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg, 400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 10 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 200-1200 mg, 200-1000 mg, 200-800 mg, 200-600 mg, 200-400 mg, 400-1200 mg, 400-1000 mg,

400-800 mg, 400-600 mg, 600-1200 mg, 600-1000 mg, 600-800 mg, 800-1200 mg, 800-1000 mg, 800-900 mg, 750-850 mg, 750-800 mg, 775-825 mg, 350-450 mg, 375-425 mg, or 375-400 mg per dose every 12 weeks.

[0038] In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 600 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 800 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 800 mg per dose every 10 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 800 mg per dose every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 760 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 760 mg per dose every 10 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 760 mg per dose every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 400 mg per dose every 4 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 400 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 400 mg per dose every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 380 mg per dose every 4 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 380 mg per dose every 8 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 380 mg per dose every 12 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 220 mg per dose every 2 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 220 mg per dose every 4 weeks. In certain embodiments of the methods described herein, the anti-PCSK9 antibody is administered subcutaneously at 220 mg per dose every 8 weeks.

[0039] In certain embodiments of the methods described herein, subjects receiving the anti-PCSK9 antibody are monitored for LDL-c levels and if their levels drop below 25 or 15

mg/dL, then their dose is adjusted down to around 50% or 25% of the initial dose by adjusting the dose and/or frequency of administration. In certain embodiments of the methods described herein, a subject is administered an initial dose of 800 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 25 mg/dL, the dose is adjusted to 200 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 800 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 15 mg/dL, the dose is adjusted to 200 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 760 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 25 mg/dL, the dose is adjusted to 200 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 760 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 15 mg/dL, the dose is adjusted to 200 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 760 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 25 mg/dL, the dose is adjusted to 190 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 760 mg of anti-PCSK9 antibody every 8 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 15 mg/dL, the dose is adjusted to 190 mg of anti-PCSK9 antibody every 8 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 400 mg of anti-PCSK9 antibody every 4 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 25 mg/dL, the dose is adjusted to 100 mg of anti-PCSK9 antibody every 4 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 400 mg of anti-PCSK9 antibody every 4 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 15 mg/dL, the dose is adjusted to 100 mg of anti-PCSK9 antibody every 4 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 380 mg of anti-PCSK9 antibody every 4 weeks, the LDL-c levels of the subject are monitored and

if the subject's LDL-c levels drop below 25 mg/dL, the dose is adjusted to 100 mg of anti-PCSK9 antibody every 4 weeks. In certain embodiments of the methods described herein, a subject is administered an initial dose of 380 mg of anti-PCSK9 antibody every 4 weeks, the LDL-c levels of the subject are monitored and if the subject's LDL-c levels drop below 15 mg/dL, the dose is adjusted to 100 mg of anti-PCSK9 antibody every 4 weeks.

[0040] In certain embodiments, any of the foregoing subcutaneous doses are administered using a subcutaneous administration device. In certain embodiments, the device is a pre-filled syringe (e.g., 0.5-mL, 1-mL, 1.25-mL, 1.5-mL, 1.75-mL, 2-mL, 2.25-mL, or 2.5-mL syringe). In certain embodiments, the device is a 1-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 1.5-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2.25-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, the device is a 2.5-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is about 200 mg/mL. In certain embodiments, more than one syringe may be used to obtain the full flat dose, e.g., one syringe, two syringes, three syringes, or four syringes. In alternative embodiments, a high volume, single use, subcutaneous infusion device may be used to obtainthe full flat dose, e.g., a dose that can administer 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL or 10 mL.

[0041] In certain embodiments, the dose is 800 mg and it is administered every 8 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 800 mg and it is administered every 8 weeks using three 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 800 mg and it is administered every 10 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 800 mg and it is administered every 10 weeks using three 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 800 mg and it is administered every 12 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 800 mg and it is administered every 12 weeks using three 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain

embodiments, the dose is 800 mg and it is administered every 8 weeks using a high volume, single use, subcutaneous infusion device containing 4 mL of an anti-PCSK9 antibody at 200 mg/mL.

[0042] In certain embodiments, the dose is 760 mg and it is administered every 8 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 760 mg and it is administered every 10 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 760 mg and it is administered every 12 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration.

[0043] In certain embodiments, the dose is 600 mg and it is administered every 8 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 600 mg and it is administered every 12 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration.

[0044] In certain embodiments, the dose is 400 mg and it is administered every 4 weeks using one 2.5 mL syringe containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 400 mg and it is administered every 4 weeks using two 2.25 mL syringes containing an anti-PCSK9 antibody at 200 mg/mL concentration. In certain embodiments, the dose is 380 mg and it is administered every 4 weeks using one 2.25 mL syringe containing an anti-PCSK9 antibody at 200 mg/mL concentration.

[0045] In certain embodiments, the methods described herein further comprise administering to the subject an effective amount of a second medicament, wherein the anti-PCSK9 antibody is the first medicament. In one embodiment, the second medicament elevates the level of LDLR protein. In another embodiment, the second medicament reduces the level of LDL-cholesterol. In another embodiment, the second medicament comprises a statin. In another embodiment, the statin is selected from the group consisting of atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and any combination thereof. In another embodiment, the second medicament elevates the level of HDL-cholesterol. In certain embodiments, the subject or the individual is human.

[0046] In one aspect, the invention concerns a method of detecting PCSK9 protein in a sample suspected of containing the PCSK9 protein, the method comprising (a) contacting the sample with the anti-PCSK9 antibody described herein; and (b) detecting formation of a

complex between the anti-PCSK9 antibody and the PCSK9 protein. In one embodiment, the anti-PCSK9 antibody is detectably labeled.

[0047] Any embodiment described herein or any combination thereof applies to any and all anti-PCSK9 antibodies, methods and uses of the invention described herein.

BRIEF DESCRIPTION OF THE FIGURES

[0048] **FIGURE 1** shows heavy chain HVR sequences, H1 (SEQ ID NOS 1, 1, 1, 1, 1, 2, 42, 3, 3, 1, respectively, in order of appearance), H2 (all disclosed as SEQ ID NO: 4), and H3 (all disclosed as SEQ ID NO: 5), and light chain HVR sequences, L1 (SEQ ID NOS: 6, 7, 7, 7, 7, 7, 7, 7 and 7, respectively, in order of appearance), L2 (SEQ ID NOS: 26, 8, 8, 8, 8, 8, 8, 8, 8 and 8, respectively, in order of appearance) and L3 (SEQ ID NOS: 9, 9, 10, 10, 11, 12, 12, 13, 33 and 14, respectively, in order of appearance), of anti-PCSK9 antibodies.

[0049] **FIGURE 2A-B** show the amino acid sequences of (A) the heavy chain variable domains (SEQ ID NOS: 15, 27, 15, 27, 27, 16, 43, 17, 17 and 27, respectively, in order of appearance) and (B) light chain variable domains (SEQ ID NOS: 18, 44, 19, 19, 20, 21, 21, 22, 34 and 23, respectively, in order of appearance) of anti-PCSK9 antibodies. Positions are numbered according to Kabat and hypervariable regions are boxed.

[0050] **FIGURE 3A-D** show dissociation constants of the anti-PCSK9 antibodies (IgG) against (A) human PCSK9, (B) murine PCSK9, (C) cyno PCSK9 and rat PCSK9, and (D) rhesus PCSK9.

[0051] **FIGURE 4.** Anti-PCSK9 antibodies inhibit binding of PCSK9 to LDLR in a competition binding ELISA. Blank (no antibody; open square) and control antibody (open circle) are shown in dashed lines. Anti-PCSK9 antibodies are shown in solid lines. IC_{50} values of anti-PCSK9 antibodies are shown in the table.

[0052] **FIGURE 5.** Different concentrations of anti-PCSK9 antibodies were incubated with 15 μg/ml PCSK9 and added to HepG2 cells for 4 hours. Cells were processed for FACS analysis of surface LDLR. The data indicate that the anti-PCSK9 antibodies effectively prevented LDLR downregulation. The positive control is cells not treated with PCSK9.

[0053] **FIGURE 6.** Western blot with anti-LDLR antibody showing that 30 μg of PCSK9 for 1 hr significantly downregulated LDLR levels in mouse liver.

[0054] **FIGURE 7.** Western blot with anti-LDLR antibody showing that all five anti-PCSK9 antibodies prevented LDLR downregulation in mouse liver. The bottom immunoblot is a pool of 4 livers (10 μg of protein from each liver) per treatment group.

[0055] **FIGURE 8** shows anti-PCSK9 antibody concentrations in sera of C57JBL/6 mice after single I.V. injection. Shown are the average concentrations of the dosing groups 0.5 mg/kg; 5 mg/kg; and 20 mg/kg (n=3).

[0056] **FIGURE 9** shows comparison of anti-PCSK9 antibody concentrations in sera of C57JBL/6 WT and $PCSK9^{-/-}$ mice after single I.V. injection of 5 mg/kg anti-PCSK9 antibody. The average concentrations of each dosing group are shown (n=3).

[0057] **FIGURE 10** shows anti-PCSK9 antibody concentrations in sera of individual cynomolgus monkey after single I.V. injection. Three dosing groups are included: 5 mg/kg; 20 mg/kg; and 60 mg/kg.

[0058] **FIGURE 11** shows anti-PCSK9 antibody concentrations in sera of cynomolgus monkeys after single I.V. injection. Shown are the average concentrations of the dosing groups 5 mg/kg, 20 mg/kg, and 60 mg/kg (n=3).

[0059] **FIGURE 12** shows total cholesterol level in the sera of mice treated with a single dose (10mg/kg body weight) of either control (Crtl) or anti-PCSK9 antibody. Cholesterol levels were measured at different days as indicated in the figure.

[0060] **FIGURE 13** shows total cholesterol level in the sera from the mice treated with single dose (10mg/kg body weight) of either control or anti-PCSK9 antibody.

[0061] **FIGURE 14** shows a schematic of the Phase I trial design including cohorts A-J. Each cohort included six patients treated with the active agent and two patients treated with placebo, for a total of 8 patients per cohort and 80 total patients.

[0062] **FIGURE 15** shows pharmacokinetic data for study cohorts A-J. Results from the single dose cohorts A-E and J are shown in the left panel and results from the multiple dose cohorts F-I are shown in the right panel. Red arrows indicate timing of drug administration.

[0063] **FIGURE 16** shows mean absolute change from baseline in LDL-c (mg/dL) levels for the single dose cohorts.

[0064] **FIGURE 17** shows mean percent change in baseline in LDL-c levels for the single dose cohorts.

[0065] **FIGURE 18** shows mean absolute change from baseline in LDL-c (mg/dL) levels for the multiple dose cohorts.

[0066] **FIGURE 19** shows mean percent change in baseline in LDL-c levels for the multiple dose cohorts.

[0067] **FIGURE 20** shows the viscosity of anti-PCSK9 as a function of protein concentration in a formulation of 200 mM arginine succinate, 0.02% PS20, pH 5.5.

[0068] **FIGURE 21** shows size exclusion chromatography (SEC) (left panel) and turbidity (right panel) analyses for control and agitated anti-PCSK9 samples containing various concentrations of Polysorbate 20 (PS20) in 2cc glass vials.

[0069] **FIGURE 22** shows oxidation of methionine and tryptophan residues in anti-PCSK9 under various conditions by peptide mapping.

[0070] **FIGURE 23** shows oxidation of methionine and tryptophan residues in and adjacent to CDRs of anti-PCSK9 under various conditions by peptide mapping.

[0071] **FIGURE 24** shows ion exchange chromatography (IEC) (left panel) and SEC (right panel) pH rate profiles for 200 mg/mL anti-PCSK9 from pH 5.0 to 6.5 (200 mM arginine succinate, 0.02% PS20 at pH 5.0-6.0 or 20 mM histidine HCL, 160 mM arginine HCl, 0.02% PS20 at pH 6.5).

[0072] **FIGURE 25** shows percent main peak (left panel) and percent high molecular weight species (HMWS) (right panel) data by SEC for anti-PCSK9 during frozen storage in HCl (200 mg/mL anti-PCSK9 in 20 mM histidine HCl, 160 mM arginine HCl, 0.02% PS20, pH 6.0) and Acetate (200 mg/mL anti-PCSK9 in 20 mM histidine acetate, 160 mM arginine acetate, 0.02% PS20, pH 6.0) formulations.

[0073] **FIGURE 26** shows counter-ion effects on 200 mg/mL anti-PCSK9 at pH 6.0 by CE-SDS (top), SEC (middle), and IEC (bottom) after 1 month at 40°C storage.

[0074] **FIGURE 27** shows the study design of a phase II clinical trial, including an overview of study dose cohorts, anti-PCSK9 antibody dose regimen, and number of patients in each arm of the trial.

[0075] **FIGURE 28** shows mean pharmacokinetics (+/- standard deviation) (left panel) and mean total PCSK9 (+/- standard error) (right panel) in patients receiving anti-PCSK9 antibody or placebo.

[0076] **FIGURE 29** shows the absolute change from baseline in direct LDL cholesterol observed in patients receiving anti-PCSK9 antibody or placebo.

[0077] **FIGURE 30** shows the relative change from baseline in direct LDL cholesterol observed in patients receiving anti-PCSK9 antibody or placebo.

[0078] **FIGURE 31** shows the absolute change from baseline in total cholesterol observed in patients receiving anti-PCSK9 antibody or placebo.

[0079] **FIGURE 32** shows the relative change from baseline in total cholesterol observed in patients receiving anti-PCSK9 antibody or placebo.

[0080] **FIGURE 33** shows the absolute change from baseline in non-HDL cholesterol in patients receiving anti-PCSK9 antibody or placebo.

[0081] **FIGURE 34** shows the relative change from baseline in non-HDL cholesterol in patients receiving anti-PCSK9 antibody or placebo.

[0082] **FIGURE 35** shows the absolute change from baseline in apolipoprotein B in patients receiving anti-PCSK9 antibody or placebo.

[0083] **FIGURE 36** shows the relative change from baseline in apolipoprotein B in patients receiving anti-PCSK9 antibody or placebo.

[0084] **FIGURE 37A** shows the proportion of patients with direct LDL-c values less than or equal to 15 mg/dL for at least one visit after receiving anti-PCSK9 antibody or placebo, and **FIGURE 37B** shows the results of experiments performed to determine the proportion of patients with direct LDL-c values less than or equal to 25 mg/dL for at least one visit after receiving anti-PCSK9 antibody or placebo.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[0085] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)); *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., 1993-8) J. Wiley and Sons; *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); *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); and *Cancer: Principles and Practice of Oncology* (V. T. DeVita et al., eds., J.B. Lippincott Company, 1993).

I. DEFINITIONS

[0086] Unless defined otherwise, 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. Singleton *et al.*, Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application. All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

[0087] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0088] Throughout the present specification and claims, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), expressly incorporated herein by reference. The "EU index as in Kabat" refers to the residue numbering of the human IgG_1 EU antibody.

[0089] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0090] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0091] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0092] The terms "anti-PCSK9 antibody", "anti-PCSK9", "PCSK9 antibody" or "an antibody that binds to PCSK9" refers to an antibody that is capable of binding PCSK9 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PCSK9. In one embodiment, the extent of binding of an anti-PCSK9 antibody to an unrelated, non-PCSK9 protein is less than about 10% of the binding of the antibody to PCSK9 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to PCSK9 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-PCSK9 antibody binds to an epitope of PCSK9 that is conserved among PCSK9 from different species.

[0093] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0094] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0095] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein. In certain embodiments, the epitope is determined based on the crystal structure of the anti-PCSK9 antibody Fab fragment bound to PCSK9.

[0096] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0097] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0098] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin,

melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0099] The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0100] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

[0101] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0102] The "Fab" fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0103] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term

includes native sequence Fc regions and variant Fc regions. In certain embodiments, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0104] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0105] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0106] "Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0107] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same

function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0108] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0109] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

[0110] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0111] The term "hypercholesterolemia," as used herein, refers to a condition in which cholesterol levels are elevated above a desired level. In certain embodiments, the LDL-cholesterol level is elevated above the desired level. In certain embodiments, the serum LDL-cholesterol levels are elevated above the desired level.

[0112] The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs

generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (*See* Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (*e.g.*, FR residues) are numbered herein according to Kabat et al., *supra*.

[0113] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0114] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0115] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

[0116] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic

acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0117] "Isolated nucleic acid encoding an anti-PCSK9 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0118] The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0119] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0120] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable

domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0121] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0122] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0123] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0124] The term "pharmaceutical formulation" or "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0125] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0126] The term "proprotein convertase subtilisin kexin type 9," "PCSK9," or "NARC-1," as used herein, refers to any native PCSK9 from any vertebrate source, including mammals such as primates (*e.g.* humans) and rodents (*e.g.,* mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed PCSK9 as well as any form of PCSK9 that results from processing in the cell or any fragment thereof. The term also encompasses naturally occurring variants of PCSK9, *e.g.*, splice variants or allelic variants.

[0127] The term "PCSK9 activity" or "biological activity" of PCSK9, as used herein, includes any biological effect of PCSK9. In certain embodiments, PCSK9 activity includes the ability of PCSK9 to interact or bind to a substrate or receptor. In certain embodiments, the biological activity of PCSK9 is the ability of PCSK9 to bind to a LDL-receptor (LDLR). In certain embodiments, PCSK9 binds to and catalyzes a reaction involving LDLR. In certain embodiments, PCSK9 activity includes the ability of PCSK9 to decrease or reduce the availability of LDLR. In certain embodiments, the biological activity of PCSK9 includes the ability of PCSK9 to increase the amount of LDL in a subject. In certain embodiments, the biological activity of PCSK9 includes the ability of PCSK9 to decrease the amount of LDLR

that is available to bind to LDL in a subject. In certain embodiments, the biological activity of PCSK9 includes the ability of PCSK9 to decrease the amount of LDLR that is available to bind to LDL. In certain embodiments, biological activity of PCSK9 includes any biological activity resulting from PCSK9 signaling.

[0128] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0129] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0130] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See, e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0131] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host

cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0132] As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

[0133] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0134] It is understood that aspect and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

II. COMPOSITIONS AND METHODS

[0135] In one aspect, the invention is based, in part, on experimental and clinical results obtained with anti-PCSK9 antibodies. Results obtained indicate that blocking biological activity of PCSK9 with anti-PCSK9 antibodies leads to a prevention of reduction in LDLR. In addition, the results demonstrate that administration of anti-PCSK9 antibody reduces total LDL-cholesterol level in a subject. Accordingly, PCSK9 antibodies of the invention, as described herein, provide important therapeutic and diagnostic agents for use in targeting pathological conditions associated with PCSK9, *e.g.*, cholesterol related disorders.

[0136] In certain embodiments, a "cholesterol related disorder" includes any one or more of the following: hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimers disease and generally dyslipidemias, which can be manifested, for example, by an elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL. Some non-limiting examples of primary and secondary dyslipidemias that can be treated using an anti-PCSK9 antibody, either alone, or in combination with one or more other agents include the metabolic syndrome, diabetes mellitus, familial combined hyperlipidemia, familial hypertriglyceridemia, familial hypercholesterolemias, including heterozygous hypercholesterolemia, homozygous hypercholesterolemia, familial defective apoplipoprotein B-100; polygenic hypercholesterolemia; remnant removal disease, hepatic lipase deficiency; dyslipidemia secondary to any of the following: dietary indiscretion, hypothyroidism, drugs

including estrogen and progestin therapy, beta-blockers, and thiazide diuretics; nephrotic syndrome, chronic renal failure, Cushing's syndrome, primary biliary cirrhosis, glycogen storage diseases, hepatoma, cholestasis, acromegaly, insulinoma, isolated growth hormone deficiency, and alcohol-induced hypertriglyceridemia. Anti-PCSK9 antibodies described herein can also be useful in preventing or treating atherosclerotic diseases, such as, for example, coronary heart disease, coronary artery disease, peripheral arterial disease, stroke (ischaemic and hemorrhagic), angina pectoris, or cerebrovascular disease and acute coronary syndrome, myocardial infarction. In certain embodiments, the anti-PCSK9 antibodies described herein are useful in reducing the risk of: nonfatal heart attacks, fatal and non-fatal strokes, certain types of heart surgery, hospitalization for heart failure, chest pain in patients with heart disease, and/or cardiovascular events because of established heart disease such as prior heart attack, prior heart surgery, and/or chest pain with evidence of clogged arteries. In certain embodiments, the anti-PCSK9 antibodies and methods described herein can be used to reduce the risk of recurrent cardiovascular events.

A. Exemplary Anti-PCSK9 Antibodies

[0137] In one aspect, the invention provides isolated antibodies that bind to PCSK9. In certain embodiments, an anti-PCSK9 antibody modulates PCSK9 activity.

[0138] In one aspect, the invention provides an anti-PCSK9 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0139] In one aspect, the invention provides an anti-PCSK9 antibody comprising six HVRs comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (e) HVR-L2

comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 or SEQ ID NO:33.

[0140] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:5. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:5 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 or SEQ ID NO:33. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:5, HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:4. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

[0141] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0142] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or

SEQ ID NO:42, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:5; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0143] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:26; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:9. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:9. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:10. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:11. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2

comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:12. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:42; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:12. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:13. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:14. In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:33.

[0144] In certain embodiments, the anti-PCSK9 antibody is humanized. In one embodiment, an anti-PCSK9 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[0145] In another aspect, an anti-PCSK9 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, or SEQ ID NO:43. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains

substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PCSK9 antibody comprising that sequence retains the ability to bind to PCSK9. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:15, SEQ ID NO:16, SEQ NO:17, SEQ ID NO:27, or SEQ ID NO:43. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PCSK9 antibody comprises the VH sequence in SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, or SEQ ID NO:43, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

[0146] In another aspect, an anti-PCSK9 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PCSK9 antibody comprising that sequence retains the ability to bind to PCSK9. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PCSK9 antibody comprises the VL sequence in SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0147] In another aspect, an anti-PCSK9 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:15 and SEQ ID NO:18, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:27 and SEQ ID NO:44, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:15 and SEQ ID NO:19, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:27 and SEQ ID NO:19, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:27 and SEQ ID NO:20, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:16 and SEQ ID NO:21, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:43 and SEQ ID NO:21, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:17 and SEQ ID NO:22, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:27 and SEQ ID NO:23, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:17 and SEQ ID NO:34, respectively, including post-translational modifications of those sequences.

[0148] In another aspect, an anti-PCSK9 antibody comprises a heavy chain sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:35. In certain embodiments, a heavy chain sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PCSK9 antibody comprising that sequence retains the ability to bind to PCSK9. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:35. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

Optionally, the anti-PCSK9 antibody heavy chain comprises the VH sequence in SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, or SEQ ID NO:43, including post-translational modifications of that sequence. In a particular embodiment, the heavy chain comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:42, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

[0149] In another aspect, an anti-PCSK9 antibody is provided, wherein the antibody comprises a light chain having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:36. In certain embodiments, a light chain sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PCSK9 antibody comprising that sequence retains the ability to bind to PCSK9. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:36. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PCSK9 antibody light chain comprises the VL sequence in SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, or SEQ ID NO:44, including post-translational modifications of that sequence. In a particular embodiment, the light chain comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

[0150] In another aspect, an anti-PCSK9 antibody is provided, wherein the antibody comprises a heavy chain as in any of the embodiments provided above, and a light chain as in any of the embodiments provided above. In one embodiment, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:35, and a light chain comprising the amino acid sequence of SEQ ID NO:36. In certain embodiments, SEQ ID NO:35 is truncated by one or two amino acids at the C-terminus, e.g., it does not contain K451, or G450 and K451. In certain embodiments, P449 in SEQ ID NO:35 is amidated.

Antibody 508.20.33b heavy chain amino acid sequence (SEQ ID NO:35):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSTAIHWVRQAPGKGLEWVARISPANGN
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARWIGSRELYIMDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Antibody 508.20.33b light chain amino acid sequence (SEQ ID NO:36):

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAYPALHTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0151] In certain embodiments, SEQ ID NO:35 is truncated by one or two amino acids at the C-terminus, e.g., it does not contain K451, or G450 and K451 (e.g., the heavy chain comprises amino acids 1-449 of SEQ ID NO:35 or amino acids 1-450 of SEQ ID NO:35). In certain embodiments, P449 in SEQ ID NO:35 is amidated.

[0152] In certain embodiments, functional epitopes can be mapped by combinatorial alanine scanning. In this process, a combinatorial alanine-scanning strategy can be used to identify amino acids in the PCSK9 protein that are necessary for interaction with anti-PCSK9 antibodies. In certain embodiments, the epitope is conformational and crystal structure of anti-PCSK9 antibody Fab fragment bound to PCSK9 may be employed to identify the epitopes. In one aspect, the invention provides an antibody that binds to the same epitope as any of the anti-PCSK9 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:19. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:19. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:20. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:16 and a VL sequence of SEQ ID NO:21. In certain embodiments, an antibody is provided that binds to the same

epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:43 and a VL sequence of SEQ ID NO:21. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:22. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:23. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:34.

[0153] In one aspect, the invention provides an anti-PCSK9 antibody, or antigen binding fragment thereof, that binds to human PCSK9 competitively with any one of the antibodies described herein. In certain embodiments, competitive binding may be determined using an ELISA assay. For example, in certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:19. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:19. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:20. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:16 and a VL sequence of SEQ ID NO:21. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:43 and a VL sequence of SEQ ID NO:21. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:22. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:23. In certain embodiments, an antibody is provided that binds to PCSK9 competitively with an anti-PCSK9 antibody comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:34.

[0154] In certain embodiments, an antibody is provided that binds to an epitope within a fragment of PCSK9 as described herein. In certain embodiments, an antibody is provided that

binds to an epitope within a fragment of PCSK9 comprising amino acids 376 to 379 of human PCSK9 amino acid sequence of SEQ ID NO:24. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue D238 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue A239 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues D238 and A239 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue E366 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue D367 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues E366 and D367 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residue H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention includes residues E366, D367 and H391 of human PCSK9. According to another embodiment, the functional and/or structural epitope of an antibody according to this invention includes residues A239 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of includes one or more of residues A239, A341, E366, D367 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of includes one or more of residues near A239, A341, E366, D367 and H391 of human PCSK9. In certain embodiments, the functional and/or structural epitope of an antibody according to this invention comprises (i) at least one residue selected from the group consisting of R194 and E195, (ii) at least one residue selected from the group consisting of D238 and A239, (iii) at least one residue selected from the group consisting of A341 and Q342, and (iv) at least one residue selected from the group consisting of E366, D367, I369, S376, T377, C378, F379, S381 and H391, of human PCSK9. In certain embodiments, the functional and/or structural epitope comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or all of the following residues: R194, E195, D238, A239, A341, Q342, E366, D367, I369, S376, T377, C378, F379, S381 and H391 of human PCSK9.

[0155] In a further aspect of the invention, an anti-PCSK9 antibody according to any of the above embodiment is a monoclonal antibody, including a chimeric, humanized or human

antibody. In one embodiment, an anti-PCSK9 antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, *e.g.*, an intact IgG_1 antibody or other antibody class or isotype as defined herein.

[0156] In a further aspect, an anti-PCSK9 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

[0157] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0158] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0159] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0160] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments

comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0161] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0162] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516 B1).

[0163] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0164] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0165] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the

antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0166] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0167] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[0168] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0169] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci

have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). *See also, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0170] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0171] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0172] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature*

348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0173] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0174] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0175] In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.* a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for PCSK9 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of PCSK9. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PCSK9. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0176] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see,e.g.*, Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991).

[0177] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.*, US 2006/0025576A1).

[0178] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to PCSK9 as well as another, different antigen (*see, e.g.*, US 2008/0069820).

7. Antibody Variants

[0179] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0180] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs

and FRs. Conservative substitutions are shown in Table A under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0181] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0182] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0183] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.,* improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0184] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0185] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0186] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0187] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0188] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0189] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0190] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.,* between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech.*

Bioeng. 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0191] Antibody variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0192] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0193] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.*

166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0194] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0195] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0196] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0197] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0198] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et

al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0199] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[0200] In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

[0201] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-

polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.*

[0202] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[0203] Anti-PCSK9 antibodies described herein may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-PCSK9 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 heavy chain variable region is provided wherein the nucleic acid comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of SEQ ID NO:38 or SEQ ID NO:39. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 light chain variable region is provided wherein the nucleic acid comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of SEQ ID NO:40 or SEQ ID NO:41. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 heavy chain variable region and an anti-PCSK9 light chain

variable region is provided, wherein the nucleic acid encoding the heavy chain variable region comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of SEQ ID NO:38 or SEQ ID NO:39 and the nucleic acid encoding the light chain variable region comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of SEQ ID NO:40 or SEQ ID NO:41. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 heavy chain variable region is provided wherein the nucleic acid comprises SEQ ID NO: 38 or 39. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 light chain variable region is provided wherein the nucleic acid comprises SEQ ID NO: 40 or 41. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 heavy chain variable region and light chain variable region is provided, wherein the nucleic acid encoding the heavy chain comprises SEQ ID NO:38 and the nucleic acid encoding the light chain comprises SEQ ID NO:40. In certain embodiments, an isolated nucleic acid encoding an anti-PCSK9 heavy chain variable region and light chain variable region is provided, wherein the nucleic acid encoding the heavy chain comprises SEQ ID NO:39 and the nucleic acid encoding the light chain comprises SEQ ID NO:41.

Antibody 508.20.33b Full Length Heavy Chain Nucleic Acid Sequence (SEQ ID NO: 38)

GAA GTTCAGCTGG TGGAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC
TGTGCAGCTT CTGGCTTCAC CTTCTCTAGT ACTGCTATTC ACTGGGTGCG TCAGGCCCCG
GGTAAGGGCC TGGAATGGGT TGCTAGGATT TCTCCTGCTA ACGGTAATAC TAACTATGCC
GATAGCGTCA AGGGCCGTTT CACTATAAGC GCAGACACAT CCAAAAACAC AGCCTACCTA
CAAATGAACA GCTTAAGAGC TGAGGACACT GCCGTCTATT ATTGTGCTCG TTGGATCGGG
TCCCGGGAGC TGTACATTAT GGACTACTGG GGTCAAGGAA CCCTGGTCAC CGTCTCCTCG
GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
GGACTCTACT CCCTCAGCAG CGTGGTGACT GTGCCCTCTA GCAGCTTGGG CACCCAGACC
TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC
AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG
GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGAAGAG
ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG
CTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA

Antibody 508.20.33b Heavy Chain Variable Region Nucleic Acid Sequence (SEQ ID NO: 39)

GAA GTTCAGCTGG TGGAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC
TGTGCAGCTT CTGGCTTCAC CTTCTCTAGT ACTGCTATTC ACTGGGTGCG TCAGGCCCCG

GGTAAGGGCC TGGAATGGGT TGCTAGGATT TCTCCTGCTA ACGGTAATAC TAACTATGCC
GATAGCGTCA AGGGCCGTTT CACTATAAGC GCAGACACAT CCAAAAACAC AGCCTACCTA
CAAATGAACA GCTTAAGAGC TGAGGACACT GCCGTCTATT ATTGTGCTCG TTGGATCGGG
TCCCGGGAGC TGTACATTAT GGACTACTGG GGTCAAGGAA CCCTGGTCAC CGTCTCCTCG

Antibody 508.20.33b Full Length Light Chain Nucleic Acid Sequence (SEQ ID NO: 40)

GA TATCCAGATG ACCCAGTCCC CGAGCTCCCT GTCCGCCTCT GTGGGCGATA GGGTCACCAT
CACCTGCCGT GCCAGTCAGG ATGTGTCCAC TGCTGTAGCC TGGTATCAAC AGAAACCAGG
AAAAGCTCCG AAGCTTCTGA TTTACTCGGC ATCCTTCCTC TACTCTGGAG TCCCTTCTCG
CTTCTCTGGT AGCGGTTCCG GGACGGATTT CACTCTGACC ATCAGCAGTC TGCAGCCGGA
AGACTTCGCA ACTTATTACT GTCAGCAAGC CTATCCGGCC CTACACACGT TCGGACAGGG
TACCAAGGTG GAGATCAAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC
TGATGAGCAG TTGAAATCTG GAACTGCTTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC
CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA ACTCCCAGGA
GAGTGTCACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA CCCTGACGCT
GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC ATCAGGGCCT
GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT

Antibody 508.20.33b Light Chain Variable Region Nucleic Acid Sequence (SEQ ID NO: 41)

GA TATCCAGATG ACCCAGTCCC CGAGCTCCCT GTCCGCCTCT GTGGGCGATA GGGTCACCAT
CACCTGCCGT GCCAGTCAGG ATGTGTCCAC TGCTGTAGCC TGGTATCAAC AGAAACCAGG
AAAAGCTCCG AAGCTTCTGA TTTACTCGGC ATCCTTCCTC TACTCTGGAG TCCCTTCTCG
CTTCTCTGGT AGCGGTTCCG GGACGGATTT CACTCTGACC ATCAGCAGTC TGCAGCCGGA
AGACTTCGCA ACTTATTACT GTCAGCAAGC CTATCCGGCC CTACACACGT TCGGACAGGG
TACCAAGGTG GAGATCAAAC GA

[0204] In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-PCSK9 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0205] For recombinant production of an anti-PCSK9 antibody, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0206] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (*See also* Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0207] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See* Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0208] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0209] Plant cell cultures can also be utilized as hosts. *See, e.g.*, US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0210] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful

mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR$^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

[0211] Anti-PCSK9 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

[0212] In one aspect, an anti-PCSK9 antibody of the invention is tested for its PCSK9 binding activity, *e.g.*, by known methods such as ELISA, Western blot, *etc*. Numerous types of competitive binding assays can be used to determine if an anti-PCSK9 antibody competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see, e.g.*, Stahli et al., 1983, *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (*see, e.g.*, Kirkland et al., 1986, *J. Immunol.* 137:3614-3619) solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see, e.g.*, Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see, e.g.*, Morel et al., 1988, *Molec. Immunol.* 25:7-15); solid phase direct biotin-avidin EIA (*see, e.g.*, Cheung, et al., 1990, *Virology* 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, *Scand. J. Immunol.* 32:77-82). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabelled test antigen binding protein and a labeled reference antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur. Additional details regarding methods for determining competitive

binding are provided in the examples herein. Usually, when a competing antigen binding protein is present in excess, it will inhibit (*e.g.*, reduce) specific binding of a reference antigen binding protein to a common antigen by at least 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or 75% or more. In certain embodiments, binding is inhibited by at least 80-85%, 85-90%, 90-95%, 95-97%, or 97% or more.

[0213] In one aspect of the invention, competition assays may be used to identify an antibody that competes with anti-PCSK9 antibody 508.20.04a, 508.20.04b, 508.20.06, 508.20.28a, 508.20.28b, 508.20.33a, 508.20.33b or 508.20.84 for binding to PCSK9. In certain embodiments, such a competing antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by anti-PCSK9 antibody 508.20.04a, 508.20.04b, 508.20.06, 508.20.28a, 508.20.28b, 508.20.33a, 508.20.33b and/or 508.20.84. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[0214] In an exemplary competition assay, immobilized PCSK9 is incubated in a solution comprising a first labeled antibody that binds to PCSK9 (*e.g.*, anti-PCSK9 antibody 508.20.04a, 508.20.04b, 508.20.06, 508.20.28a, 508.20.28b, 508.20.33a, 508.20.33b or 508.20.84) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to PCSK9. The second antibody may be present in a hybridoma supernatant. As a control, immobilized PCSK9 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to PCSK9, excess unbound antibody is removed, and the amount of label associated with immobilized PCSK9 is measured. If the amount of label associated with immobilized PCSK9 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to PCSK9. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

[0215] In one aspect, assays are provided for identifying anti-PCSK9 antibodies thereof having biological activity. Biological activity of the anti-PCSK9 antibodies may include, *e.g.*, blocking, antagonizing, suppressing, interfering, modulating and/or reducing one or more

biological activities of PCSK9. Antibodies having such biological activity *in vivo* and/or *in vitro* are provided.

[0216] In certain embodiments, anti-PCSK9 antibody binds human PCSK9 and prevents interaction with the LDLR. In certain embodiments, anti-PCSK9 antibody binds specifically to human PCSK9 and/or substantially inhibits binding of human PCSK9 to LDLR by at least about 20%-40%, 40-60%, 60-80%, 80-85%, or more (for example, by measuring binding in an *in vitro* competitive binding assay). In certain embodiments, the invention provides isolated anti-PCSK9 antibodies which specifically bind to PCSK9 and which antagonize the PCSK9-mediated effect on LDLR levels when measured *in vitro* using the LDLR down regulation assay in HepG2 cells disclosed herein.

D. Immunoconjugates

[0217] The invention also provides immunoconjugates comprising an anti-PCSK9 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0218] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0219] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0220] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0221] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0222] The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

[0223] In certain embodiments, any of the anti-PCSK9 antibodies provided herein is useful for detecting the presence of PCSK9 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample is blood, serum or other liquid samples of biological origin. In certain embodiments, a biological sample comprises a cell or tissue.

[0224] In one embodiment, an anti-PCSK9 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of PCSK9 in a biological sample is provided. In certain embodiments, the method comprises detecting the presence of PCSK9 protein in a biological sample. In certain embodiments, PCSK9 is human PCSK9. In certain embodiments, the method comprises contacting the biological sample with an anti-PCSK9 antibody as described herein under conditions permissive for binding of the anti-PCSK9 antibody to PCSK9, and detecting whether a complex is formed between the anti-PCSK9 antibody and PCSK9. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-PCSK9 antibody is used to select subjects eligible for therapy with an anti-PCSK9 antibody, *e.g.* where PCSK9 or LDL-cholesterol is a biomarker for selection of patients.

[0225] Exemplary disorders that may be diagnosed using an antibody of the invention include cholesterol related disorders (which includes "serum cholesterol related disorders"), including any one or more of the following: hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimers disease and generally dyslipidemias, which can be manifested, for example, by an elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL. In one aspect, the invention provides a method for treating or preventing hypercholesterolemia, and/or at least one symptom of dyslipidemia,

atherosclerosis, cardiovascular disease (CVD) or coronary heart disease, in an individual comprising administering to the individual an effective amount of anti-PCSK9 antibody. In certain embodiments, the invention provides an effective amount of an anti-PCSK9 antibody for use in treating or preventing hypercholesterolemia, and/or at least one symptom of dyslipidemia, atherosclerosis, CVD or coronary heart disease, in a subject. The invention further provides the use of an effective amount of an anti-PCSK9 antibody that antagonizes extracellular or circulating PCSK9 in the manufacture of a medicament for treating or preventing hypercholesterolemia, and/or at least one symptom of dyslipidemia, atherosclerosis, CVD or coronary heart disease, in an individual.

[0226] In certain embodiments, labeled anti-PCSK9 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

[0227] This invention also encompasses compositions, including pharmaceutical formulations, comprising an anti-PCSK9 antibody, and polynucleotides comprising sequences encoding an anti-PCSK9 antibody. In certain embodiments, compositions comprise one or more antibodies that bind to PCSK9, or one or more polynucleotides comprising sequences encoding one or more antibodies that bind to PCSK9. These compositions may further comprise suitable carriers, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

[0228] Pharmaceutical formulations of an anti-PCSK9 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; 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 polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0229] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0230] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide statin. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0231] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) 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's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0232] 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.

[0233] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0234] In one aspect, the invention provides a composition comprising an anti-PCSK9 antibody at about 100 to about 225 mg/mL, arginine succinate at about 180 to about 220 mM, polysorbate at about 0.01% to about 0.03%, and pH at about 5.2 to about 5.8. In certain embodiments, the composition is suitable for subcutaneous administration. In certain embodiments, the viscosity of the composition is less than about 25 cP at 25°C, less than about 20 cP at 25°C, less than about 15 cP at 25°C, less than about 12 cP at 25°C, or less than about 10 cP at 25°C. In certain embodiments, the composition is stable for at least one month, at least two months, at least three months, at least four months, at least five months, or at least six months at 2-8°C. In some embodiments, the composition is in a 0.5-mL, 1-mL, 1.25-mL, 1.5-mL, 1.75-mL, 2-mL, 2.25-mL , or 2.5-mL pre-filled syringe. In certain embodiments, the antibody in the composition is about any of 110, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, and 225 mg/mL, including concentrations between any of these concentrations. In certain embodiments, arginine succinate in the composition is about any of 180, 185, 190, 200, 210 and 220 mM, including concentrations between any of these concentrations. In certain embodiments, polysorbate (e.g., polysorbate 20, polysorbate 80) in the composition is about any of 0.01%, 0.015%, 0.02%, 0.025%, and 0.03%, including concentrations between any of these concentrations. In certain embodiments, the composition has a pH at any of 5.0, 5.2, 5.4, 5.5, 5.6, 5.8, 5.9, 6.0, 6.1 and 6.2, including pH between any of these values. In certain

embodiments, the anti-PCSK9 antibody in the composition is at about 150 mg/mL, arginine succinate in the composition is at about 200 mM, and polysorbate 20 in the composition is about 0.02%, and pH at about 5.5.

[0235] In one aspect, the invention provides a composition comprising an anti-PCSK9 antibody at about 150 to about 225 mg/mL, histidine acetate at about 10 to about 30 mM, arginine acetate at about 150 to about 170 mM, polysorbate at about 0.01% to about 0.03%, and pH at about 5.8 to about 6.2. In certain embodiments, the composition is suitable for subcutaneous administration. In certain embodiments, the viscosity of the composition is less than about 25 cP at 25°C, less than about 20 cP at 25°C, less than about 15 cP at 25°C, less than about 12 cP at 25°C, or less than about 10 cP at 25°C. In certain embodiments, the composition is stable for at least one month, at least two months, at least three months, at least four months, at least five months, or at least six months at 2-8°C. In some embodiments, the composition is in a 0.5-mL, 1-mL, 1.25-mL, 1.5-mL, 1.75-mL, 2-mL, 2.25-mL , or 2.5-mL pre-filled syringe. In certain embodiments, the antibody in the composition is about any of 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, and 225 mg/mL, including concentrations between any of these concentrations. In certain embodiments, histidine acetate in the composition is about any of 10, 15, 20, 25, and 30 mM, including concentrations between these concentrations. In certain embodiments, arginine acetate in the composition is about any of 150, 155, 160, 165, and 170 mM, including concentrations between any of these concentrations. In certain embodiments, polysorbate (e.g., polysorbate 20, polysorbate 80) in the composition is about any of 0.01%, 0.015%, 0.02%, 0.025%, and 0.03%, including concentrations between any of these concentrations. In certain embodiments, the composition has a pH at any of 5.8, 5.9, 6.0, 6.1 and 6.2, including pH between any of these values. In certain embodiments, the anti-PCSK9 antibody in the composition is at about 200 mg/mL, histidine acetate in the composition is at about 20 mM, arginine acetate in the composition is at about 160 mM, and polysorbate 20 in the composition is about 0.02%, and pH at about 6.0.

[0236] Also provided herein is a subcutaneous administration device containing the anti-PCSK9 antibody in a composition described herein, for delivering to an individual a flat dose in the range of 200 mg to 1200 mg of the antibody. A complete dose for one administration may be in one or more of the devices. In certain embodiments, the concentration of the antibody in the device is about 200 mg/mL. In certain embodiments, the device is a pre-filled

syringe (e.g., 0.5-mL syringe, 1-mL syringe, 1.25-mL syringe, 1.5-mL syringe, 1.75-mL syringe, 2-mL syringe, 2.25-mL syringe, or 2.5-mL syringe) or a high volume, single use, subcutaneous infusion device (e.g., for delivery of from 1-10 mL, 2-8 mL, 3-6 mL, 4-5 mL, or 4, 5, 6, 7, 8, 9, or 10 mL).

G. Therapeutic Methods and Compositions

[0237] Any of the anti-PCSK9 antibodies provided herein may be used in therapeutic methods.

[0238] In one aspect, an anti-PCSK9 antibody for use as a medicament is provided. In another aspect, an anti-PCSK9 antibody for use in treating conditions associated with cholesterol related disorder is provided. In certain embodiments, an anti-PCSK9 antibody for use in treating conditions associated with elevated level of LDL-cholesterol is provided. In certain embodiments, an anti-PCSK9 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in a method of treating an individual having conditions associated with elevated level of LDL-cholesterol comprising administering to the individual an effective amount of the anti-PCSK9 antibody. In certain embodiments, the methods and uses described herein further comprise administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, statin. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in reducing LDL-cholesterol level in a subject. In further embodiments, the invention provides an anti-PCSK9 antibody for use in lowering serum LDL-cholesterol level in a subject. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in increasing availability of LDLR in a subject. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in inhibiting binding of PCSK9 to LDLR in a subject. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in a method of reducing LDL-cholesterol level in an individual comprising administering to the individual an effective of the anti-PCSK9 antibody to reduce the LDL-cholesterol level. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in a method of lowering serum LDL-cholesterol level in an individual comprising administering to the individual an effective of the anti-PCSK9 antibody to lower the serum LDL-cholesterol level. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in a method of increasing availability of LDLR in an individual comprising administering to the individual

an effective of the anti-PCSK9 antibody to increase availability of LDLR. In certain embodiments, the invention provides an anti-PCSK9 antibody for use in a method of inhibiting binding of PCSK9 to LDLR in an individual comprising administering to the individual an effective of the anti-PCSK9 antibody to inhibit the binding of PCSK9 to LDLR. An "individual" or "subject" according to any of the embodiments described herein is preferably a human.

[0239] In a further aspect, the invention provides for the use of an anti-PCSK9 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cholesterol related disorder. In certain embodiments, the cholesterol related disorder is hypercholesterolemia. In another embodiment, the medicament is for use in a method of treating hypercholesterolemia comprising administering to an individual having hypercholesterolemia an effective amount of the medicament.

[0240] In certain embodiments, the disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of PCSK9 activity. In certain embodiments, diseases or disorders that are generally addressable (either treatable or preventable) through the use of statins can also be treated. In certain embodiments, disorders or disease that can benefit from the prevention of cholesterol synthesis or increased LDLR expression can also be treated by anti-PCSK9 antibodies of the present invention. In certain embodiments, individuals treatable by the anti-PCSK9 antibodies and therapeutic methods of the invention include individuals indicated for LDL apheresis, individuals with PCSK9-activating mutations (gain of function mutations, "GOF"), individuals with heterozygous Familial Hypercholesterolemia (heFH), individuals with primary hypercholesterolemia who are statin intolerant or statin uncontrolled, and individuals at risk for developing hypercholesterolemia who may be preventably treated. Other indications include dyslipidemia associated with secondary causes such as Type 2 diabetes mellitus, cholestatic liver diseases (primary biliary cirrhosis), nephrotic syndrome, hypothyroidism, obesity, and the prevention and treatment of atherosclerosis and cardiovascular diseases. In certain embodiments, the individuals treatable by the anti-PCSK9 antibodies and therapeutic methods described herein include individuals with LDL-c levels of 90-250 mg/dL and with coronary heart disease (CHD) or a CHD risk equivalent as described in detail in Example 12.

[0241] In certain embodiments, the methods described herein comprise administering an anti-PCSK9 antibody to an individual suffering from coronary heart disease. In certain embodiments, an individual with coronary heart disease has a history of documented myocardial infarction. In certain embodiments, an individual with coronary heart disease refers to an individual who has had a prior coronary revascularization procedure (e.g., percutaneous coronary intervention or coronary artery bypass graft). In certain embodiments, an individual with coronary heart disease refers to an individual having at least one coronary atherosclerotic lesion with ≥ 50% diameter stenosis (e.g., as determined by coronary angiography including invasive coronary angiography or cardiac computed tomography coronary angiography).

[0242] In certain embodiments, the methods described herein comprise administering an anti-PCSK9 antibody to an individual having at least one CHD risk equivalent. In certain embodiments, an individual with a CHD risk equivalent is an individual having one or more forms of clinical atherosclerotic disease, such as, for example, peripheral arterial disease (e.g., ankle/brachial blood pressure index of <0.85, prior percutaneous or surgical peripheral arterial revascularization procedure, prior non-traumatic amputation of a lower extremity due to peripheral artery disease, or ≥ 50% diameter stenosis on prior vascular imaging), carotid artery disease (e.g., carotid atherosclerotic lesion with ≥ 50% diameter stenosis or prior cutaneous or surgical carotid revascularization procedure), prior ischemic stroke, or abdominal aortic aneurysm. In certain embodiments, an individual with a CHD risk equivalent is an individual having type II diabetes. In certain embodiments, an individual with a CHD risk equivalent is an individual having type I diabetes coupled with organ damage (e.g., retinopathy, neuropathy, or nephropathy including microalbuminuria). In certain embodiments, an individual with a CHD risk equivalent is an individual having moderate to severe chronic kidney disease.

[0243] In certain embodiments, the methods described herein comprise administering an anti-PCSK9 antibody to an individual having one or more of the following risk factors: age (≥ 45 years for men or ≥ 55 years for women), smoking (within 1 month), hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or taking an antihypertensive medication), low HDL cholesterol (< 40 mg/dL), or a family history of premature CHD.

[0244] In certain embodiments, the methods and uses described herein further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, statin. In certain embodiments, the additional therapeutic agent is for preventing and/or treating atherosclerosis and/or cardiovascular diseases. In certain embodiment, the additional therapeutic agent is for use in a method of reducing the risk of recurrent cardiovascular events. In certain embodiments, the additional therapeutic agent is for elevating the level of HDL-cholesterol in a subject.

[0245] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-PCSK9 antibodies provided herein, *e.g.*, for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-PCSK9 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-PCSK9 antibodies provided herein and at least one additional therapeutic agent, *e.g.*, statin.

[0246] Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, such additional therapeutic agent elevates the level of LDLR. In certain embodiments, an additional therapeutic agent is a LDL-cholesterol lowering drugs such as statin, *e.g.*, atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or any combination thereof, *e.g.*, VYTORIN®, ADVICOR® or SIMCOR®. In certain embodiments, an additional therapeutic agent is a HDL-cholesterol raising drugs.

[0247] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the anti-PCSK9 antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

[0248] An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.*, by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various

dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0249] Anti-PCSK9 antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0250] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (*e.g.* 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient

receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered.

[0251] In certain embodiments, a flat-fixed dosing regimen is used to administer anti-PCSK9 antibody to an individual. Depending on the type and severity of the disease an exemplary flat-fixed dosage might range from 10 to 1200 mg of anti-PCSK9 antibody. One exemplary dosage of the antibody would be in the range from about 10 mg to about 1000 mg. Another exemplary dosage of the antibody would be in the range from about 100 mg to about 600 mg. Another exemplary dosage of the antibody would be in the range from about 200 mg to about 800 mg. Another exemplary dosage of the antibody would be in the range from about 350 mg to about 400 mg. Another exemplary dosage of the antibody would be in the range from about 750 mg to about 800 mg. In certain embodiments, 150 mg, 200 mg, 220 mg, 300 mg, 380 mg, 400 mg, 500 mg, 600 mg, 700 mg, 760 mg, 800 mg, 1000 mg, 1140 mg, or 1200 mg of anti-PCSK9 antibody is administered to an individual. In certain embodiments, the flat dose of the anti-PCSK9 antibody is administered every 2 weeks, every 4 weeks, every 6 weeks, every 8 weeks, every 10 weeks, or every 12 weeks. In certain embodiments, the flat dose of the anti-PCSK9 antibody is administered no more frequently than once every 2 weeks, every 4 weeks, every 6 weeks, every 8 weeks, every 10 weeks, or every 12 weeks. In certain embodiments, the flat dose of the anti-PCSK9 antibody is administered every month, every 1.5 months, every 2 months, every 2.5 months, or every 3 months. In certain embodiments, the flat dose of the anti-PCSK9 antibody is administered no more frequently than once every month, every 1.5 months, every 2 months, every 2.5 months, or every 3 months. In certain embodiments, the anti-PCSK9 antibody is administered subcutaneously. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0252] In certain embodiments, the flat, fixed, subcutaneous dose to be administered is provided in a volume that is less than or equal to 5 mL, 4.5 mL, 4 mL, 3.8 mL, 3.5 mL, 3 mL, 2.5 mL, 2 mL, 1.9 mL, 1.5 mL, or 1 mL. In certain embodiments, the flat, fixed, subcutaneous dose is 800 mg in a total volume of less than or equal to 4 mL. In certain embodiments, the flat, fixed, subcutaneous dose is 760 mg in a total volume of less than or equal to 3.8 mL. In certain embodiments, the flat, fixed, subcutaneous dose is 600 mg in a total volume of less than or equal to 3 mL. In certain embodiments, the flat, fixed, subcutaneous dose is 400 mg in a total volume of less than or equal to 2 mL. In certain

embodiments, the flat, fixed, subcutaneous dose is 380 mg in a total volume of less than or equal to 1.9 mL.

[0253] In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described herein is reduced by at least about 45%, at least about 50%, at least about 55%, or at least about 60% from baseline. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 45%, at least about 50%, at least about 55%, or at least about 60% from baseline, and maintains at the reduced level for at least two weeks, at least one month, at least two months, or three months after last dosing. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 45%, at least about 50%, at least about 55%, or at least about 60% from baseline within about 1 week, about 10 days, or about 2 weeks of the initial dose. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 45%, at least about 50%, at least about 55%, or at least about 60% from baseline within about 1 week, about 10 days, or about 2 weeks of the initial dose, and maintains at the reduced level for at least two weeks, at least one month, at least two months, or three months after last dosing. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 45% and maintains at the reduced level for at least about six weeks, at least about 7 weeks or at least about 1.5 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 45% within about 1 week from the initial dose and maintains at the reduced level for at least about six weeks, at least about 7 weeks or at least about 1.5 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 50% and maintains at the reduced level for at least about four weeks or at least about 1 month. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 50% within about 10 days from the initial dose and maintains at the reduced level for at least about four weeks or at least about 1 month. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 50% and maintains at the reduced level for at least about eight weeks or at least about 2 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 50% within about 10 days from the initial dose and maintains at the reduced level for at least about eight weeks or

at least about 2 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 55% and maintains at the reduced level for at least about two weeks. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced at least about 55% within about 2 weeks of the initial dose and maintains at the reduced level for at least about two weeks. As used herein, a "baseline" level (such as baseline level for LDL-cholesterol level) in an individual refers to the level before an administration of an anti-PCSK9 antibody described herein to the individual. In certain embodiments, the baseline may be a mean or average of two or more measurements obtained before administration of an anti-PCSK9 antibody.

[0254] In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described herein is reduced by at least about 60 mg/dL, at least about 70 mg/dL, at least about 75 mg/dL, at least about 80 mg/dL, or at least about 90 mg/dL from baseline. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 60 mg/dL, at least about 70 mg/dL, at least about 75 mg/dL, at least about 80 mg/dL, or at least about 90 mg/dL from baseline, and maintains at the reduced level for at least two weeks, at least one month, at least two months, or three months after last dosing. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 60 mg/dL, at least about 70 mg/dL, at least about 75 mg/dL, at least about 80 mg/dL, or at least about 90 mg/dL from baseline within about 1 week, about 10 days, or about 2 weeks of the initial dose. In some embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 60 mg/dL, at least about 70 mg/dL, at least about 75 mg/dL, at least about 80 mg/dL, or at least about 90 mg/dL from baseline within about 1 week, about 10 days, or about 2 weeks of the initial dose, and maintains at the reduced level for at least two weeks, at least one month, at least two months, or three months after last dosing. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 60 mg/dL or 70 mg/dL and maintains at the reduced level for at least about six weeks, at least about 7 weeks or at least about 1.5 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 60 mg/dL or 70 mg/dL within about 1 week from the initial dose and maintains at the reduced level for at least about six weeks, at least about 7 weeks or at least about 1.5 months. In certain embodiments, the LDL-cholesterol level in the individual treated by the

methods described is reduced by at least about 80 mg/dL and maintains at the reduced level for at least about four weeks or at least about 1 month. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 80 mg/dL within about 10 days from the initial dose and maintains at the reduced level for at least about four weeks or at least about 1 month. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 90 mg/dL and maintains at the reduced level for at least about two weeks. In certain embodiments, the LDL-cholesterol level in the individual treated by the methods described is reduced by at least about 90 mg/dL within about 2 weeks of the initial dose and maintains at the reduced level for at least about two weeks.

[0255] In certain embodiments, the reduction in LDL-cholesterol levels is maintained within a certain range between dosings. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 45%, at least about 50%, at least about 55%, or at least about 60% from baseline and do not increase beyond about 40%, 45%, 50%, 55%, or 60% below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 45% from baseline and do not increase beyond about 40% or 45% below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 50% from baseline and do not increase beyond about 40%, 45%, or 50% below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 55% from baseline and do not increase beyond about 40%, 45%, 50%, or 55% below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 60% from baseline and do not increase beyond about 40%, 45%, 50%, 55%, or 60% below baseline before the next dosing of the anti-PCSK9 antibody.

[0256] In certain embodiments, the reduction in LDL-cholesterol levels is maintained within a certain range between dosings. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least

about 60 mg/dL, at least about 70 mg/dL, at least about 75 mg/dL, at least about 80 mg/dL, or at least about 90 mg/dL below baseline and do not increase beyond about 55 mg/dL, 60 mg/dL, 65 mg/dL, 70 mg/dL, 75 mg/dL, 80 mg/dL or 90 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 60 mg/dL below baseline and do not increase beyond about 55 mg/dL or 60 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 70 mg/dL below baseline and do not increase beyond about 55 mg/dL, 60 mg/dL, 65 mg/dL, or 70 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 75 mg/dL below baseline and do not increase beyond about 55 mg/dL, 60 mg/dL, 65 mg/dL, 70 mg/dL, or 75 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 80 mg/dL below baseline and do not increase beyond about 55 mg/dL, 60 mg/dL, 65 mg/dL, 70 mg/dL, 75 mg/dL, or 80 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody. In certain embodiments, upon administration of a dose of an anti-PCSK9 antibody, LDL-cholesterol levels are reduced to a nadir of at least about 90 mg/dL below baseline and do not increase beyond about 55 mg/dL, 60 mg/dL, 65 mg/dL, 70 mg/dL, 75 mg/dL, 80 mg/dL or 90 mg/dL below baseline before the next dosing of the anti-PCSK9 antibody.

[0257] In one embodiment, an anti-PCSK9 antibody is administered to a subject at a dose of 800 mg every 8 weeks, wherein the level of LDL-cholesterol in the subject is reduced by at least 50% below baseline within 10 days and does not increase to more than 40% or 45% below baseline before the next dose. In one embodiment, an anti-PCSK9 antibody is administered to a subject at a dose of 760 mg every 8 weeks, wherein the level of LDL-cholesterol in the subject is reduced by at least 45% below baseline within 14 days and does not increase to more than 35% or 40% below baseline before the next dose. In one embodiment, an anti-PCSK9 antibody is administered to a subject at a dose of 400 mg every 4 weeks, wherein the level of LDL-cholesterol in the subject is reduced by at least 50% below baseline within 10 days and does not increase to more than 45% or 50% below baseline

before the next dose. In one embodiment, an anti-PCSK9 antibody is administered to a subject at a dose of 380 mg every 4 weeks, wherein the level of LDL-cholesterol in the subject is reduced by at least 50% below baseline within 10 days and does not increase to more than 45% or 50% below baseline before the next dose.

[0258] In certain embodiments, subjects receiving the anti-PCSK9 antibody are monitored for LDL-c levels and if their levels drop below 25 or 15 mg/dL, then their dose is adjusted down to around 50% or 25% of the initial dose, by reducing the total amount of antibody administered to around 50% or 25% of the initial dose administered and keeping the frequency of injections the same, by keeping the total amount of antibody administered the same but decrease the frequency by 2-fold or 4-fold (e.g., from once every 4 weeks to once every 8 weeks or 16 weeks), or a combination thereof (e.g., by reducing the dose and changing the frequency of administration). In certain embodiments, an anti-PCSK9 antibody is administered to a subject at an initial dose of 800 mg every 8 weeks. The subject is monitored and if the LDL-c levels of the subject drop below 25 or 15 mg/dL, then the subject is switched to a dose of 400 mg every 8 weeks, 400 mg every 16 weeks, 380 mg every 8 weeks, 380 mg every 16 weeks, 200 mg every 8 weeks, 200 mg every 4 weeks, 190 mg every 8 weeks, 190 mg every 4 weeks, 760 mg every 16 weeks or 4 months, or 760 mgs every 24 weeks or 6 month 800 mg every 16 weeks or 4 months, or 800 mgs every 24 weeks or 6 months. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 800 mg every 8 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 800 mg every 8 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In certain embodiments, an anti-PCSK9 antibody is administered to a subject at an initial dose of 760 mg every 8 weeks. The subject is monitored and if the LDL-c levels of the subject drop below 25 or 15 mg/dL, then the subject is switched to a dose of 380 mg every 8 weeks, 380 mg every 16 weeks, 200 mg every 4 weeks, 200 mg every 8 weeks, 190 mg every 8 weeks, 190 mg every 4 weeks, 760 mg every 16 weeks or 4 months, or 760 mgs every 24 weeks or 6 months. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 760 mg every 8 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 190 mg or 200 mg every 8 weeks. In one

embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 760 mg every 8 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 190 mg or 200 mg every 8 weeks. In certain embodiments, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks. The subject is monitored and if the LDL-c levels of the subject drop below 25 or 15 mg/dL, then the subject is switched to a dose of 200 mg every 4 weeks, 200 mg every 8 weeks, 100 mg every 4 weeks, 400 mg every 8 weeks, 400 mgs every 16 weeks or 3 months, 50 mgs every 2 weeks, or 25 mgs every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 100 mg every 4 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 100 mg every 4 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 50 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 50 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 25 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 400 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 25 mg every 2 weeks. In certain embodiments, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks. The subject is monitored and if the LDL-c levels of the

subject drop below 25 mg/dL or 15 mg/dL, then the subject is switched to a dose of 200 mg every 4 weeks, 200 mg every 8 weeks, 190 mg every 4 weeks, 100 mg every 4 weeks, 90 mg every 4 weeks, 380 mg every 8 weeks, 380 mgs every 16 weeks or 3 months, 50 mg every 2 weeks, or 25 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 100 mg every 4 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 100 mg every 4 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 200 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 190 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 190 mg every 8 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 50 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 50 mg every 4 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 25 mg/dL, the subject is switched to a dose of 25 mg every 2 weeks. In one embodiment, an anti-PCSK9 antibody is administered to a subject at an initial dose of 380 mg every 4 weeks, the subject is monitored and if the subject's LDL-c levels drop below 15 mg/dL, the subject is switched to a dose of 25 mg every 2 weeks.

[0259] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-PCSK9 antibody.

H. Articles of Manufacture and Kits

[0260] In another aspect of the invention, an article of manufacture or kit containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. In certain embodiments, the article of manufacture or kit comprises a container containing one or more of the anti-PCSK9 antibodies or the compositions described herein. In certain embodiments, the article of manufacture or kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PCSK9 antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture or kit may comprise (a) a first container with a composition contained therein, wherein the composition comprises an anti-PCSK9 antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In certain embodiments, the second container comprises a second therapeutic agent, wherein the second therapeutic agent is a cholesterol-lowering drug of the "statin" class. In certain embodiments, the statin is and/or comprises atorvastatin (*e.g.,* LIPITOR® or Torvast), fluvastatin (*e.g.*, LESCOL®), lovastatin (*e.g.*, MEVACOR®, ALTOCOR™, or ALTOPREV®), mevastatin (pitavastatin (*e.g.*, LIVALO® or PITAVA®), pravastatin (*e.g.*, PRAVACHOL®, SELEKTINE®, LIPOSTAT®), rosuvastatin (*e.g.*, CRESTOR®), simvastatin (*e.g.*, ZOCOR®, LIPEX®), or any combination thereof, *e.g.*, VYTORIN®, ADVICOR® or SIMCOR®.

[0261] The article of manufacture or kit in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular

condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0262] It is understood that any of the above articles of manufacture or kit may include an immunoconjugate of the invention in place of or in addition to an anti-PCSK9 antibody.

III. EXAMPLES

[0263] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: Generation of Anti-PCSK9 Antibodies

[0264] Residue numbers are according to Kabat (Kabat et al., Sequences of proteins of immunological interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

Library Sorting and Screening to Identify Anti-PCSK9 Antibodies

[0265] Biotinylated human PCSK9 generated in-house was used as antigen for library sorting. The phage libraries were sorted five rounds against biotinylated PCSK9 in solution phase. For the first round of sorting, 20μg/mL biotinylated PCSK9 was added to antibody phage libraries VH (*see, e.g.*, Lee et al., *J. Immunol. Meth.* 284:119-132, 2004) and VH/VL (see Liang et al., JMB. 366: 815-829, 2007) pre-blocked with phage blocking buffer PBST (phosphate-buffered saline (PBS) and 1% (w/v) bovine serum albumin (BSA) and 0.05% (v/v) TWEEN® 20) and incubated overnight at room temperature. The following day 120μl of PBST/BSA pre-absorbed DYNABEADS® MyOne™ Streptavidin T1 (Invitrogen, Carlsbad, CA) was added to each library and incubated for 1 hour at room temperature. The beads were then washed three times with PBT (PBS with 0.05% TWEEN® 20), and bound phage were eluted with 1mL 50mM HCl and 500mM NaCl for 30 minutes and neutralized with 400μL of 1 M Tris base (pH7.5). Recovered phages were amplified in *E. coli* XL-1 Blue cells. During the subsequent selection rounds, incubation of antibody phage with the biotinylated PCSK9 was reduced to 2-3 hours, and the phage bound antigen was captured for

30 minutes on neutravidin-coated (Catalog #89890, 10μg/ml, Fisher Scientific, Waltham, MA) or streptavidin-coated (Catalog #21125, 10μg/ml, Fisher Scientific, Waltham, MA) Nunc 96 well Maxisorp™ immunoplates. The stringency of plate washing was gradually increased.

[0266] After 5 rounds of panning, significant enrichment was observed. 96 clones were picked each from VH and VH/VL library sorting to determine whether they specifically bound to human PCSK9. The variable regions of these clones were PCR sequenced to identify unique sequence clones. Unique phage antibodies that bind human PCSK9 at least 5x above background were chosen and reformatted to full length IgGs for evaluation in *in vitro* cell assay.

[0267] Clones of interest were reformatted into IgGs by cloning VL and VH regions of individual clones into the LPG3 and LPG4 vector respectively, transiently expressing in mammalian CHO cells, and purifying with a protein A column.

Construct Libraries for Affinity Improvement of Clones Derived from the VH Library

[0268] Phagemid pW0703 (derived from phagemid pV0350-2b (Lee et al., *J. Mol. Biol* 340, 1073-1093 (2004)), containing stop codon (TAA) in all CDR-L3 positions and displaying monovalent Fab on the surface of M13 bacteriophage served as the library template for grafting heavy chain variable domains (VH) of clones of interest from the VH library for affinity maturation. Both hard and soft randomization strategies were used for affinity maturation. For hard randomization, one light chain library with selected positions of the three light chain CDRs was randomized using amino acids designed to mimic natural human antibodies and the designed DNA degeneracy was as described in Lee et al. (*J. Mol. Biol* 340, 1073-1093 (2004)). For soft randomization, residues at positions 91-96 of CDR-L3, 30-33, 35 of CDR-H1, 50, 52, 53-54, 56, and 58 of CDR-H2, 95–100, 100A, and 100C of CDR-H3, were targeted; and three different combinations of CDR loops, H1/L3, H2/L3, and H3/L3, were selected for randomization. To achieve the soft randomization conditions, which introduced the mutation rate of approximately 50% at the selected positions, the mutagenic DNA was synthesized with 70-10-10-10 mixtures of bases favoring the wild type nucleotides (Gallop et al., *Journal of Medicinal Chemistry* 37:1233-1251 (1994)).

Phage Sorting Strategy to Generate Affinity Improvement

[0269] For affinity improvement selection, phage libraries were subjected to five rounds of solution sorting with increasing stringency. For the first round of solution sorting, 3 O.D./ml in 1% BSA and 0.05% TWEEN® 20 of phage input were incubated with 100 nM biotinylated PCSK9 (the concentration is based on parental clone phage IC50 value) in 100µl buffer containing 1% SUPERBLOCK® (Pierce Biotechnology) and 0.05% TWEEN® 20 for 2 hours at room temperature. The mixture was further diluted 10X with 1% SUPERBLOCK®, and 100µl/well was applied to neutravidin-coated wells (10µg/ml) for 30 minutes at room temperature with gentle shaking. The wells were washed with PBS-0.05% TWEEN® 20 ten times. To determine background binding, control wells containing phage were captured on neutravidin-coated plates. Bound phage was eluted with 150µl/well 50mM HCl, 500mM KCl for 30 minutes, and subsequently neutralized by 50µl/well of 1M Tris pH8, titered, and propagated for the next round. Four more rounds of solution sorting were carried out together with increasing selection stringency. The first couple of rounds were for on-rate selection by decreasing biotinylated target protein concentration from 100nM to 1nM, and the last two rounds were for off-rate selection by adding excess amounts of non-biotinylated target protein (300 to 1000 fold more) to compete off weaker binders at room temperature.

High Throughput Affinity Screening ELISA (Single Spot Competition)

[0270] Colonies were picked from the fifth round of screening. Colonies were grown overnight at 37°C in 150µl/well of 2YT media with 50µg/ml carbenicillin and 1E10/ml KO7 in 96-well plate (Falcon). From the same plate, a colony of XL-1 infected parental phage was picked as control. 96-well Nunc Maxisorp™ plates were coated with 100µl/well of neutravidin (10µg/ml) in PBS at 4°C overnight. The plates were blocked with 150µl of 1% BSA and 0.05% TWEEN® 20 in PBS for 1 hour.

[0271] 35µl of the phage supernatant was diluted with 35µl of ELISA (enzyme linked immunosorbent assay) buffer (PBS with 0.5% BSA, 0.05% TWEEN® 20) with or without 15nM PCSK9 and let incubate for 1 hour at room temperature in an F plate (NUNC). 35µl of 3µg/ml biotinylated-PCSK9 was then added to each well and incubated for 15 minutes at room temperature. 95µl of mixture was transferred side by side to the neutravidin coated plates. The plate was gently shaken for 15 min to allow the capture of biotinylated-PCSK9 bound phage to the plate. The plate was washed ten times with PBS-0.05% TWEEN® 20. The binding was quantified by adding horseradish peroxidase (HRP)-conjugated anti-M13

antibody in ELISA buffer (1:2500) and incubated for 30 minutes at room temperature. The plates were washed with PBS-0.05% TWEEN® 20 ten times. Next, 100µl/well of a 1:1 ratio of 3,3',5,5'-tetramethylbenzidine (TMB) Peroxidase substrate and Peroxidase Solution B (H_2O_2) (Kirkegaard-Perry Laboratories (Gaithersburg, MD)) was added to the well and incubated for 5 minutes at room temperature. The reaction was stopped by adding 100µl 0.1M Phosphoric Acid (H_3PO_4) to each well and allowed to incubate for 5 minutes at room temperature. The O.D. (optical density) of the yellow color in each well was determined using a standard ELISA plate reader at 450 nm. The O.D. reduction (%) was calculated by the following equation:

OD_{450nm} reduction (%) = [(OD_{450nm} of wells with competitor) / (OD_{450nm} of well with no competitor)]*100

[0272] In comparison to the OD_{450nm} reduction (%) of the well of parental phage (100%), clones that had the OD_{450nm} reduction (%) lower than 50% were picked for sequence analysis. Unique clones were selected for phage preparation to determine binding affinity (phage IC_{50}) against PCSK9 by comparison to parental clone (clone 508.20b). Then the most affinity-improved clones (508.20.04b, 508.20.06, 508.20.28b, 508.20.33b and 508.20.84) were reformatted into human IgG_1 for antibody production and further BIAcore binding kinetic analysis and other *in vitro* or *in vivo* assay. *See* **Figures 1 and 2**.

Example 2: Characterization of Anti-PCSK9 Antibodies by BIAcore

[0273] Binding affinities of anti-PCSK9 antibodies were measured by Surface Plasmon Resonance (SRP) using a BIAcore™-3000 instrument. Anti-PCSK9 human antibodies were captured by mouse anti-human Fc antibody (Catalog # BR-1008-39, GE Healthcare, Piscataway, NJ) coated on CM5 biosensor chips to achieve approximately 200 response units (RU). For kinetics measurements, two-fold serial dilutions (500nM to 0.245nM) of human, murine, rhesus, and cyno PCSK9 (Genentech, South San Francisco, CA) were injected in PBT buffer (PBS with 0.05% TWEEN® 20) at 25°C with a flow rate of 30µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (K_D) was calculated as the ratio k_{off}/k_{on}. *See* **Figure 3**.

Example 3: LDLR-PCSK9 Binding Assay

[0274] A competition binding ELISA was performed to investigate the activity of anti-PCSK9 antibodies in blocking human PCSK9 binding to LDLR. Briefly, 1 μg/mL of soluble human LDLR extracellular domain (R&D Systems, Minneapolis, MN) was coated on 384-well MaxiSorp™ plate (NALGENE® NUNC™ International, Rochester, NY) at 4°C overnight. Then 0.25 μg/mL of biotinylated human PCSK9 pre-mixed with different concentrations of anti-PCSK9 antibodies and control antibodies were added to the plate and incubated for 2 hour at room temperature. The binding of PCSK9 to coated LDLR was detected by adding streptavidin-HRP (GE Healthcare) and substrate 3, 3′, 5, 5′-tetramethyl benzidine (TMBE-1000, Moss, Inc., Pasadena, MD). The binding results (OD) were plotted against antibody concentration and IC_{50} values were generated using KaleidaGraph software. *See* **Figure 4**.

Example 4: Antibodies Prevent LDLR Downregulation on HepG2 Cells

[0275] HepG2 cells were seeded at 1 x 10^5 into a 48-well plate. The next day, the media was changed to 10% lipoprotein deficient serum (LPDS, Frederick, Maryland). The following day, 15 μg/ml PCSK9 plus/minus anti-PCSK9 antibody were added to cells for 4 hours at 37°C. Cells were rinsed with PBS and detached using 2.5 mM EDTA. Cells were incubated with 1:20 anti-LDLR (Progen Biotechnik, Heidelberg, Germany) for 15 minutes, washed with PBS and incubated with 1:200 goat anti-mouse ALEXAFLUOR® 488 from Invitrogen (Carlsbad, CA) for 15 minutes. Cells were washed and resuspended in PBS plus 10 μg/ml propidium iodide. The samples were then analyzed with a dual laser flow cytometer (FACScan™, Becton Dickinson, Franklin Lakes, NJ). The data suggest all five of the anti-PCSK9 antibodies (508.20.04b, 508.20.06, 508.20.28b, 508.20.33b and 508.20.84) prevent downregulation of LDLR. *See* **Figure 5**.

Example 5: LDLR Downregulation in Mouse Liver

[0276] Normal C57/BL6 mice (Charles River, Wilmington, MA) were treated with 3, 30 or 60 μg of PCSK9 by I.V. administration. Using the PROTEOEXTRACT® Native Membrane Protein Extraction Kit from Calbiochem (Gibbstown, NJ) according to the manufacturer's instructions, liver from each mouse was harvested 15 min, 1 hr or 4 hrs after PCSK9 I.V.

administration and proteins extracted. As a control, 5 mice were treated with vehicle only and 8 μg of each liver lysate were pooled for analysis. Lysates were analyzed by SDS-PAGE on 8% tris-gly gel (Invitrogen, Carlsbad, CA). Proteins were transferred to nitrocellulose membrane using IBLOT® (Invitrogen). The membrane was blocked with 5% nonfat milk for 1 hour and then incubated with 1:500 anti-LDLR (Abcam, Cambridge, MA) in 5% nonfat milk overnight at 4°C. The next day, the membrane was washed three times with TBS-T, incubated with 1:5000 anti-rabbit HRP (GE Healthcare, Piscataway, NJ) for 1 hour and washed with TBS-T three times. Proteins were visualized using ECL-Plus (GE Healthcare) and exposed to XAR film (KODAK®, Rochester, NY). After an overnight exposure, the membrane was washed with TBS-T, incubated with 1:500 anti-transferrin receptor antibody (Invitrogen) for 1 hour, washed with TBS-T, incubated with 1:5000 anti-mouse HRP (GE Healthcare) for 1 hour, washed with TBS-T and visualized with ECL-Plus. Western blot with anti-LDLR antibody shows that 30 μg of PCSK9 for 1 hour significantly downregulated LDLR levels in mouse liver. *See* **Figure 6**.

Example 6: Antibodies Prevent Liver LDLR Downregulation

[0277] Normal C57/BL6 (Charles River) mice were injected with vehicle or 5 mg/kg anti-PCSK9 antibodies 24 hours prior to treatment with 30 μg PCSK9 for 1 hour. Liver from each mouse was harvested using the PROTEOEXTRACT® Native Membrane Protein Extraction Kit (Calbiochem) according to the manufacturer's instructions. Lysates were analyzed by SDS-PAGE on 8% bis-tris gel. Proteins were transferred to nitrocellulose membrane using IBLOT® (Invitrogen). The membrane was blocked with 5% nonfat milk for 1 hour and then incubated with 1:500 anti-LDLR (Abcam) in 5% nonfat milk overnight at 4°C. The next day, the membrane was washed three times with TBS-T, incubated with 1:5000 anti-rabbit HRP (GE Healthcare) for 1 hour and washed three times with TBS-T. Proteins were visualized using ECL-Plus (GE Healthcare) and exposed to XAR film (KODAK®). Western blot with anti-LDLR antibody show that all five anti-PCSK9 antibodies (508.20.84, 508.20.33b, 508.20.04b, 508.20.28b, 508.20.06) prevented LDLR downregulation in mouse liver. *See* **Figure 7**.

Example 7: Pharmacokinetics of Anti-PCSK9 Antibody

[0278] Anti-PCSK9 antibody concentrations in mouse PK study samples were determined using anti-human IgG Fc ELISA. Briefly, donkey anti-human IgG Fc (Jackson ImmunoResearch, West Grove, PA) was used to coat assay plates and goat anti-human IgG Fc HRP conjugate (Jackson ImmunoResearch, West Grove, PA) was used as detection antibody. The assay was able to measure anti-PCSK9 antibody in up to 10% mouse serum matrix with assay range of 0.31 - 20 ng/mL. *See* **Figures 8 and 9**.

[0279] Serum anti-PCSK9 antibody concentrations in cynomolgus monkey PK study samples were determined by anti-PCSK9 antibody ELISA using recombinant human PCSK9 (Genentech, Inc. South San Francisco, CA) as capture and goat anti-human IgG (H+L) HRP as detection antibody. The assay was able to measure anti-PCSK9 antibody in up to 2% cynomolgus monkey serum matrix with assay range of 0.313-50 ng/mL. *See* **Figures 10 and 11**.

Example 8: Antibodies Reduce Serum Cholesterol Level in Mice

[0280] Eight weeks old male C57BL/6J mice were purchased commercially from Jackson Laboratory. The mice were on housing for one week at the holding room before the start of the experiment. All mice were pre-bled under anesthesia and total cholesterol levels from the mice were determined using INFINITY™ Cholesterol Reagent (Fisher Diagnostics, Middletown, VA). The mice were randomized into 6 different groups with the same level of average cholesterol level. All mice received a single dose of 10mg/kg body weight of either control antibody or anti-PCSK9 antibodies. The mice were bled on day 3, day 7, day 10 and day 15 and serum total cholesterol levels were determined using INFINITY™ Cholesterol Reagent (Fisher Diagnostics, Middletown, VA).

[0281] All five anti-PCSK9 antibodies (508.20.04b, 508.20.06, 508.20.28b, 508.20.33b, 508.20.84) showed a reduction in total cholesterol levels when a single dose of 10mg/kg was administered. The administration of anti-PCSK9 antibody resulted in a significant reduction in total cholesterol level on day 3 and up to day 10 when compared to the mice receiving control antibody. *See* **Figure 12**.

Example 9: Enhancement of Statin Effectiveness

[0282] This experiment demonstrates that a combination of anti-PCSK9 antibody and statin results in a greater reduction in total cholesterol level compared to anti-PCSK9 antibody alone or statin alone treatments. *See e.g.*, **Figure 13**. Eight weeks old male C57BL/6J mice was purchased from Jackson Laboratory. The mice were grouped into 2 different groups. The non-statin mice received control diet, while statin groups received 0.2% of lovastatin in the diet for 2 weeks prior to antibody administration (Bioserve, Frenchtown, NJ). All the mice were pre-bled and mice were randomized based on equal average cholesterol level. Mice were bled on day 3 and the total cholesterol levels were assayed using INFINITY™ Cholesterol Reagent (Fisher Diagnostics, Middletown, VA).

[0283] The anti-PCSK9 antibodies showed significant cholesterol lowering effect. Statin alone treatment resulted in modest reduction in total cholesterol level, compared to non-statin groups. The combination of statin plus anti-PCSK9 antibody resulted in an additional reduction compared to anti-PCSK9 alone in total cholesterol level. *See* **Figure 13**.

Example 10: X-Ray Crystal Structure of PCSK9 Bound to Fab Fragment of Anti-PCSK9 Antibody

Protein purification and crystallization

[0284] 210 g of frozen cell paste from 10 L *E. coli* expression were thawed in 1 L of lysis buffer (PBS/25mM EDTA/1mM PMSF). Cells were disrupted by Tissuemizer (30 seconds) and the resulting slurry was passed through a microfluidizer twice. Insoluble matter was pelleted by centrifugation. Clarified lysate (250 mL at a time) was loaded onto a Protein G column (cat#17-0618-05, GE Healthcare) at 5 mL/min. The column was then washed with 100 mL of lysis buffer before eluting the bound Fab fragment of anti-PCSK9 antibody with 150 mL of elution buffer (0.58% acetic acid). 25 mL fractions were collected during elution. Fractions containing Fab fragment of anti-PCSK9 antibody were pooled after SDS PAGE analysis.

[0285] 5 mL prepacked SPHP column (GE Healthcare, cat# 17-1152-01) were equilibrated with 50ml of Buffer A (20mM MES pH5.5). Pooled fractions from the prior step were loaded onto the column at 3 mL/min. The column was washed with Buffer A to baseline. Bound Fab fragment was eluted with buffer B (20mM MES pH 5.5, 1M NaCl) using a gradient from 0% to 100% buffer B in 20 column volumes. 2 mL fractions were collected during elution. The fractions containing the protein (determined using SDS-PAGE) were

pooled and concentrated to 5 mL before loaded onto a 320 mL S75 gel filtration column that had been pre-equilibrated with sizing buffer (20 mM Hepes 7.2, 150 mM NaCl). The sizing buffer was run continuously at 1.5 mL/min for 220 mins while collecting 2 mL fractions. The peak fractions (A280) were analyzed using SDS-PAGE.

[0286] Human PCSK9 (Genbank EF692496) complementary deoxyribonucleic acids (cDNAs) containing a histidine $(His)_8$ C-terminal tag (SEQ ID NO:32) were inserted into a mammalian expression vector (pRK5) with a cytomegalovirus (CMV) promoter using standard molecular biology techniques. Protein was expressed by transient transfection of Chinese hamster ovary (CHO) cells and purified from conditioned media using affinity chromatography on a nickel-nitrilotriacetic-agarose column (Qiagen) followed by gel filtration on a SEPHACRYL® S-200 column (GE Healthcare). The correct masses of purified proteins were verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the accuracy of amino acid sequences were confirmed by N-terminal sequencing.

[0287] The purified Fab fragment of anti-PCSK9 antibody and 6.9 mg of PCSK9 protein were mixed in 2-fold molar excess of the Fab fragment and incubated at 4°C for 1 hour before concentration to 5 mL. The concentrated mixture was then loaded onto a Superdex 200 size exclusion column (cat# 17-1071-01, GE Healthcare) pre-equilibrated with sizing buffer. The sizing buffer was continuously run at 1.5 mL/min for 220 mins while collecting 2 mL fractions. The peak fractions (A280) containing both PCSK9 and Fab fragment of anti-PCSK9 antibody (SDS-PAGE) were pooled and concentrated to 20 mg/mL. The concentrated complex was then used to set up crystallization trials. Initial crystals were formed from a 1:1 mixture between protein and reservoir containing 1.3 M potassium/ sodium phosphate at pH 7 using sitting drops. Crysals were optimized by varying the protein:reservoir ratio in hanging drops. A selected crystal was treated with mother liquor supplumented with 25% glycerol and preserved in liquid nitrogen.

Structure Determination of the PCSK9:Fab Fragment of Anti-PCSK9 Antibody Complex

[0288] Diffraction data extending to about 3.5 Å resolution were collected at synchrotron beamline SSRL 7-1 and integrated and scaled in space group I222. Approximate phases were obtained by the method of molecular replacement, using the previously reported structure of PCSK9 (Hampton *et al.*, *PNAS* 104:14609-9 (2007), pdb accession code 2QTW) and the

previously reported structure of an antibody Fv fragment (Eigenbrot *et al.*, *J Mol Biol* 229:969-95 (1993), pdb accession code 1FVC). The constant region of Fab fragment of anti-PCSK9 antibody was placed as a rigid body using a part of a previously reported homologous structure (Eigenbrot et al. *supra*, pdb accession code 1FVD) after partial refinement had improved phases. The final refined structure has crystallographic R-values of 25 & 30%. Data collection and refinement statistics appear in Table 1 below.

Table 1.

Data collection

space group	I222
unit cell (Å,°)	***a***= 92.283, ***b***= 142.523, ***c***= 253.983
V_M (Å^3/Dalton)	2.8
Resolution (Å)	40 – 3.5 (3.63 – 3.50)
Rsym[a,b]	0.184 (0.807)
Number of observations	157526
Unique reflections	21579
Completeness (%)[b]	100 (100)
I/σI[b]	11 (2.6)
Wilson B (Å^2)	58

Refinement

Resolution (Å)	40 – 3.5
Number of reflections (F>0σ(F))	20644
Final R[c], R_{FREE}	0.247, 0.295
complexes/asymmetric unit	1
protein residues	994
solvent molecules	0
atoms	7463
Mean B-factor (Å^2)	86
Rmsd bonds (Å)	0.007
Rmsd angles (°)	1.1
Rmsd bonded Bs (Å^2)	2.4/1.9
Number of TLS groups	4
Ramachandran (%)	81.5/16.8/0.6/1.1

[a] Rsym = Σ||I| - |<I>||/Σ|<I>|, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.
[b] In parenthesis, for the highest resolution shell.
[c] R = Σ|Fo-Fc|/Σ|Fo|, where Fo and Fc are observed and calculated structure factor amplitudes, respectively. R_{FREE} is calculated as R for reflections sequestered from refinement.

Determination of Epitope on PCSK9 from the X-Ray Structure

[0289] A 4 Å criterion was applied using the molecular analysis program PyMOL. PCSK9 residues within 4 Å of any part of the Fab fragment of anti-PCSK9 antibody were determined as an epitope. Based on the analysis, the epitope comprises one or more of the following residues: R194, E195, D238, A239, A341, Q342, E366, D367, I369, S376, T377, C378, F379, S381 and H391 of human PCSK9.

Example 11: Human Clinical Trial, Single and Multiple Ascending Doses

[0290] A randomized, double-blind, placebo-controlled, single and multiple dose study was conducted to evaluate, primarily, the safety and tolerability of single and multiple (four weekly) doses of study drug (YW508.20.33b reformatted into human IgG_1 having a heavy chain with SEQ ID NO: 35 and a light chain with SEQ ID NO: 36) administered by subcutaneous (SC) injection to healthy volunteers with elevated serum low-density lipoprotein cholesterol (LDL-c) concentration. 80 healthy adult volunteers (men and women) with elevated serum LDL-c concentrations (130-220 mg/dL) were randomized into 10 cohorts each containing 8 subjects. Subjects in each cohort were randomized to receive either study drug or placebo (6 active and 2 placebo subject per cohort).

[0291] The cohorts were dosed as shown in Figure 14 and the Table 2. All doses were administered subcutaneously using syringes, typically in the abdomen or thigh. The drug product was formulated as 150 mg/mL antibody in 200 mM arginine succinate, 0.02% polysorbate 20, pH 5.5. For the multiple dose cohorts, study drug was administered once per week for four consecutive weeks. The statin cohorts (H and I), were initially administered atorvastatin at 20 mg once a day orally for at least 7 days, followed by a safety and tolerability assessment. If the 20 mg dose was well tolerated, the dose was increased to 40 mg daily and continued for a minimum of 21 days prior to initiation of study drug on Day 1. Subjects in cohorts H and I continued atorvastatin (40 mg PO daily) until and including Day 35. Treatment was discontinued for any subject whose direct LDL-c level fell below 25 mg/dL at any point during the study.

Table 2. Overview of Study Dose Cohorts.

Cohort	**Dose (mg)**	**Total doses administered**	**Follow-up duration[a]**	**Atorvastatin**
A	10	1	8 weeks	No
B	40	1	8 weeks	No

Cohort	Dose (mg)	Total doses administered	Follow-up duration[a]	Atorvastatin
C	150	1	12 weeks	No
D	300	1	12 weeks	No
E	600	1	16 weeks	No
F	40	4	16 weeks	No
G	150	4	16 weeks	No
H	40	4	16 weeks	Yes
I	150	4	16 weeks	Yes
J	800	1	16 weeks	No

a = Time between first dose of study drug and final study visit.

[0292] Subjects were followed for 8 to 16 weeks following initiation of study drug with frequent safety, PK and PD assessments. The following data were evaluated: safety outcomes (adverse events, abnormalities in hematology, clinical chemistry, and urinalysis, and incidence of anti-therapeutic antibodies), pharmacokinetic (PK) profile (including C_{max}, total serum apparent clearance (CL/F), apparent volume of distribution (V/F), total exposure (AUC), t_{max}, $t_{1/2}$, and dose proportionality (based on AUC)), pharmacodynamics outcomes (percent and absolute reduction from baseline in LDL-c at day 15 in single dose cohorts and day 36 in multiple dose cohorts), and percent and absolute change from baseline over time in total cholesterol, LDL-c, HDL-c, non-HDL-c, triglycerides, and lipid particle sub-fractions.

[0293] Early results from the study have not identified a drug-related, clinically significant pattern of adverse events. There were no serious or severe adverse events, no discontinuations for adverse events, and no dose-limiting toxicities. The tested doses have not defined a maximum tolerated dose. Two moderate adverse events have been reported: one headache (study drug-treated subject in the 10-mg single dose cohort) and one radius fracture (study drug-treated subject in the 600-mg single dose cohort). Five study drug-treated subjects, all in multiple dose cohorts and treated with concomitant atorvastatin, were discontinued from study drug therapy because of LDL-c levels below the protocol-specified threshold of 25 mg/dL. There were no associated adverse events in these subjects.

[0294] As shown in Figure 15 (left panel), there was a dose related increase in exposure from 10-600 mg for study drug. No differences in PK were observed between statin treated and untreated groups (Figure 15, right panel). There was a saturable clearance of study drug with a Km of 5.94 ug/mL.

[0295] As shown in Figures 16-19 and Tables 3 and 4, study drug produced clinically meaningful LDL-c reductions in healthy volunteers, alone and in combination with statin.

Pharmacodynamic (PD) data showed a dose-dependent reduction in LDL-c that was statistically significant in all cohorts except the 10 mg single dose cohort. LDL-c decreased by 80–90 mg/dL in the highest dose groups (300–800 mg in the single dose cohorts), from an average baseline LDL-c of 160–170 mg/dL. Similar reductions in LDL-c levels were seen between atorvastatin (cohorts H and I) and non-statin cohorts (cohorts F and G) (see Figures 18 and 19 and Tables 3 and 4). The differences between cohorts I and G (at day 10) and F and H (at day 36) are not statistically significant. As shown in Figures 16 and 17, at doses ≥ 300 mg, the maximal LDL-c effect appears to saturate but the duration of the effect lengthens. The data support monthly or less frequent dosing.

Table 3. Absolute Change in LDL-c Levels from Baseline in Single and Multiple Dose Cohorts.

	Mean (SD) Change in LDL (mg/dL)		
Arm	**Active**	**Placebo**	**P-value[a]**
Single Dose (at day 15)			
A (10 mg)	-18 (21)	-5.6 (15)	0.22
B (40 mg)	-45 (32)		0.03
C (150 mg)	-61 (17)		<0.001
D (300 mg)	-88 (28)		<0.001
E (600 mg)	-82 (22)		<0.001
J (800 mg)	-91 (14)		<0.001
Multiple Dose (at day 36)			
F (40 mg x 4)	-50 (28)	-9.7 (13)	0.016
G (150 mg x 4)	-71 (26)		0.001
H (A[b] + 40 mg x 4)	-38 (10)	-5 (14)	0.009
I (A[b] + 150 mg x 4)	N/A[c]	-15 (21)	N/A[c]

a = The differences between cohorts I and G (at day 10) and F and H (at day 36) are not statistically significant. b = A is Atorvastatin. c = Multiple subjects in cohort I (150 mg x 4 + Atorvastatin) were discontinued after day 10 due to LDL levels falling below the protocol threshold of <25 mg/dL.

Table 4. Percent Change in LDL-c Levels from Baseline in Single and Multiple Dose Cohorts.

	Mean % (SD) Change in LDL		
Arm	**Active**	**Placebo**	**P-value**
Single Dose (at day 15)			
A (10 mg)	-9.4 (11)	-3.7 (10)	0.3
B (40 mg)	-23 (12)		0.008
C (150 mg)	-37 (11)		<0.001
D (300 mg)	-53 (10)		<0.001
E (600 mg)	-51 (18)		<0.001

J (800 mg)	-58 (4)		<0.001
Multiple Dose (at day 36)			
F (40 mg x 4)	-34 (19)	-6.2 (8)	0.016
G (150 mg x 4)	-49 (10)		0.001
H (A^a + 40 mg x 4)	-48 (17)	-5.7 (16)	0.005
I (A^a + 150 mg x 4) (at day 10)	-65 (13)	-12 (24)	0.014

a = A is Atorvastatin.

Example 12: Human Clinical Trial in Patients with Coronary Heart Disease (CHD) or at High Risk of CHD

[0296] A randomized, double-blind, placebo-controlled, study of study drug (YW508.20.33b reformatted into human IgG_1 having a heavy chain with SEQ ID NO: 35 and a light chain with SEQ ID NO: 36) will be conducted to evaluate the safety and efficacy of study drug on top of standard-of-care (SOC) statin in patients with LDL-c levels of 90-250 mg/dL and either coronary heart disease (CHD) or a CHD risk equivalent. Approximately 224 patients (adult men and women) with serum LDL-c concentrations of 90-250 mg/dL and either CHD or a CHD risk equivalent will be randomized to one of five study arms to be administered study drug or a placebo arm, as set forth below in Table 5. All doses will be administered subcutaneously using syringes. The drug product is formulated as 150 mg/mL antibody in 200 mM arginine succinate, 0.02% polysorbate 20, pH 5.5.

Table 5. Overview of Study Dose Cohorts.

	Study Drug Dose Regimen		**Planned Number of Patients**	
Arm	**Dose (mg)**	**Frequency (weeks)**	**Active Drug**	**Placebo**
A	400	4	56	--
B	200	8	14	--
C	400	8	28	--
D	800	8	56	--
E	800	12	14	--
F	Placebo	--	--	56
(A-F) total	--	--	168	56

[0297] The study will include consecutive periods for screening (0–4 weeks), run-in (0–6 weeks, if necessary), treatment (24 weeks; Days 1–169), and follow-up (12 weeks). The study completion visit at the end of the follow-up period (Day 253) will occur 16 weeks after the final dose of study drug (Day 141). All patients, regardless of treatment assignment, will receive SOC treatment, including statins unless statins are not tolerated. All patients will

continue SOC statin therapy throughout the treatment and follow-up periods, at the same dose they were receiving during the run-in period and at enrollment. Other prescription and over-the-counter (OTC) lipid-modifying therapies are not permitted. Patients who have been taking a stable dose of SOC statin therapy (or no statin and have documented intolerance to two or more statins) and no other lipid-modifying therapy for at least 4 weeks (or 6 weeks in the case of fibrates) at the time of screening will not require a run-in period.

[0298] Patients will be monitored to determine efficacy based on absolute change from baseline in LDL-c concentration at day 169. In addition, patients will be monitored to determine secondary efficacy outcomes including absolute change from baseline in LDL-c concentration for each arm at the nadir for that arm; average value over time of the change in LDL-c (absolute and percent change) for each arm, up to Day 169, weighted by the number of weeks between consecutive LDL-c measurements; percent change from baseline in LDL-c concentration at Day 169 and at the nadir for each arm; percent and absolute change from baseline in LDL-c concentration at all other designated timepoints; and percent and absolute change from baseline in total cholesterol, non–HDL-c, and apolipoprotein B at Day 169 and at the nadir for each arm. Finally, patients will also be monitored for safety including incidence, nature, and severity of adverse events; incidence and nature of changes in vital signs, physical findings, and clinical laboratory results during and following study drug administration; and incidence of anti-therapeutic antibodies directed against study drug.

[0299] The safety of low LDL-c values will be assessed regularly in a blinded, exploratory manner. Study drug will be withheld from patients with two consecutive LDL-c values of < 15 mg/dL. This will not be considered an adverse event. Such patients will be treated with placebo instead, in blinded fashion, until LDL-c increases to ≥ 50 mg/dL, after which these patients will be switched to the lowest dosage (200 mg every 8 weeks). All doses of active drug or placebo will be given according to the study drug administration schedule, that is, on Days 1, 29 (± 2 days), 57 (± 2 days), 85(± 2 days), 113 (± 4 days), and 141 (± 4 days) only.

[0300] The primary efficacy outcome measure is the change from baseline in LDL-c at Day 169. Baseline LDL-c is defined as the average of the last two measurements collected before the first dose of study drug. The treatment comparisons between the study drug doses and between each of the study drug doses and placebo will be based on an analysis of covariance (ANCOVA), which will be performed through a linear regression model adjusting for two covariates: baseline LDL-c concentration (< 120 mg/dL, ≥ 120 mg/dL) and diabetes status

(yes, no). The confidence intervals, as well as the least-square estimates from the ANCOVA models, will be used to aid in the interpretation of the study results.

[0301] The eligibility criteria define a population of patients with high cardiovascular and CHD risk based on risk categories in the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) and National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) lipid-lowering guidelines. The study aims to enroll patients who qualify for a therapeutic target LDL-c level of 70 mg/dL according to these guidelines, but who have not come close to this goal despite SOC statin therapy, either because SOC is insufficient or because statins were not tolerated. These patients are in need of additional safe and effective LDL-c–lowering therapies.

[0302] CHD refers to a history of documented myocardial infarction, prior coronary revascularization procedure (percutaneous coronary intervention or coronary artery bypass graft), or prior coronary angiography (invasive coronary angiography or cardiac computed tomography coronary angiography) demonstrating at least one coronary atherosclerotic lesion with $\geq 50\%$ diameter stenosis.

[0303] A CHD risk–equivalent condition is at least one of the following:

1. One or more forms of clinical atherosclerotic disease:
    a. Peripheral arterial disease (previously documented ankle/brachial blood pressure index < 0.85, prior percutaneous or surgical peripheral arterial revascularization procedure, prior non-traumatic amputation of a lower extremity due to peripheral artery disease, or $\geq 50\%$ diameter stenosis on prior vascular imaging),
    b. Carotid artery disease (previously documented carotid atherosclerotic lesion with $\geq 50\%$ diameter stenosis on imaging or prior cutaneous or surgical carotid revascularization procedure),
    c. Prior ischemic stroke, documented by CT or MRI brain imaging, not due to embolism of cardiac origin (e.g., atrial fibrillation, valvular disease, or left ventricular mural thrombus) in the opinion of the investigator, or
    d. Abdominal aortic aneurysm with prior surgical or endovascular repair.
2. Diabetes mellitus type 2,
3. Diabetes mellitus type 1 with target organ damage (retinopathy, neuropathy, or nephropathy including microalbuminuria, as determined by the investigator),

4. Moderate to severe chronic kidney disease (manifested by an estimated glomerular filtration rate of 15–60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease equation consistently over at least three measurements spanning at least 3 months, including screening laboratories), or

5. Two or more of the CHD risk factors listed below AND either an absolute 10-year risk of a CHD event ≥ 20% (as determined by the National Cholesterol Education Program Adult Treatment Panel III guideline modification of the Framingham risk score) or a 10-year risk of a first fatal atherosclerotic event ≥ 10% (determined by the Systemic Coronary Risk Estimation system):

a. Age ≥ 45 years for men or ≥ 55 years for women,

b. Current cigarette smoking (within 1 month),

c. Hypertension (screening systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or taking an antihypertensive medication to treat hypertension)

d. Low HDL cholesterol (< 40 mg/dL), or

e. Family history of premature CHD (myocardial infarction or coronary revascularization in a male first-degree relative < 55 years of age or in a female first-degree relative < 65 years of age).

[0304] Standard-of-care statin therapy refers to a therapy meeting one of the following conditions: (1) high-dose simvastatin (40 mg daily), atorvastatin (40–80 mg daily), or rosuvastatin (20–40 mg daily), (2) low-dose simvastatin, atorvastatin, or rosuvastatin and documented intolerance of a high dose of that statin or of any dose of another statin, (3) other statin (any dose) and documented intolerance of simvastatin, atorvastatin, or rosuvastatin (any dose), or (4) no statin and documented intolerance of at least two statins (any statin, any dose).

[0305] Diabetes status will be determined based on the presence of any one of the following, according to patient medical record or history, or to screening laboratory test results: (1) HbA_{1c} > 6.5%, (2) fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L), (3) prior 2-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (the test should be performed as described by the World Health Organization, with use of a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water), or (4) currently on an oral or injectable therapy for a diagnosis of diabetes mellitus.

Example 13: Development of Stable, High Concentration Antibody Formulation

[0306] Initial clinical studies (see Examples 11 and 12) were carried out using a formulation of anti-PCSK9 antibody (YW508.20.33b reformatted into human IgG_1 having a heavy chain with SEQ ID NO: 35 and a light chain with SEQ ID NO: 36) formulated at 150 mg/mL antibody in 200 mM arginine succinate, 0.02% (w/v) polysorbate 20 at pH 5.5. However, a formulation with a higher protein concentration (≥200 mg/mL) and increased stability was desired to facilitate administration of higher subcutaneous doses that could be delivered monthly or less frequently.

Viscosity of anti-PCSK9 Formulations

[0307] The viscosity of a 200 mM arginine succinate, 0.02% (w/v) PS20, pH 5.5 anti-PCSK9 formulation was evaluated at various protein concentrations. At each protein concentration, the viscosity was measured at 5, 15, 25 and 40°C using a rheometer (Anton Paar Physica MCR 501) with a shear rate of 1000 1/s.

[0308] Viscosity is an important parameter for subcutaneous dosing of drug solution. A desirable viscosity limit for subcutaneous delivery using a syringe is <10 cP at ambient temperature. The viscosity of anti-PCSK9 at 100 to 300 mg/mL in 200 mM arginine succinate, 0.02% (w/v) PS20, pH 5.5 is presented in Table 6. For anti-PCSK9, viscosity is protein concentration and temperature dependent. As protein concentration increases, viscosity also increases. However, at each concentration, the viscosity can be lowered by increasing temperature. By increasing the protein concentration over 200 mg/mL, viscosity of anti-PCSK9 increased exponentially (Figure 20). Therefore, anti-PCSK9 at 200 mg/mL was selected as the target concentration.

Table 6. Viscosity of anti-PCSK9 from 100 to 300 mg/mL antibody concentration.

Temp (°C)	**Viscosity (cP)[1]**						
	100 mg/mL	150 mg/mL	200 mg/mL	225 mg/mL	250 mg/mL	275 mg/mL	300 mg/mL
5	4.6 ± 0.27	8.0 ± 0.16	18.6 ± 0.17	50.2 ± 0.99	76.9 ± 0.83	306 ± 5.8	603 ± 9.2
15	3.1 ± 0.10	5.2 ± 0.08	11.7 ± 0.15	31.9 ± 0.33	46.7 ± 0.91	179 ± 2.8	357 ± 4.8
25	2.5 ± 0.06	3.8 ± 0.09	8.3 ± 0.13	22.6 ± 0.14	31.0 ± 0.84	115 ± 0.6	225 ± 4.1
40	1.8 ± 0.01	2.7 ± 0.10	5.7 ± 0.26	15.5 ± 0.2	18.8 ± 0.63	74.8 ± 4.3	135 ± 3.2

[1]200 mM arginine succinate, 0.02% PS20, pH 5.5.

Agitation Study

[0309] An agitation study was performed to assess the minimum amount of surfactant required to prevent or minimize aggregation of anti-PCSK9 at 150 mg/mL in 200 mM arginine succinate, pH 5.5. Polysorbate 20 (PS20) was added to the formulation to achieve concentrations of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1% (w/v). All samples were sterile filtered, and 0.5 mL of each sample was filled into 2-cc glass vial. Samples were agitated using Glas-Col benchtop shaker set at 50 cycles/min with a sample displacement of 11 cm for 24 hours at room temperature (RT). The appropriate sample controls (no shaking) in the corresponding configuration were placed in the same vicinity of the shaker. All samples were analyzed by size-exclusion chromatography (SEC) and turbidity by UV measurement at 340-360 nm absorbance (abs).

[0310] The results are presented in Figure 21. Without PS20 in the formulation, the 24-hour agitated sample (at room temperature) had obvious visible changes when compared to the unshaken control vial. The agitated sample had a milky appearance with an increase in turbidity and a 6% decrease in SEC main peak. With the addition of ≥0.01% PS20 to the formulation, no differences were observed by SEC and turbidity measurement between the control (without agitation) and agitated samples in the vials. These results suggest that the use of 0.01% PS20 was sufficient to prevent agitation-induced aggregate formation of anti-PCSK9 at 200 mg/mL. However, a concentration of 0.02% PS20 was selected as the target concentration to account for potential degradation of the surfactant during product storage.

Oxidation Potential of Anti-PCSK9 Formulations

[0311] Oxidation of anti-PCSK9 was determined by trypsin-peptide map and the site(s) of oxidation was characterized by LC-MS. Oxidation of anti-PCSK9 was induced by elevated temperature, light and oxidizing agents such as hydrogen peroxide and 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH). The degradation conditions for preparing the oxidative samples are summarized in Table 7. These oxidized anti-PCSK9 samples were also evaluated for possible potency loss due to oxidation by measuring its ability to inhibit PCSK9 binding to low density lipoprotein receptor domain Fc ($LDLR_D$-Fc) fusion protein as described in Example 3.

[0312] For peptide mapping, samples were reduced with 1M dithiothreitol, alkylated with 2.9 M iodoacetamide, and buffer exchanged before digestion. Trypsin was used for a 1.5 hour

digestion at 37°C using an enzyme to protein ratio of 1:25. The digestion was quenched with 10% trifluoroacetic acid (TFA) to a final pH 2-3. The resulting peptide digestion mixture was analyzed by reverse-phase liquid chromatography with detection by mass spectrometry (LC-MS) with a LTQ Orbitrap XL. The peptide map utilized a linear gradient from 0-40% over 160 minutes at 0.25 mL/min in conjunction with a Phenomenex Jupiter C18 column (5 μm, 2x250 mm, 300Å) maintained at 55°C. Mobile phases A and B consisted of 0.1% TFA in water and 0.09% TFA in acetonitrile respectively. Peptides were also detected at 214 and 280 nm abs before MS analysis. LC-MS data was processed by Mascot software to identify peptides and respective oxidation sites of anti-PCSK9. Amount of oxidation in a sample was expressed as "total oxidation per site" or accumulative oxidation since Trp and Met produce multiple oxidation products and/or oxidation states.

[0313] Methionine (Met/M) and Tryptophan (Trp/W) are the two common amino acid residues that are easily oxidized in protein drug products. W_{99} and M_{108} located in the complementarity-determining region (CDR) III of the heavy chain and the three Trp residues (W_{36}, W_{111} and W_{486}) adjacent to the CDRs are the potential oxidation sites of anti-PCSK9. Oxidation of these amino acid residues may result in loss of drug potency due to their proximity to the CDRs. Peptide mapping analysis of the degraded samples revealed that oxidation of anti-PCSK9 mainly occurred at M_{256}, M_{362}, M_{432} and M_{455} residues of the Fc portion (Figure 22). When anti-PCSK9 was degraded by exposing to light (room or UV) and oxidizing agents such as H_2O_2 and AAPH, the relative amount of oxidation per site for Met or Trp residues in/adjacent to the CDR was less than 3% with no significant impact on potency (Figure 23). Therefore, anti-PSCK9 is considered not susceptible to oxidation and the use of antioxidants in the protein formulation is not necessary.

Table 7. Anti-PCSK9 Degradation Conditions for Oxidation Analysis.

Degradation Mode	**Exposure Condition**	**Expected Degradation**
Thermal	2 weeks @ 40°C	Oxidation
Light	24 hours of Room Light	Photo-oxidation
	1.2 million lux hours	
Oxidizing Agents	1000 ppM H_2O_2 (24 hours @ 5°C)	Methionine Oxidation
	5 mM AAPH (24 hours @ 40°C)	Methionine + Tryptophan Oxidation

pH Profile and Excipient Studies

[0314] The effect of formulation pH and excipients on anti-PCSK9 was evaluated at a protein concentration of 200 mg/mL. A pH range of 5.0 to 6.5 in formulations containing arginine succinate, histidine HCl or histidine acetate as buffer species and arginine HCl or arginine acetate as solubilizers were assessed for accelerated stability at 40°C (see Table 9) and viscosity at 5°C and 25°C (see Table 8). The following assays were used for the assessment: SEC, ion-exchange chromatography (IEC), capillary electrophoresis- sodium dodecyl sulfate (CE-SDS) and potency. A total of seven formulations were evaluated.

[0315] IEC was performed on an Agilent 1100 HPLC and utilized a Dionex ProPac™ WCX-10 column (4 x 250 mm) with mobile phase A (20 mM HEPES, pH 7.9) and gradient from 1%-34% mobile phase B (20 mM HEPES, 100 mM NaCl, pH 7.9) in 50 minutes at a flow-rate of 0.9 mL/min. The column was maintained at 35°C. The sample load was 40 µg, and the separation was monitored at 280nm abs.

Effect of pH

[0316] The effect of pH on stability of anti-PCSK9 at 200 mg/mL in 200 mM arginine succinate, 0.02% PS20 was evaluated from pH 5.0, 5.5 and 6.0. As analyzed by SEC, IEC and CE-SDS, increasing the formulation pH from 5.0 to 6.0 increased the stability of anti-PCSK9 after 1 month at 40°C. Compared to pH 5.0 and 5.5, the formulation at pH 6.0 had less acidic and basic peak formation as determined by IEC. The formulation at pH 6.0 also had a decrease in high molecular weight species (HMWS) as determined by SEC and low molecular weight species by determined by both SEC and CE-SDS. For the formulation at pH 6.5, anti-PCSK9 was formulated at 200 mg/mL in 20 mM histidine HCl, 160 mM arginine HCl, and 0.02% PS20. The degradation rates of anti-PCSK9 at 40°C for all formulations at pH 5.0 to 6.5 are shown in Table 9 and the pH rate profiles for IEC and SEC are presented in Figure 24. Based on the pH rate profiles and degradation rates, a target pH 6.0 was selected.

Table 8. Viscosity of anti-PCSK9 at 200 mg/mL in Various Formulations.

Formulation	**Buffer**	**Stabilizer/Excipients**	**pH**	**Viscosity (cP)**	
				5°C	**25°C**
1	200 mM Arginine	0.02% PS20	5.0	18.2	7.7

2	Succinate		5.5	**18.6**	**8.3**
3			6.0	16.4	7.9
4	20 mM Histidine HCl	160 mM Arginine HCl, 0.02% PS20	6.0	18.0	7.6
5			6.5	17.5	7.3
6	20 mM Histidine Acetate	160 mM Arginine Acetate, 0.02% PS20	5.5	16.4	7.7
7			6.0	15.9	7.6

Effect of Buffer Species

[0317] The effect of buffer species on accelerated stability of 200 mg/mL anti-PCSK9 at pH 6.0 was evaluated in formulations containing the following three buffer systems: (1) 160 mM arginine succinate, (2) 20 mM histidine HCl and 160 mM arginine HCl, and (3) 20 mM histidine acetate and 160 mM arginine acetate. All three formulations contained 0.02% PS20. After 1 month at 40°C, anti-PCSK9 had comparable CE-SDS profiles among the three buffer systems (Figure 26, top panel). No differences were observed by SEC between histidine HCl/arginine HCI and histidine acetate/arginine acetate buffer systems, while the use of the arginine succinate buffer had a slight increase in a HMWS Peak (Figure 26, middle panel). By IEC analysis, the use of histidine HCI/arginine HCl buffer system in the formulation had less acidic peak formation when compared to histidine acetate/arginine acetate buffer system and arginine succinate buffer (Figure 26, bottom panel). However, the overall degradation rates of anti-PCSK9 at 40°C determined by SEC, IEC and CE-SDS are comparable in all three buffer systems at pH 6.0 (Table 9).

Table 9. Degradation Rate for 200 mg/mL anti-PCK9 at 40ºC in Various Formulations.

% Loss/Month at 40ºC	200 mM Arginine Succinate			20 mM HisHCl, 160 mM ArgHCl		20 mM HisAce, 160 mM ArgAce	
	pH 5.0	pH 5.5	pH 6.0	pH 6.0	pH 6.5	pH 5.5	pH 6.0
SEC Main Peak	3.8	2.7	2.2	2.1	2.9	2.1	2.1
IEC Main Peak	33	22	19	15.9	18	20.9	19
CE-SDS Main Peak	5.1	4.5	3.6	3.8	3.5	4.0	4.3

[0318] The stability of anti-PCSK9 in two formulations (histidine HCl, pH 6.0 and histidine acetate, pH 6.0) in a 1 mL syringe was also evaluated.

[0319] At 5°C, both formulations were stable for up to 6 months (Tables 10 and 11). At accelerated and stress conditions, formation of acidic variants and aggregation are the major degradation routes for anti-PCSK9 in liquid formulation. At 30°C/65% relative humidity (RH) and 40°C/75%RH, the protein degraded faster in histidine acetate than histidine HCl at pH 6.0 as determined by IEC (Table 11). No differences in aggregation rate were observed by SEC and CE-SDS for either formulation under the same storage conditions (Table 12). No increase in oxidation was observed for both lead formulations when stored at 5°C for up to 6 months. Although there was a slight increase in oxidation of Met256 (~2%) in the Fc portion in both formulations after 6 months at 30°C/65% RH, increase in oxidation of other Met and Trp residues was not observed. Loss of potency was not observed in either formulation for up to 6 months at 5°C and 30°C/65%RH. Similar results were obtained using a 2.25 mL syringe.

Table 10. Stability Data for 200 mg/mL anti-PCSK9 in 20 mM Histidine HCl, 160 mM Arginine HCl, 0.02% PS20, pH 6.0 in a 1-mL Syringe.

			IEC			SEC				
Temp °C/ %RH	Timepoint Days/ Months	Strength mg/mL	% Acidic	% Main Peak	% Basic	% HMWS	% Main Peak	% LMWS	CE-SDS % Main Peak	Potency % Relative Potency
NA	T = 0/0	209	11.6	73.1	15.1	0.7	99.2	0	96.1	114
5	28/1	210	11.9	73.5	14.5	0.6	99.3	0	96.2	101
5	61/2	206	11.7	72.9	15.3	0.6	99.3	0	96.0	100
5	91/3	208	11.6	73.3	15.0	0.6	99.3	0	96.0	101
5	183/6	208	12.4	71.9	15.6	0.7	99.2	0	95.3	103
30/65	28/1	210	15.0	69.5	15.3	0.7	99.1	0.1	95.6	NT
30/65	61/2	206	19.0	64.0	16.9	0.9	98.8	0.2	94.7	91
30/65	91/3	204	21.4	61.9	16.5	1.0	98.5	0.4	94.0	84
30/65	183/6	209	33.9	48.7	17.3	1.4	97.7	0.8	91.2	92
40/75	7/0.25	206	15.0	68.6	16.3	0.8	99.0	0.1	95.5	NT
40/75	14/0.5	206	18.9	64.3	16.7	0.9	98.8	0.2	95.0	NT
40/75	28/1	209	25.9	57.4	16.6	1.1	98.4	0.4	93.6	105

NT = not tested.

Table 11. Stability Data for 200 mg/mL anti-PCSK9 in 20 mM Histidine Acetate, 160 mM Arginine Acetate, 0.02% PS20, pH 6.0 in a 1-mL syringe.

			IEC			SEC				
Temp (°C)	Timepoint Days/ Months	Strength mg/mL	% Acidic	% Main Peak	% Basic	% HMWS	% Main Peak	% LMWS	% Main Peak	% Relative Potency
NA	T = 0/0	211	11.8	72.7	15.4	0.6	99.3	0	96.2	100
5	28/1	203	12.1	72.8	14.9	0.6	99.4	0	96.2	106
5	61/2	208	11.7	72.8	15.4	0.6	99.3	0	96.0	97
5	91/3	208	11.6	72.9	15.3	0.6	99.3	0	96.0	92
5	183/6	207	12.5	71.6	15.8	0.7	99.2	0	95.6	98
30/65	28/1	209	16.8	67.5	15.6	0.7	99.1	0.1	95.6	NT
30/65	61/2	210	21.6	61.8	16.4	0.9	98.8	0.2	94.7	98
30/65	91/3	205	26.1	57.4	16.3	1.0	98.6	0.3	94.3	87
30/65	183/6	205	41.4	42.5	16.0	1.5	97.6	0.8	91.1	91
40/75	7/0.25	206	17.0	66.6	16.2	0.8	99.0	0.1	95.4	NT
40/75	14/0.5	204	22.5	60.0	16.2	0.9	98.8	0.2	94.5	NT
40/75	28/1	196	31.8	51.9	16.1	1.1	98.4	0.4	93.5	106

NT = not tested.

Table 12. Degradation Rates for anti-PCSK9 in a 1-mL Syringe at Accelerated Stability Conditions.

% Change Per Month		Histidine HCl[1]	Histidine Acetate[2]
IEC	30°C/65%RH	4.0	5.0
	40°C/75%RH	16.7	22.0
SEC	30°C/65%RH	0.2	0.2
	40°C/75%RH	0.8	0.9
CE-SDS	30°C/65%RH	0.8	0.8
	40°C/75%RH	2.6	2.8

[1]Histidine HCl = 200 mg/mL anti-PCSK9 in 20 mM histidine HCl, 160 mM arginine HCl, 0.02% PS20, pH 6.0
[2]Histidine Acetate = 200 mg/mL anti-PCSK9 in 20 mM histidine acetate, 160 mM arginine acetate, 0.02% PS20, pH 6.0

Frozen Stability

[0320] Anti-PCSK9 was formulated at 200 mg/mL in the following two formulations: (1) 20 mM histidine HCl, 160 mM arginine HCl, 0.02% PS20, pH 6.0; and (2) 20 mM histidine acetate, 160 mM arginine acetate, 0.02% PS20, pH 6.0. For each formulation, 20 mL of the drug solution was filled into 25-cc 316L stainless steel minicans. All minicans were then placed at -20°C for up to 6 months for stability analysis.

[0321] No difference was observed by IEC, CE-SDS and potency for both formulations for up to 6 months at -20°C. However, aggregates increased by 1.4% in the histidine HCl after 6 months of frozen storage when compared to only a 0.5% increase in aggregates in the histidine acetate formulations under the same storage condition (see Figure 25). Due to the faster rate of aggregation with the histidine HCl formulation under frozen storage conditions, the histidine acetate formulation was selected as the preferred buffer.

Effect of Sucrose on Frozen Stability

[0322] Sucrose was evaluated for its effect on stabilizing anti-PCSK9 during frozen storage. Using a lab-scale Millipore Tangential Flow Filtration (TFF) system equipped with LCGC10 cartridges, anti-PCSK9 was tested in the following two sucrose-containing formulations: (1) 200 mg/mL anti-PCSK9 in 20 mM histidine HCl, 130 mM arginine HCl, 60 mM sucrose, 0.02% PS20 (w/v), pH 6.0; and (2) 200 mg/mL anti-PCSK9 in 20 mM histidine acetate, 100 mM arginine acetate, 60 mM sucrose, 0.02% PS20 (w/v), pH 6.0. Samples of anti-PCSK9 in the two formulations were placed at -20°C for up to 3 months and analyzed by SEC for aggregation.

[0323] The addition of sucrose (60 mM) had no effect on reducing aggregation of aPCSK9 in the histidine acetate formulation, but it did help to slow down aggregation in the histidne HCl formulation by 0.7% over 3 months at -20°C. However, the addition of sucrose also increased the viscosity from 7-8 cP to 11-13 cP at 25°C for both formulations, which was undesirable for a subcutaneous formulation. Therefore, sucrose was not selected as a stabilizer for the formulation.

[0324] Based on the results described above, a liquid formulation consisting of 200 mg/mL anti-PCSK9 in 20 mM histidine acetate, 160 mM arginine acetate, 0.02% PS20 (w/v), pH 6.0 was selected. This formulation has optimal stability at 2-8°C and at -20°C for storage and improved stability when compared to the initial formulation at pH 5.5.

Example 14: Human Clinical Trial in Patients with Coronary Heart Disease (CHD) or at High Risk of CHD

[0325] This Example describes a phase II clinical study and Figures 27-37 show interim results for at least 50% of patients at 12 weeks. The study enrolled 248 patients, including 183 patients treated with study drug and 64 patients treated with placebo. One patient dropped out prior to the first treatment and 13 patients discontinued treatment prior to day 85 of the study. 234 patients completed at least 12 weeks of the study.

[0326] A ~3:1 randomized, double-blind, placebo-controlled, study of study drug (YW508.20.33b reformatted into human IgG_1 having a heavy chain with SEQ ID NO: 35 and a light chain with SEQ ID NO: 36) was conducted to evaluate the safety and efficacy of study drug on top of standard-of-care (SOC) statin in patients with fasting serum LDL-c (direct) levels of 90-250 mg/dL and either coronary heart disease (CHD) or a CHD risk equivalent. Additional eligibility criteria included weight $\geq$ 45 kg (100 lb); body mass index of 18-37 kg/m^2; and age between 18 and 80. The randomization was stratified by LDL-c > 120 mg/dL and diabetes status.

[0327] The eligibility criteria for this phase II clinical study defined a population of patients with high cardiovascular and CHD risk based on risk categories in the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) and National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) lipid-lowering guidelines. This study enrolled patients who qualified for a therapeutic target LDL-c level of 70 mg/dL according to these guidelines, but who had not come close to this goal despite stable SOC statin therapy, either because SOC is insufficient or because statins were not tolerated.

[0328] Briefly, CHD refers to a history of documented myocardial infarction, prior coronary revascularization procedure (percutaneous coronary intervention or coronary artery bypass graft), or prior coronary angiography (invasive coronary angiography or cardiac computed tomography coronary angiography) demonstrating at least one coronary atherosclerotic lesion with $\geq$ 50% diameter stenosis.

[0329] A patient with a CHD risk–equivalent condition had at least one of the following:

1. One or more forms of clinical atherosclerotic disease:
    a. Peripheral arterial disease (previously documented ankle/brachial blood pressure index < 0.85, prior percutaneous or surgical peripheral arterial revascularization procedure, prior non-traumatic amputation of a lower extremity due to peripheral artery disease, or $\geq$ 50% diameter stenosis on prior vascular imaging),

b. Carotid artery disease (previously documented carotid atherosclerotic lesion with ≥ 50% diameter stenosis on imaging or prior cutaneous or surgical carotid revascularization procedure),

c. Prior ischemic stroke, documented by CT or MRI brain imaging, not due to embolism of cardiac origin (e.g., atrial fibrillation, valvular disease, or left ventricular mural thrombus) in the opinion of the investigator, or

d. Abdominal aortic aneurysm with prior surgical or endovascular repair.

2. Diabetes mellitus type 2,

3. Diabetes mellitus type 1 with target organ damage (retinopathy, neuropathy, or nephropathy including microalbuminuria, as determined by the investigator),

4. Moderate to severe chronic kidney disease (manifested by an estimated glomerular filtration rate of 15–60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease equation consistently over at least three measurements spanning at least 3 months, including screening laboratories), or

5. Two or more of the CHD risk factors listed below AND either an absolute 10-year risk of a CHD event ≥ 20% (as determined by the National Cholesterol Education Program Adult Treatment Panel III guideline modification of the Framingham risk score) or a 10-year risk of a first fatal atherosclerotic event ≥ 10% (determined by the Systemic Coronary Risk Estimation system):

a. Age ≥ 45 years for men or ≥ 55 years for women,

b. Current cigarette smoking (within 1 month),

c. Hypertension (screening systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or taking an antihypertensive medication to treat hypertension)

d. Low HDL cholesterol (< 40 mg/dL), or

e. Family history of premature CHD (myocardial infarction or coronary revascularization in a male first-degree relative < 55 years of age or in a female first-degree relative < 65 years of age).

[0330] Diabetes status was determined based on the presence of any one of the following, according to patient medical record or history, or to screening laboratory test results: (1) HbA_{1c} > 6.5%, (2) fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L), (3) prior 2-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (the test

should be performed as described by the World Health Organization, with use of a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water), or (4) currently on an oral or injectable therapy for a diagnosis of diabetes mellitus.

[0331] Exclusion criteria included: planned coronary, carotid or peripheral arterial revascularization procedure or surgery during study; uncontrolled clinically significant medical disease as listed in the protocol within 3 months or screening; any acquired or congenital immunosuppression; any organ transplant other than the corneal transplant; life expectancy <2 years, in the investigator's judgment; fasting serum triglyceride levels >= 400 mg/dL; history of alcoholism or drug addiction with a year of screening; use of illicit drugs with 3 months of screening; pregnancy or not willing to use highly effective contraception; history of anaphylaxis or anaphylactic reactions.

[0332] 248 patients (adult men and women) with serum LDL-c concentrations of 90-250 mg/dL and either CHD or a CHD risk equivalent were randomized to one of five study arms and were administered study drug or a placebo arm (Arm F). Patients in the first study arm (Arm A) were administered 400 mg of anti-PCSK9 antibody every 4 weeks; patients in the second study arm (Arm B) were administered 200 mg of anti-PCSK9 antibody every 8 weeks; patients in the third study arm (Arm C) were administered 400 mg of anti-PCSK9 antibody every 8 weeks; patients in the fourth study arm (Arm D) were administered 800 mg of anti-PCSK9 antibody every 8 weeks; and patients in the fifth study arm (Arm E) were administered 800 mg of anti-PCSK9 antibody every 12 weeks. An overview of study dose cohorts, study drug dose regimen, and number of patients per arm are provided in Figure 27. All doses were administered subcutaneously using syringes. The drug product is formulated as 150 mg/mL antibody in 200 mM arginine succinate, 0.02% polysorbate 20, pH 5.5.

[0333] The demographics of the patients in the study are set forth below in Table 13, indicating no difference by arm. The patients' baseline characteristics are set forth below in Table 14, indicating no difference by arm.

Table 13: Patient Demographics

(Mean (SD), unless noted)

	400 mg /4W	200 mg /8W	400 mg /8W	800 mg /8W	800 mg /12W	Placebo	mITT
	(n=57)	(n=23)	(n=30)	(n=50)	(n=23)	(n=64)	(n=247)
Age (years)	66 (8.5)	63 (10.0)	63 (8.1)	64 (8.9)	64 (7.2)	63 (7.8)	64 (8.4)

Weight (kg)	89 (15.4)	89 (15.3)	85 (11.7)	83 (17.7)	83 (17.1)	87 (15.1)	86 (15.6)
BMI (kg/m)	31 (4.3)	30 (4.5)	30 (4.2)	29 (5.2)	29 (3.7)	30 (5.0)	30 (4.7)
Female (%)	24 (42%)	8 (35%)	16 (53%)	24 (48%)	10 (44%)	24 (38%)	106 (43%)
Hispanic (%)	1 (2%)	1 (4%)	1 (3%)	1 (2%)	1 (4%)	5 (8%)	10 (4%)
Race: White (%)	55 (97%)	19 (83%)	27 (90%)	44 (88%)	23 (100%)	59 (92%)	227 (92%)
Race: Black (%)	1 (2%)	2 (9%)	2 (7%)	5 (10%)	0	3 (5%)	13 (5%)
Race: Asian (%)	0	0	1 (3%)	1 (2%)	0	1 (2%)	3 (1%)
Race: Other (%)	1 (2%)	1 (4%)	0	0	0	1 (2%)	3 (1%)
Race: Native (%)	0	1 (4%)	0	0	0	0	1 (0.4%)

Table 14: Patient Baseline Characteristics

(Mean (SD), unless noted)

	400 mg /4W	**200 mg /8W**	**400 mg /8W**	**800 mg /8W**	**800 mg /12W**	**Placebo**	**mITT**
	(n=57)	**(n=23)**	**(n=30)**	**(n=50)**	**(n=23)**	**(n=64)**	**(n=247)**
Pre-diabetic (% FBG ≥ 100 mg/dl)	68%	65%	60%	54%	65%	59%	62%
Statin use (%)	88%	78%	73%	76%	74%	89%	82%
LDL-c ≥ 120 (%)	46%	48%	60%	54%	52%	45%	50%
LDL-c (mg/dL)	123 (31.3)	123 (25.3)	133 (35.2)	127 (31.5)	134 (43.8)	122 (31.4)	126 (32.7)
Median LDL-c (mg/dL)	117	117	123	118	123	111	117
Triglyceride (mg/dL)	156 (66.3)	146 (60.0)	152 (54.3)	173 (90.8)	144 (37.0)	141 (63.1)	153 (67.8)
Median Trig. (mg/dL)	142	132	142	149	145	132	142
Family history of CHD (% yes)	26 (46%)	5 (22%)	13 (43%)	20 (40%)	9 (39%)	18 (28%)	91 (37%)
Smoker: never (%)	23 (40%)	6 (26%)	13 (43%)	17 (34%)	7 (30%)	25 (39%)	91 (37%)

[0334] As shown in Figure 27, the study includes consecutive periods for screening (0–4 weeks), run-in (0–6 weeks, if necessary), treatment (24 weeks; Days 1–169), and follow-up (12 weeks). The study completion visit at the end of the follow-up period (Day 253) occurs 16 weeks after the final dose of study drug (Day 141). All patients, regardless of treatment assignment, received standard-of-care (SOC) treatment, including statins unless statins were

not tolerated. SOC statin therapy refers to a therapy meeting one of the following conditions: (1) high-dose simvastatin (40 mg daily), atorvastatin (40–80 mg daily), or rosuvastatin (20–40 mg daily), (2) low-dose simvastatin, atorvastatin, or rosuvastatin and documented intolerance of a high dose of that statin or of any dose of another statin, (3) other statin (any dose) and documented intolerance of simvastatin, atorvastatin, or rosuvastatin (any dose), or (4) no statin and documented intolerance of at least two statins (any statin, any dose). All patients continue SOC statin therapy throughout the treatment and follow-up periods, at the same dose they were receiving during the run-in period and at enrollment. Other prescription and over-the-counter (OTC) lipid-modifying therapies (*e.g.,* red yeast rice, omega-3 fatty acid supplements, etc.) are not permitted. Patients who had been taking a stable dose of SOC statin therapy (or no statin and had documented intolerance to two or more statins) and no other lipid-modifying therapy for at least 4 weeks (or 6 weeks in the case of fibrates) at the time of screening did not require a run-in period.

[0335] All doses of active drug or placebo are given according to the study drug administration schedule, that is, on Days 1, 29 (± 2 days), 57 (± 2 days), 85(± 2 days), 113 (± 4 days), and 141 (± 4 days) only. See Figure 28. Patients are monitored to determine efficacy based on absolute change from baseline in LDL-c concentration at day 169. In addition, patients are monitored to determine secondary efficacy outcomes including absolute change from baseline in LDL-c concentration for each arm at the nadir for that arm; average value over time of the change in LDL-c (absolute and percent change) for each arm, up to Day 169, weighted by the number of weeks between consecutive LDL-c measurements; percent change from baseline in LDL-c concentration at Day 169 and at the nadir for each arm; percent and absolute change from baseline in LDL-c concentration at all other designated timepoints; and percent and absolute change from baseline in total cholesterol, non–HDL-c, and apolipoprotein B at Day 169 and at the nadir for each arm.

[0336] The primary efficacy outcome measure includes the change from baseline in LDL-c at Day 169. Baseline LDL-c is defined as the average of the last two measurements collected before the first dose of study drug. The treatment comparisons between the study drug doses and between each of the study drug doses and placebo were based on an analysis of covariance (ANCOVA), which was performed through a linear regression model adjusting for two covariates: baseline LDL-c concentration (< 120 mg/dL, ≥ 120 mg/dL) and diabetes status (yes, no). The confidence intervals, as well as the least-square estimates from the

ANCOVA models, were used to aid in the interpretation of the study results. The secondary efficacy outcome measures include absolute change in LDL-c at nadir and all time points; weighted average of change in LDLc per week; percent change from baseline in LDL-c at Day 169, nadir and all visits; absolute and percent change in total cholesterol, non-HDL-c, and apolipoprotein B at Day 169 and at the nadir.

[0337] Table 15 below shows patient disposition after 12 weeks of treatment.

Table 15: Disposition after 12 weeks of treatment.

	400 mg /4W	**200 mg /8W**	**400 mg /8W**	**800 mg /8W**	**800 mg /12W**	**Placebo**	**ITT***
	(n=57)	**(n=23)**	**(n=30)**	**(n=51)**	**(n=23)**	**(n=64)**	**(n=248)**
Completed study	0	0	0	0	0	0	0
Discontinued study	2 (4%)	0	1 (3%)	3 (6%)	0	1 (2%)	7 (3%)
Discontinued drug	3 (5%)	0	1 (3%)	4 (8%)	0	2 (3%)	10 (4.0%)
Adverse event	1 (2%)	0	0	0	0	0	1 (0.4%)
Protocol violation	2 (4%)	0	0	2 (4%)	0	0	4 (1.6%)
Subject choice	0	0	1 (3%)	1 (2%)	0	1 (2%)	3 (1.2%)
Sponsor choice	0	0	0	1 (2%)	0	0	1 (0.4%)
Other	0	0	0	0	0	1 (2%)	1 (0.4%)

[0338] Interim data of this study are summarized in Table 16 below and in Figures 28-36.

Table 16: Patients' Total Cholesterol, non-HDL-c, and Apolipoprotein B, Measured from Baseline to Nadir

	400 mg /4W (n=57)	*200 mg /8W (n=23)*	*400 mg /8W (n=30)*	*800 mg /8W (n=50)*	*800 mg /12W (n=23)*	*Placebo (n=63)*
TC, mean absolute change (mg/dL)	-99.9	-73.9	-92.3	-102.0	-92.3	-24.4
Reduction from placebo	**74.9**	**48.7**	**64.7**	**75.5**	**66.2**	
95% confidence interval	65.2, 84.6	36.0, 61.5	52.9, 76.4	65.5, 85.5	53.4, 79.0	
TC, mean relative change (%)	-49.4	-37.7	-43.6	-48.6	-44.8	-12.4
Reduction from placebo	**36.7**	**25.2**	**30.8**	**35.8**	**32.1**	
95% confidence interval	32.6, 40.8	19.8, 30.7	25.7, 35.8	31.6, 40.1	26.6, 37.6	

Non-HDLc, mean abs. ch. (mg/dL)	-101.6	-76.2	-96.3	-103.3	-95.0	-24.1
Reduction from placebo	**76.7**	**51.3**	**68.7**	**76.9**	**69.0**	
95% confidence interval	66.9, 86.5	38.4, 64.1	56.9, 80.5	66.9, 87.0	56.1, 81.9	
Non-HDLc, mean rel. change (%)	-67.3	-52.1	-60.5	-65.9	-59.8	-16.6
Reduction from placebo	**50.3**	**35.5**	**43.7**	**49.1**	**43.0**	
95% confidence interval	45.2, 55.4	28.8, 42.2	37.5, 49.8	43.9, 54.3	36.3, 49.7	
Apo-B, mean abs. change (mg/dL)	-64.3	-48.5	-59.1	-65.6	-62.8	-15.8
Reduction from placebo	**48.1**	**32.3**	**41.4**	**48.5**	**46.0**	
95% confidence interval	42.0, 54.3	24.2, 40.4	33.9, 48.8	42.2, 54.9	37.8, 54.1	
Apo-B, mean relative change (%)	-63.1	-49.2	-55.8	-62.7	-58.3	-15.7
Reduction from placebo	**47.0**	**33.4**	**39.9**	**46.8**	**42.3**	
95% confidence interval	42.2, 51.7	27.1, 39.6	34.2, 45.7	41.9, 51.7	36.1, 48.6	

[0339] Figure 28 provides mean pharmacokinetics (+/- standard deviation) (left panel) and mean total PCSK9, e.g. both drug-bound and free PCSK9 (+/- standard error) (right panel).

[0340] Figure 29 shows the absolute change from baseline in direct LDL cholesterol observed in patients receiving anti-PCSK9 antibody or placebo. Figure 30 shows the relative change from baseline in direct LDL cholesterol observed in patients receiving anti-PCSK9 antibody or placebo. Patients receiving 400 mg of anti-PCSK9 antibody every 4 weeks and patients receiving 800 mg of anti-PCSK9 antibody every 8 weeks exhibited the highest reduction in direct LDL-c. This effect was observed within a week of treatment. Patients receiving 800 mg of anti-PCSK9 antibody every 12 weeks exhibited the lowest reduction in direct LDL-c.

[0341] Figure 31 shows the absolute change from baseline in total cholesterol observed in patients participating in this study. Figure 32 shows the relative change from baseline in total cholesterol observed in patients receiving anti-PCSK9 antibody or placebo. Patients receiving 400 mg of anti-PCSK9 antibody every 4 weeks and patients receiving 800 mg of anti-PCSK9 antibody every 8 weeks exhibited the highest reduction in total cholesterol. This effect was observed within a week of treatment. Patients receiving 800 mg of anti-PCSK9 antibody every 12 weeks exhibited the lowest reduction in total cholesterol.

[0342] Figure 33 shows the absolute change from baseline in non-HDL cholesterol in patients participating in this study. Figure 34 shows the relative change from baseline in non-

HDL cholesterol in patients participating in this study. Patients receiving 400 mg of anti-PCSK9 antibody every 4 weeks and patients receiving 800 mg of anti-PCSK9 antibody every 8 weeks exhibited the highest reduction in non-HDL cholesterol. This effect was observed within a week of treatment. Patients receiving 800 mg of anti-PCSK9 antibody every 12 weeks exhibited the lowest reduction in non-HDL cholesterol.

[0343] Figure 35 shows the absolute change from baseline in apolipoprotein B in patients participating in this study. Figure 36 shows the relative change from baseline in apolipoprotein B in patients participating in this study. Patients receiving 400 mg of anti-PCSK9 antibody every 4 weeks and patients receiving 800 mg of anti-PCSK9 antibody every 8 weeks exhibited the highest reduction in apolipoprotein B. This effect was observed within a week of treatment. Patients receiving 800 mg of anti-PCSK9 antibody every 12 weeks exhibited the lowest reduction in apolipoprotein B.

[0344] Conclusions regarding the efficacy of study drug are summarized here. The highest dose-dependent reduction in LDL-c on Day 85, at nadir, and AUC was observed in patients receiving 400 mg of anti-PCSK9 antibody every 4 weeks and in patients receiving 800 mg of anti-PCSK9 antibody every 8 weeks. The smallest dose-dependent reduction in LDL-c based on Day 85 analyses was observed in patients receiving 800 mg of anti-PCSK9 antibody every 12 weeks. The smallest dose-dependent reduction in LDL-c based on nadir and AUC analyses was observed in patients receiving 200 mg of anti-PCSK9 antibody every 8 weeks. The reduction was evident within a week of treatment. Dose-dependent reduction in total cholesterol, non-HDL-c, and apolipoprotein-B was observed on Day 85 and at nadir, and the reduction was also evident within a week of treatment.

[0345] Finally, patients were also monitored for safety including incidence, nature, and severity of adverse events; incidence and nature of changes in vital signs, physical findings, and clinical laboratory results during and following study drug administration; and incidence of anti-therapeutic antibodies directed against study drug.

[0346] The safety of low LDL-c values was assessed regularly in a blinded, exploratory manner. Figure 37A shows the proportion of patients with direct LDL-c values less than or equal to 15 mg/dL for at least one visit after receiving anti-PCSK9 antibody or placebo, and Figure 37B shows the proportion of patients with direct LDL-c values less than or equal to 25 mg/dL for at least one visit after receiving anti-PCSK9 antibody or placebo. The highest percentage of patients with LDL-c $\leq$ 15 mg/dL or LDL-c $\leq$ 25 mg/dL were receiving either

400 mg of drug every four weeks or 800 mg of drug every 8 weeks. The lowest percent of patients with LDL-c $\leq$ 25 mg/dL were receiving 200 mg of drug every 8 weeks. Study drug was withheld from patients with two consecutive LDL-c values of $<$ 15 mg/dL. This was not considered an adverse event. Such patients were treated with placebo instead, in blinded fashion, until LDL-c increased to $\geq$ 50 mg/dL, after which these patients were switched to the lowest dosage (200 mg every 8 weeks).

[0347] Conclusions regarding the safety of study drug are summarized here. Briefly, anti-PCSK9 antibody was well tolerated in patients aged 37-80 with elevated baseline LDL-c (90-250 mg/dL), diagnosed with CHD or a CHD risk equivalent, and who were taking stable doses of statins or were statin-intolerant. Injection-site reactions were more common among patients receiving study drug (25%) vs. placebo (9%). Only 2 injection-site reactions were moderate (1 placebo, 1 study drug), and the rest were mild in severity. No other clinically significant imbalances of treatment-emergent events were observed between study drug-treated and placebo-treated patients. No clinically relevant imbalances in laboratory abnormalities were observed. No safety signals were determined. No deaths were reported, and no new safety concerns were observed. No patterns were detected in safety laboratory results.

[0348] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An anti-PCSK9 antibody comprising a heavy chain and light chain variable domain comprising six hypervariable region (HVR) sequences:

(i) HVR-H1 comprising GFTFX$_1$X$_2$X$_3$X$_4$IH (SEQ ID NO:28), wherein X$_1$ is S or T; X$_2$ is G, R or S; X$_3$ is H, T or Y; X$_4$ is A or T;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising RASQDVSX$_1$AVA (SEQ ID NO:29), wherein X$_1$ is S or T;

(v) HVR-L2 comprising SASX$_1$LYS (SEQ ID NO:30), wherein X$_1$ is F or S; and

(vi) HVR-L3 comprising QQAYX$_1$X$_2$X$_3$X$_4$T (SEQ ID NO:37), wherein X$_1$ is P, R or T; X$_2$ is A, I, S or T; X$_3$ is L, P or Q; X$_4$ is A, H, P or S.

2. The antibody of claim 1, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

3. The antibody of claim 2, further comprising (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:33.

4. The antibody of claim 1, comprising (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:33.

5. The antibody of claim 1, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:3;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:33.

6. The antibody of claim 1, comprising a VH sequence of SEQ ID NO:17.
7. The antibody of claim 1, comprising a VL sequence of SEQ ID NO:34.
8. The antibody of claim 1, comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:34.
9. The antibody of any one of claims 1 to 8, wherein the antibody is a monoclonal antibody.
10. The antibody of any one of claims 1 to 8, wherein the antibody is humanized.
11. The antibody of any one of claims 1 to 8, wherein the antibody is a human antibody.
12. The antibody of any one of claims 1 to 8, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv or $(Fab')_2$ fragment.
13. The antibody of any one of claims 1 to 8, wherein at least a portion of the framework sequence is a human consensus framework sequence.
14. The antibody of claim 1, comprising (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:35 and a light chain comprising the amino acid sequence of SEQ ID NO:36, (ii) a heavy chain comprising amino acids 1-450 of SEQ ID NO:35 and a light chain comprising the amino acid sequence of SEQ ID NO:36, (iii) a heavy chain comprising amino acids 1-449 of SEQ ID NO:35 and a light chain comprising the amino acid sequence of SEQ ID NO:36, or (iv) the heavy and light chain of any one of (i), (ii), or (iii) wherein P449 of SEQ ID NO:35 is amidated.
15. An anti-PCSK9 antibody comprising (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO:33, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4.
16. An anti-PCSK9 antibody comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:34.
17. An isolated nucleic acid encoding the anti-PCSK9 antibody of any one of claims 1 to 16.
18. A vector comprising the nucleic acid of claim 17.
19. The vector of claim 18, wherein the vector is an expression vector.
20. A host cell comprising the vector of claim 18 or 19.
21. The host cell of claim 20, wherein the host cell is prokaryotic.
22. The host cell of claim 20, wherein the host cell is eukaryotic.

23. A method for making an anti-PCSK9 antibody, said method comprising culturing the host cell of claim 20 under conditions suitable for expression of the nucleic acid encoding the anti-PCSK9 antibody.

24. The method of claim 23, further comprising recovering the anti-PCSK9 antibody produced by the host cell.

25. An anti-PCSK9 antibody produced by a method comprising culturing the host cell of claim 20 under conditions suitable for expression of the nucleic acid encoding the anti-PCSK9 antibody, and recovering the anti-PCSK9 antibody produced by the host cell.

26. A pharmaceutical composition comprising the anti-PCSK9 antibody of any one of claims 1-16 and 25 and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising an anti-PCSK9 antibody at 150 to 225 mg/mL, histidine acetate at 10 to 30 mM, arginine acetate at 150 to 170 mM, polysorbate at 0.01% to 0.03%, and pH at 5.8 to 6.2.

28. The composition of claim 27, wherein the anti-PCSK9 antibody or antibody fragment in the composition is at 200 mg/mL, histidine acetate in the composition is at 20 mM, arginine acetate in the composition is at 160 mM, and polysorbate 20 in the composition is 0.02%, and pH at 6.0.

29. The composition of claim 27, wherein the composition is suitable for subcutaneous administration.

30. The composition of any one of claims 27-29, wherein the viscosity of the composition is less than 10 cP at 25°C.

31. The composition of any one of claims 27-30, wherein the anti-PCSK9 antibody comprises a variable domain comprising one, two, three, four, five, or six hypervariable region (HVR) sequences selected from the group consisting of:

(i) HVR-H1 comprising $GFTFX_1X_2X_3X_4IH$ (SEQ ID NO:28), wherein X_1 is S or T; X_2 is G, R or S; X_3 is H, T or Y; X_4 is A or T;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising $RASQDVSX_1AVA$ (SEQ ID NO:29), wherein X_1 is S or T;

(v) HVR-L2 comprising $SASX_1LYS$ (SEQ ID NO:30), wherein X_1 is F or S; and

(vi) HVR-L3 comprising $QQSYX_1X_2X_3X_4T$ (SEQ ID NO:31) or $QQAYX_1X_2X_3X_4T$ (SEQ ID NO:37), wherein X_1 is P, R or T; X_2 is A, I, S or T; X_3 is L, P or Q; X_4 is A, H, P or S.

32. The composition of any one of claims 27-30, wherein the anti-PCSK9 antibody comprises a heavy chain and light chain variable domain comprising the following six hypervariable region (HVR) sequences:

(i) HVR-H1 comprising $GFTFX_1X_2X_3X_4IH$ (SEQ ID NO:28), wherein X_1 is S or T; X_2 is G, R or S; X_3 is H, T or Y; X_4 is A or T;

(ii) HVR-H2 comprising RISPANGNTNYADSVKG (SEQ ID NO:4);

(iii) HVR-H3 comprising WIGSRELYIMDY (SEQ ID NO:5);

(iv) HVR-L1 comprising $RASQDVSX_1AVA$ (SEQ ID NO:29), wherein X_1 is S or T;

(v) HVR-L2 comprising $SASX_1LYS$ (SEQ ID NO:30), wherein X_1 is F or S; and

(vi) HVR-L3 comprising $QQSYX_1X_2X_3X_4T$ (SEQ ID NO:31) or $QQAYX_1X_2X_3X_4T$ (SEQ ID NO:37), wherein X_1 is P, R or T; X_2 is A, I, S or T; X_3 is L, P or Q; X_4 is A, H, P or S.

33. The composition of claim 32, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:5.

34. The composition of claim 33, wherein the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

35. The composition of claim 32, wherein the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:26; and (c)

HVR-L3 comprising the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:33.

36. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:10.

37. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:11.

38. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:12.

39. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:3;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

40. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

41. The composition of claim 32, wherein the antibody comprises:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:3;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:5;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:33.

42. The composition of claim 32, wherein the antibody comprises a VH sequence of SEQ ID NO:15, SEQ ID NO:27, SEQ ID NO:16, or SEQ ID NO:17.

43. The composition of claim 32, wherein the antibody comprises a VL sequence of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:34.

44. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:18.

45. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:19.

46. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:20.

47. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:16 and a VL sequence of SEQ ID NO:21.

48. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:22.

49. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:27 and a VL sequence of SEQ ID NO:23.

50. The composition of claim 32, wherein the antibody comprises comprising a VH sequence of SEQ ID NO:17 and a VL sequence of SEQ ID NO:34.

51. A subcutaneous administration device containing the composition of any one of claims 26-50, for delivering to an individual a flat dose in the range of 200 to 1200 mg of the antibody.
52. The device of claim 51, wherein the device is a pre-filled syringe.
53. The device of claim 51, wherein the device is a 1-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is 200 mg/mL.
54. The device of claim 51, wherein the device is a 2.25-mL pre-filled syringe and the antibody concentration in the pre-filled syringe is 200 mg/mL.
55. A method of reducing LDL-cholesterol level in a subject, said method comprising administering to the subject an effective amount of the anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50.
56. A method of treating cholesterol related disorder in a subject, said method comprising administering to the subject an effective amount of the anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50.
57. A method of treating hypercholesterolemia in a subject, said method comprising administering to the subject an effective amount of the anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50.
58. The method of any one of claims 55-57, wherein the anti-PCSK9 antibody is administered subcutaneously at 200 mg, 380 mg, 400 mg, 600 mg, 760 mg, or 800 mg per dose every 4 weeks, every 6 week, every 8 weeks, every 10 weeks, or every 12 weeks.
59. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 200 mg.
60. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 380 mg.
61. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 400 mg.
62. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 600 mg.
63. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 760 mg.

64. The method of claims 58, wherein the anti-PCSK9 antibody is administered subcutaneously at 800 mg.

65. The method of any one of claims 59-64, wherein the anti-PCSK9 antibody is administered every 4 weeks.

66. The method of any one of claims 59-64, wherein the anti-PCSK9 antibody is administered every 6 weeks.

67. The method of any one of claims 59-64, wherein the anti-PCSK9 antibody is administered every 8 weeks.

68. The method of any one of claims 59-64, wherein the anti-PCSK9 antibody is administered every 10 weeks.

69. The method of any one of claims 59-64, wherein the anti-PCSK9 antibody is administered every 12 weeks.

70. The method of any one of claims 55-69, further comprising administering to the subject an effective amount of a second medicament, wherein the anti-PCSK9 antibody is the first medicament.

71. The method of claim 70, wherein the second medicament elevates the level of LDLR.

72. The method of claim 70, wherein the second medicament reduces the level of LDL-cholesterol.

73. The method of claim 70, wherein the second medicament comprises a statin.

74. The method of claim 73, wherein the statin is selected from the group consisting of atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and any combination thereof.

75. The method of claim 70, wherein the second medicament elevates the level of HDL-cholesterol.

76. A method of inhibiting binding of PCSK9 to LDLR in a subject, said method comprising administering to the subject an effective amount of the anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50.

77. A method of reducing LDL-cholesterol level in a subject, said method comprising administering to the subject subcutaneously an effective amount of an anti-PCSK9 antibody at 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months.

78. The method of claim 67, wherein the LDL-cholesterol level is reduced by at least 45% from baseline and maintains at the reduced level for at least one month after last dosing.

79. A method of treating cholesterol related disorder in a subject, said method comprising administering to the subject subcutaneously an effective amount of an anti-PCSK9 antibody at 400 mg to 1000 mg per dose subcutaneously every 4 weeks to every 12 weeks or every month to every 3 months.

80. A method of treating hypercholesterolemia in a subject, said method comprising administering to the subject subcutaneously an effective amount of an anti-PCSK9 antibody at 400 mg to 1000 mg per dose every 4 weeks or every 12 weeks or every month to every 3 months.

81. An anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for reducing LDL-cholesterol level in a subject.

82. An anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for treating a cholesterol related disorder in a subject.

83. An anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for treating hypercholesterolemia in a subject.

84. An anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for inhibiting binding of PCSK9 to LDLR in a subject.

85. A subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for reducing LDL-cholesterol level in a subject.

86. A subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for treating a cholesterol related disorder in a subject.

87. A subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for treating hypercholesterolemia in a subject.

88. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for reducing LDL-cholesterol level in a subject.

89. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for treating a cholesterol related disorder in a subject.

90. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for treating hypercholesterolemia in a subject.

91. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for inhibiting binding of PCSK9 to LDLR in a subject.

92. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for reducing LDL-cholesterol level in a subject.

93. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for treating a cholesterol related disorder in a subject.

94. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for treating hypercholesterolemia in a subject.

95. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for the manufacture of a medicament for reducing LDL-cholesterol level in a subject.

96. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for the manufacture of a medicament for treating a cholesterol related disorder in a subject.

97. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for the manufacture of a medicament for treating hypercholesterolemia in a subject.

98. Use of an anti-PCSK9 antibody of any one of claims 1-16 and 25, or the composition of any one of claims 26-50, for the manufacture of a medicament for inhibiting binding of PCSK9 to LDLR in a subject.

99. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for the manufacture of a medicament for reducing LDL-cholesterol level in a subject.

100. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3

months for the manufacture of a medicament for treating a cholesterol related disorder in a subject.

101. Use of a subcutaneous dosage of an anti-PCSK9 antibody for administration of 400 mg to 1000 mg per dose every 4 weeks to every 12 weeks or every month to every 3 months for the manufacture of a medicament for treating hypercholesterolemia in a subject.

	CDR H1									
	26	27	28	29	30	31	32	33	34	35
508.20a	G	F	T	F	T	G	Y	A	I	H
508.20b	G	F	T	F	T	G	Y	A	I	H
508.20.04a	G	F	T	F	T	G	Y	A	I	H
508.20.04b	G	F	T	F	T	G	Y	A	I	H
508.20.06	G	F	T	F	T	G	Y	A	I	H
508.20.28a	G	F	T	F	T	R	H	T	I	H
508.20.28b	G	F	T	F	T	R	H	T	I	N
508.20.33a	G	F	T	F	S	S	T	A	I	H
508.20.33b	G	F	T	F	S	S	T	A	I	H
508.20.94	G	F	T	F	T	G	Y	A	I	H

	CDR H2																
	50	51	52	A	53	54	55	56	57	58	59	60	61	62	63	64	65
508.20a	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20b	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.04a	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.04b	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.06	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.28a	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.28b	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.33a	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.33b	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G
508.20.94	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G

	CDR H3													
	95	96	97	98	99	100	A	B	C	D	E	F	101	102
508.20a	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20b	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.04a	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.04b	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.06	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.28a	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.28b	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.33a	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.33b	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.94	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y

	CDR L1											CDR L2							CDR L3								
	24	25	26	27	28	29	30	31	32	33	34	50	51	52	53	54	55	56	89	90	91	92	93	94	95	96	97
508.20a	R	A	S	Q	D	V	S	S	A	V	A	S	A	S	S	L	Y	S	Q	Q	S	Y	T	T	P	P	T
508.20b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	T	T	P	P	T
508.20.04a	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	A	T
508.20.04b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	A	T
508.20.06	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	S	P	A	T
508.20.28a	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	R	I	Q	P	T
508.20.28b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	R	I	Q	P	T
508.20.33a	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	L	H	T
508.20.33b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	A	Y	P	A	L	H	T
508.20.94	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	S	T

FIGURE 1

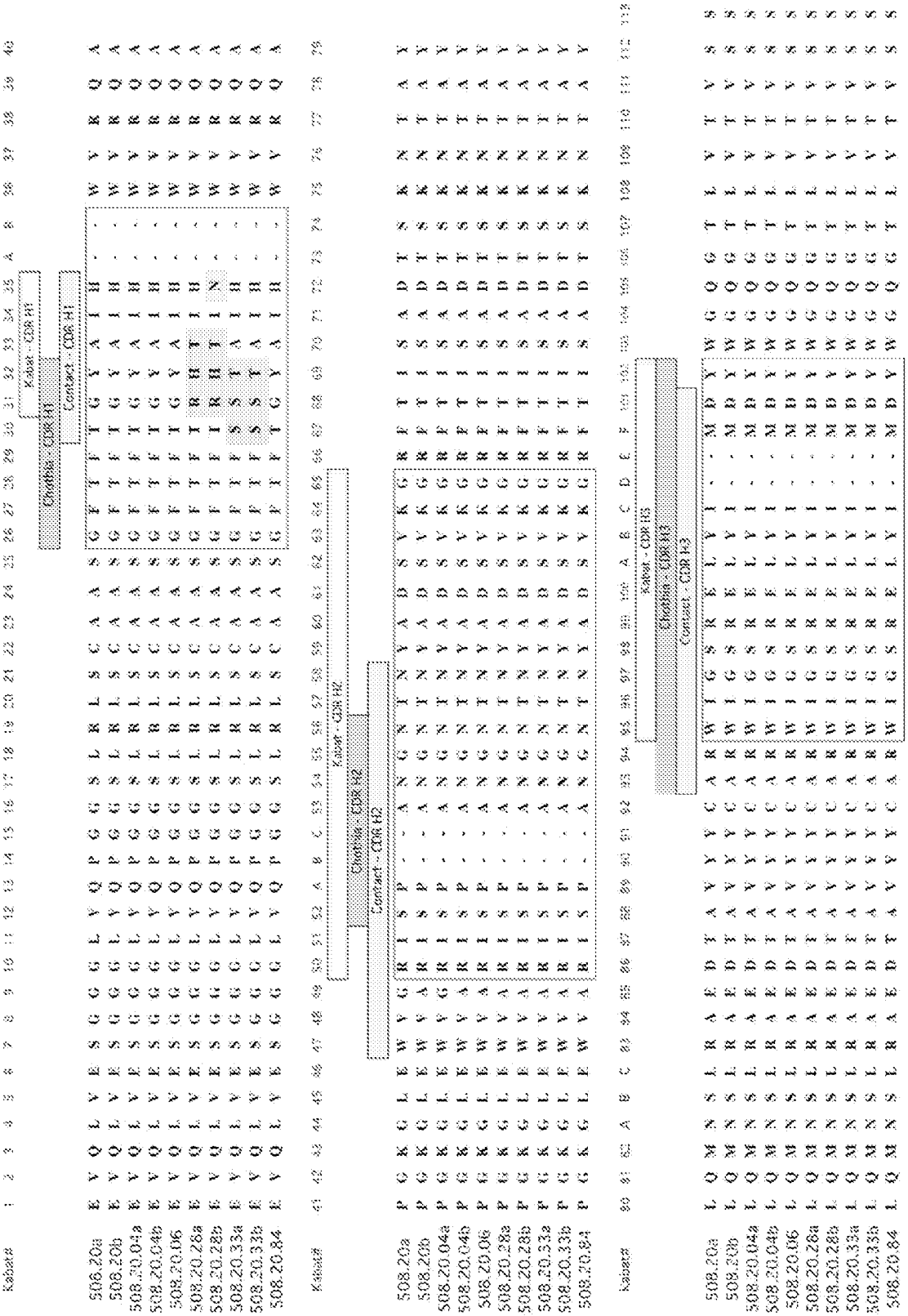

Sequence alignment - Heavy Chain
Kabat - CDR H1
Chothia - CDR H1
Contact - CDR H1
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3
508.20a
508.20b
508.20.04a
508.20.04b
508.20.06
508.20.28a
508.20.28b
508.20.33a
508.20.33b
508.20.84

FIGURE 2A

Sequence alignment - Light Chain

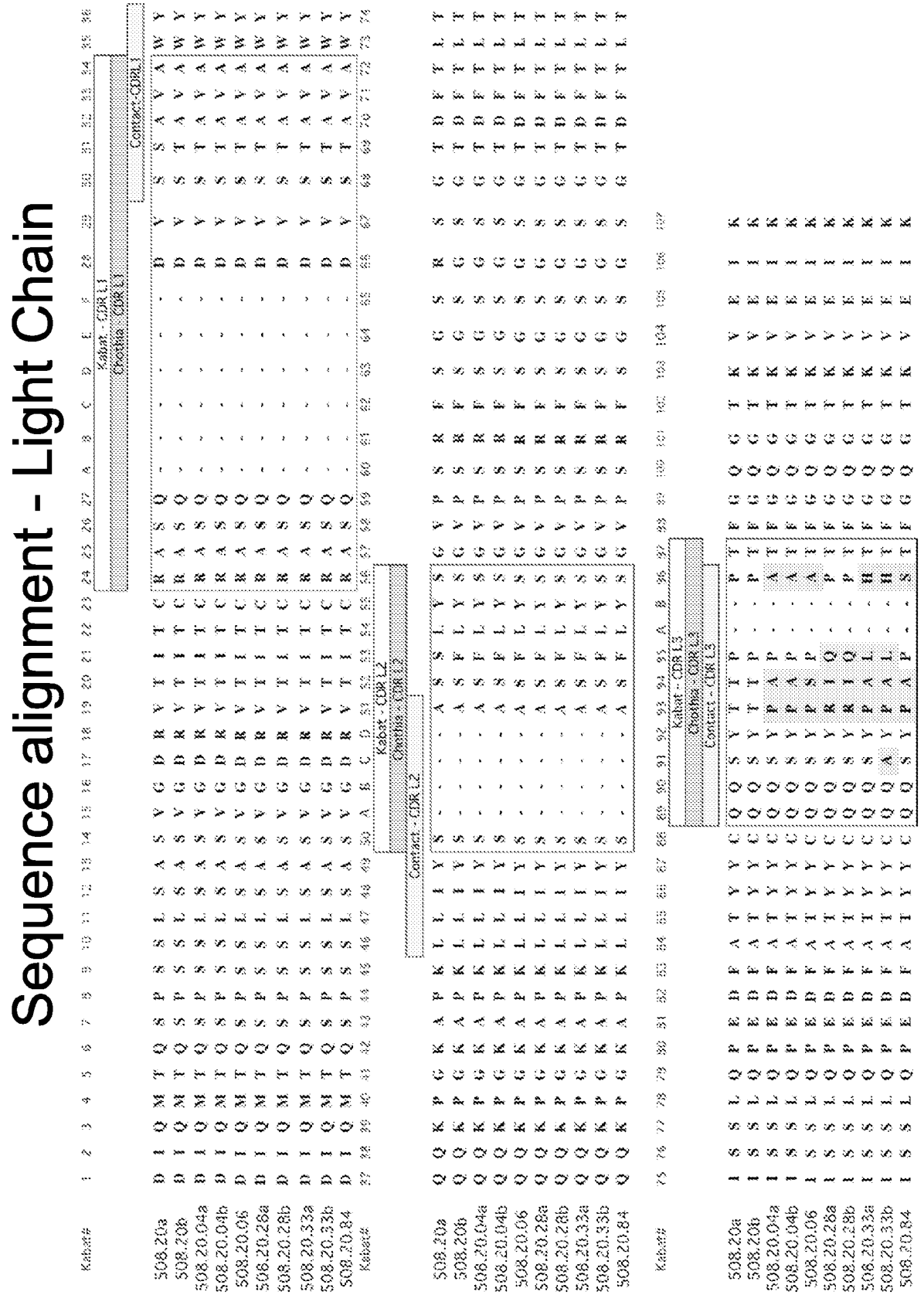


FIGURE 2B

FIGURE 3A

	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.04b	7.47E+04	5.65E-05	0.757
YW508.20.06	1.04E+05	6.51E-05	0.628
YW508.20.28b	5.98E+04	2.09E-05	0.349
YW508.20.33b	5.26E+04	2.15E-05	0.408
YW508.20.84	7.46E+04	3.59E-05	0.481

FIGURE 3B

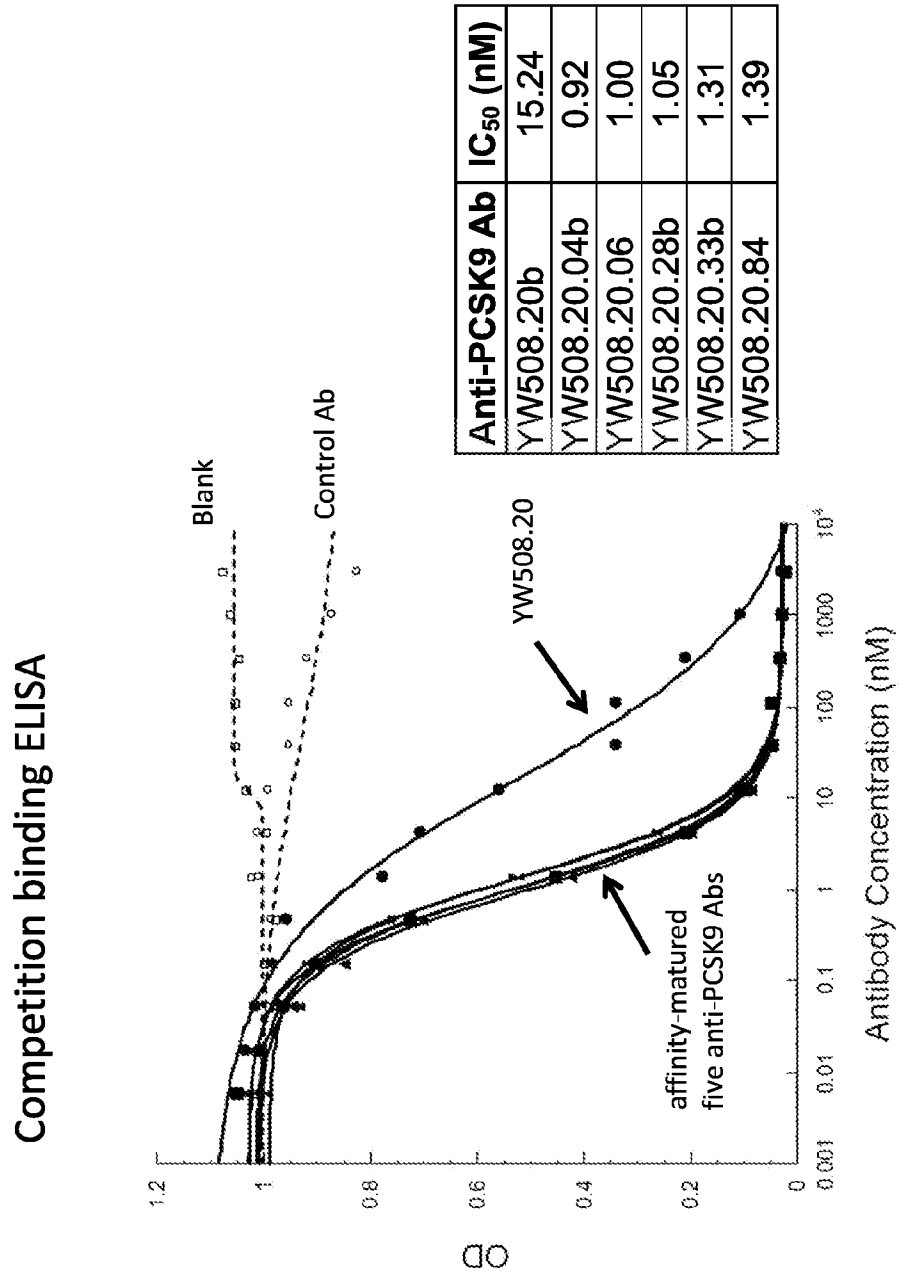
	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.04b	2.70E+05	3.17E-05	0.117
YW508.20.06	2.50E+05	5.02E-05	0.201
YW508.20.28b	1.52E+05	4.90E-05	0.323
YW508.20.33b	1.57E+05	1.94E-06	0.0123
YW508.20.84	1.92E+05	2.59E-05	0.135

FIGURE 3C

YW508.20.33b	Ka (1/Ms)	Kd (1/s)	KD (pM)
Cyno	2.62E+04	1.63E-06	62.4
Rat	6.81E+04	1.24E-06	18.2

FIGURE 3D

	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.33b	8.35E+03	3.42E-05	4.09



Anti-PCSK9 Ab	IC_{50} (nM)
YW508.20b	15.24
YW508.20.04b	0.92
YW508.20.06	1.00
YW508.20.28b	1.05
YW508.20.33b	1.31
YW508.20.84	1.39

FIGURE 4

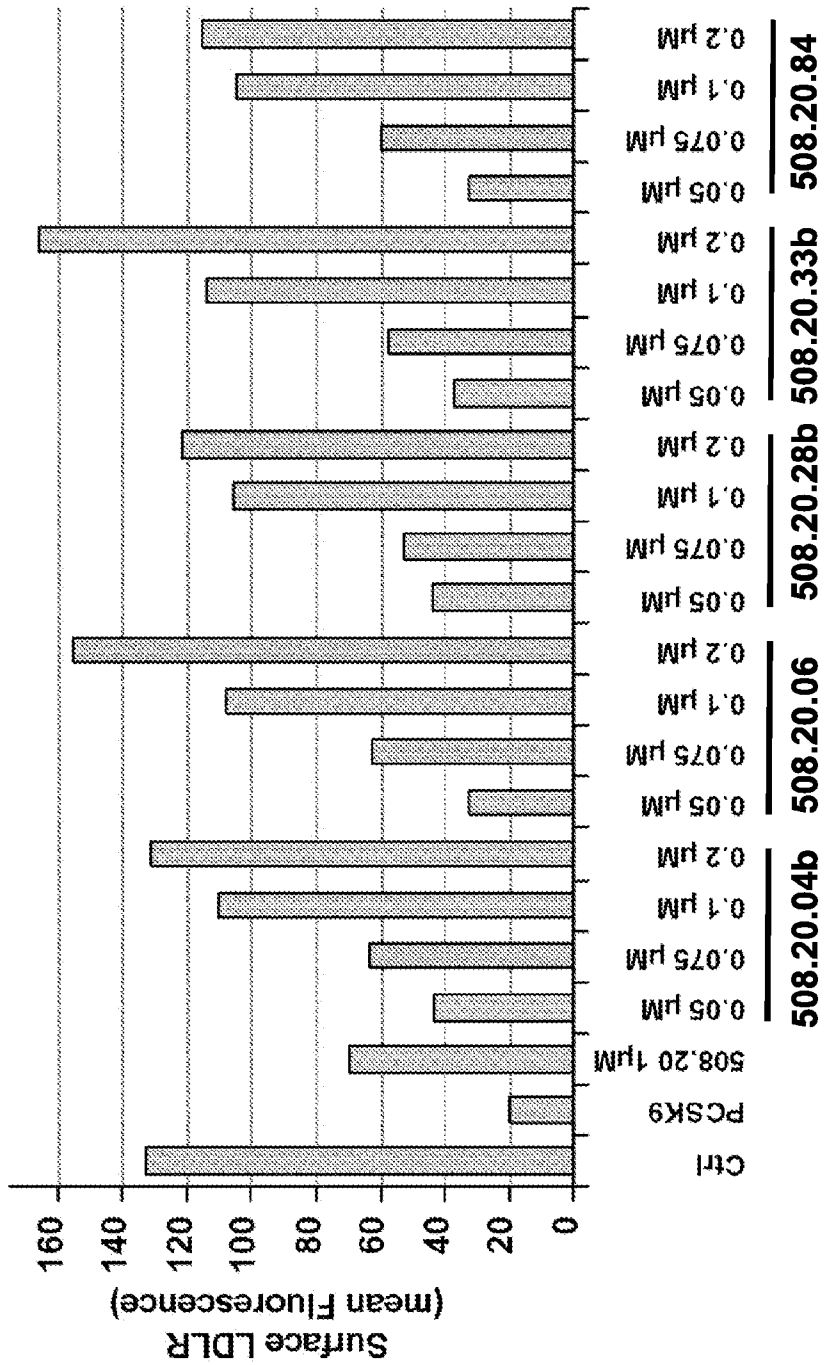

Surface LDLR
(mean Fluorescence)
160
140
120
100
80
60
40
20
0
Ctrl
PCSK9
508.20 1μM
508.20.04b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.06
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.28b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.33b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.84
0.05 μM
0.075 μM
0.1 μM
0.2 μM

FIGURE 5

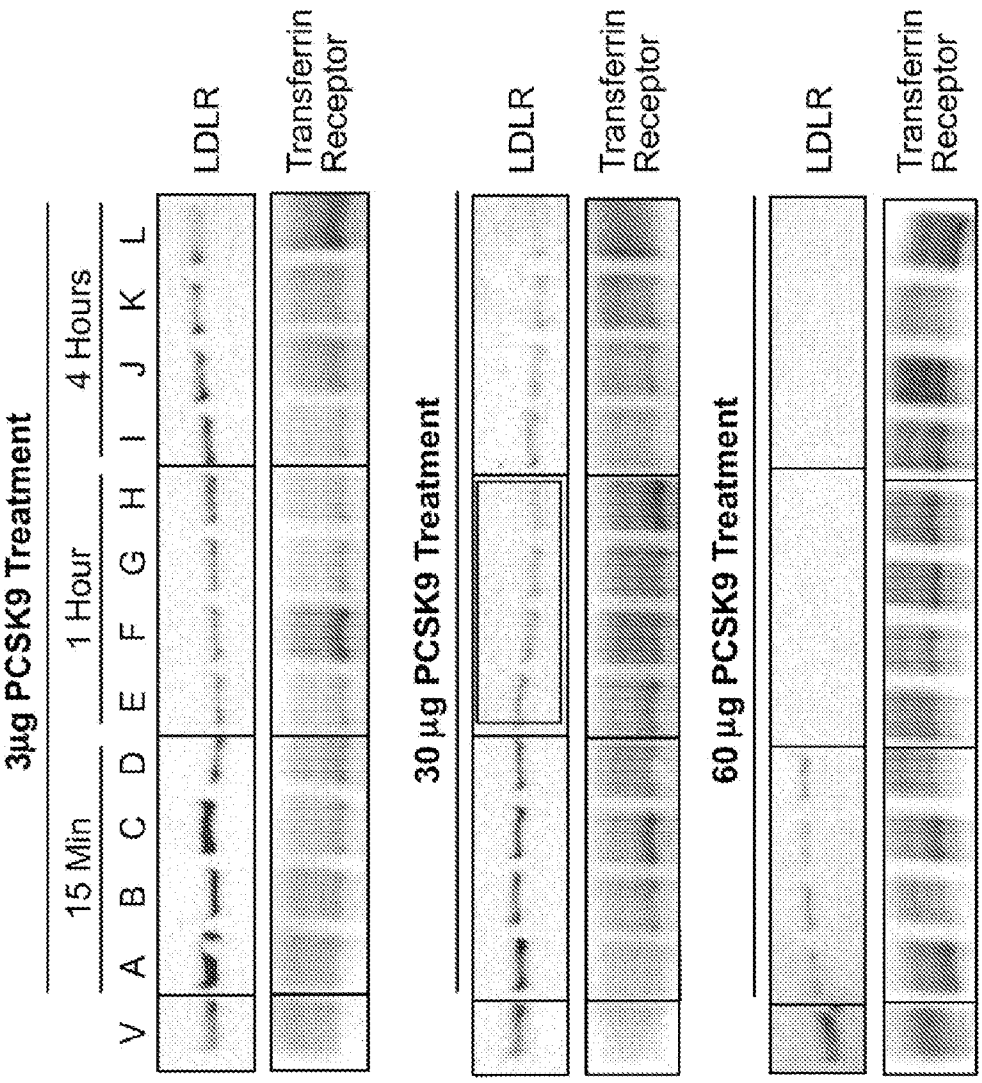
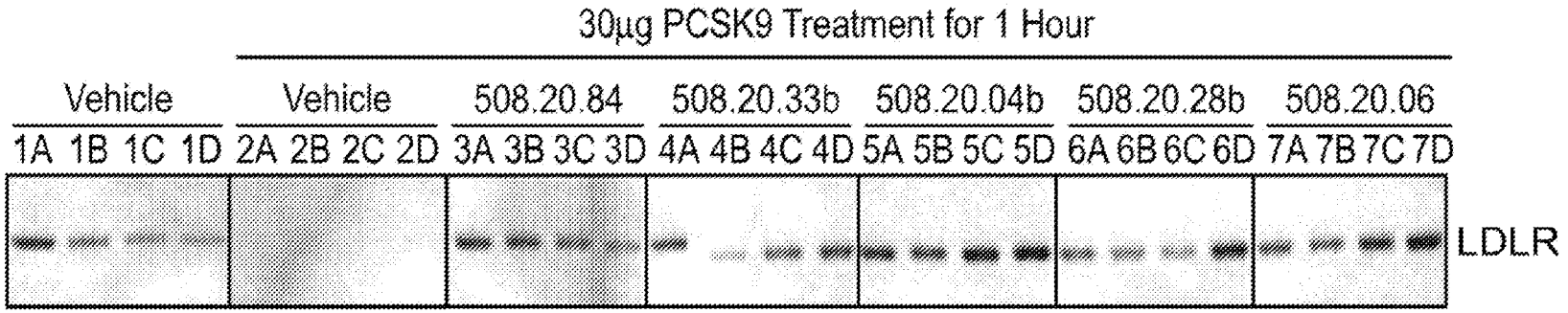

3µg PCSK9 Treatment
15 Min
1 Hour
4 Hours
V A B C D E F G H I J K L
LDLR
Transferrin Receptor
30 µg PCSK9 Treatment
LDLR
Transferrin Receptor
60 µg PCSK9 Treatment
LDLR
Transferrin Receptor

FIGURE 6



Group 1: Vehicle
Group 2: Vehicle + PCSK9
Group 3: 5 mg/kg 508.20.84 + PCSK9
Group 4: 5 mg/kg 508.20.33b + PCSK9
Group 5: 5 mg/kg 508.20.04b + PCSK9
Group 6: 5 mg/kg 508.20.28b + PCSK9
Group 7: 5 mg/kg 508.20.06 + PCSK9

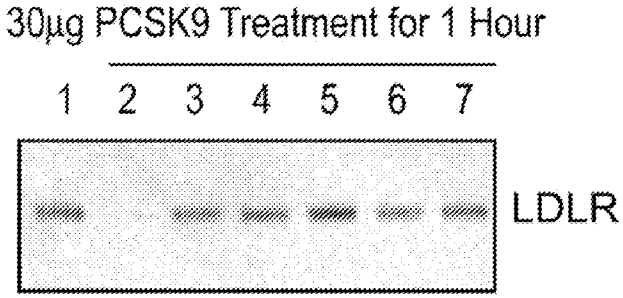


FIGURE 7

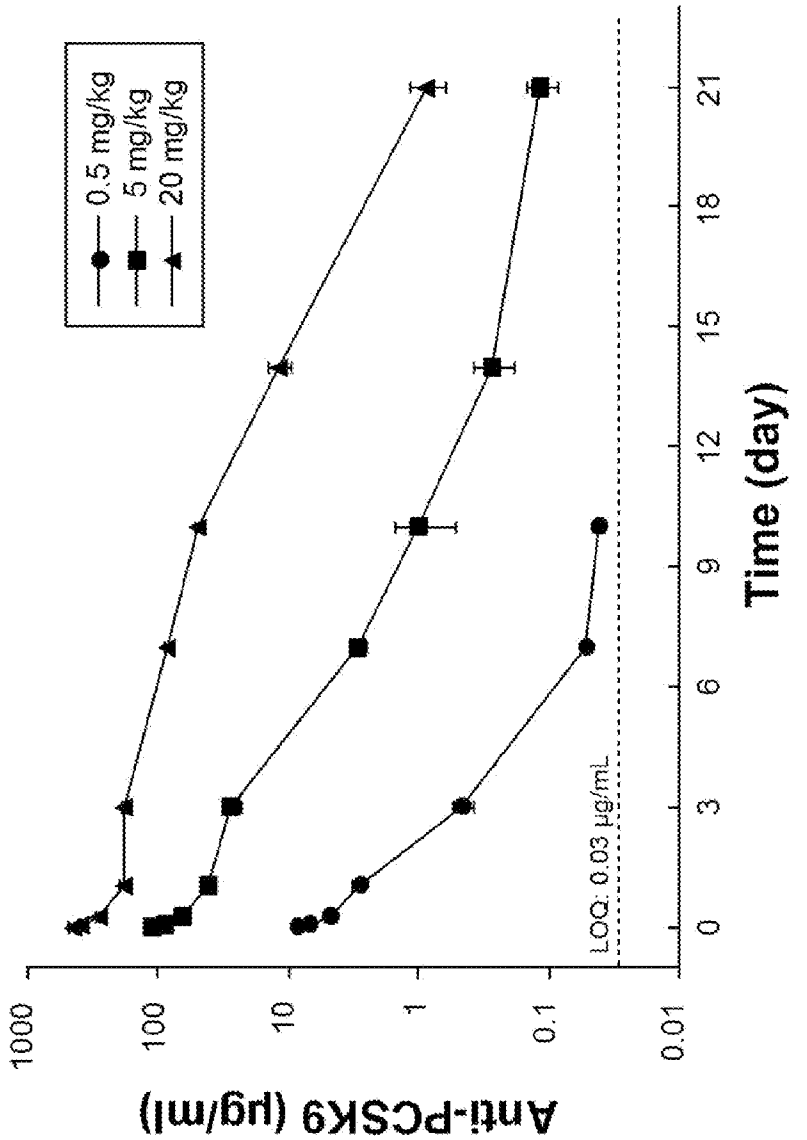

0.5 mg/kg
5 mg/kg
20 mg/kg
Anti-PCSK9 (µg/ml)
1000
100
10
1
0.1
0.01
LOQ: 0.03 µg/mL
0
3
6
9
12
15
18
21
Time (day)

FIGURE 8

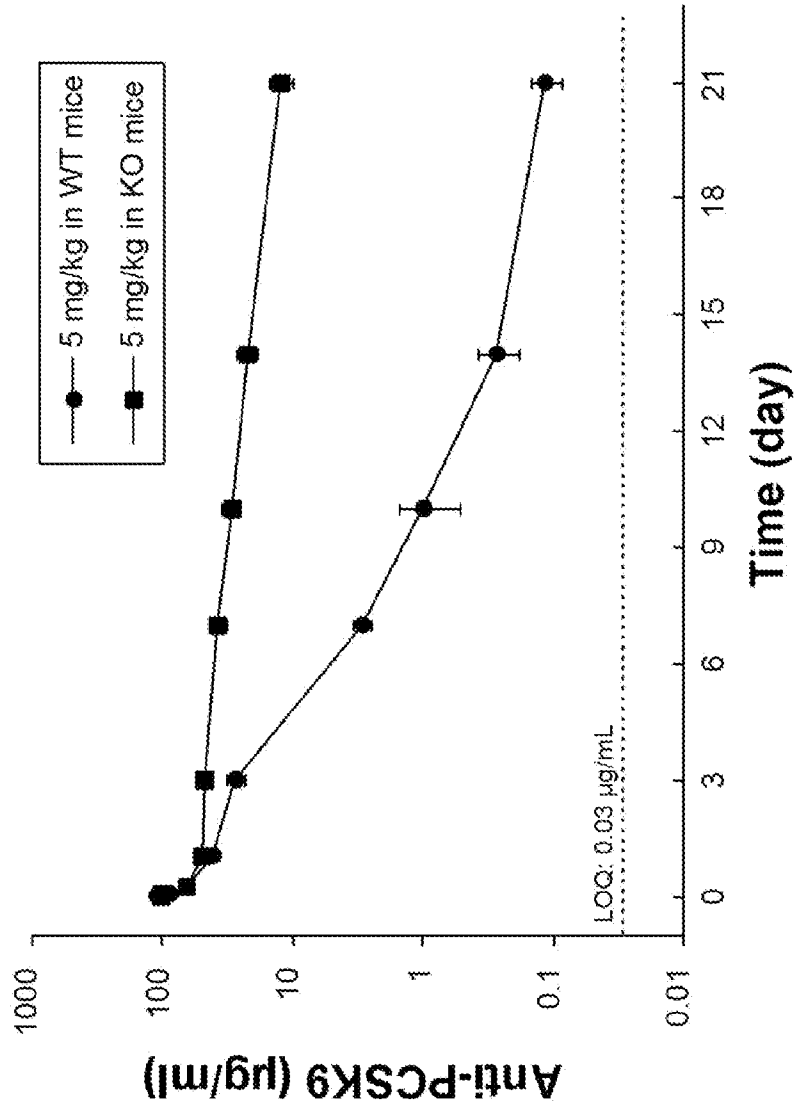

5 mg/kg in WT mice
5 mg/kg in KO mice
Anti-PCSK9 (µg/ml)
1000
100
10
1
0.1
0.01
LOQ: 0.03 µg/mL
0
3
6
9
12
15
18
21
Time (day)

FIGURE 9

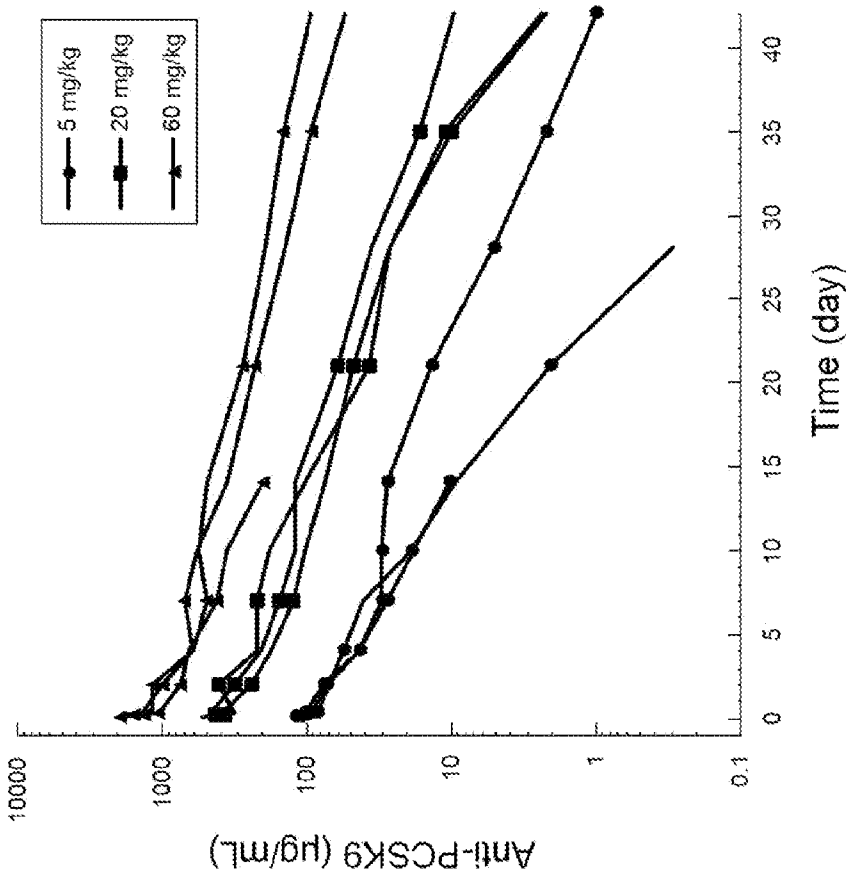

5 mg/kg
20 mg/kg
60 mg/kg
Anti-PCSK9 (µg/mL)
10000
1000
100
10
1
0.1
0
5
10
15
20
25
30
35
40
Time (day)

FIGURE 10

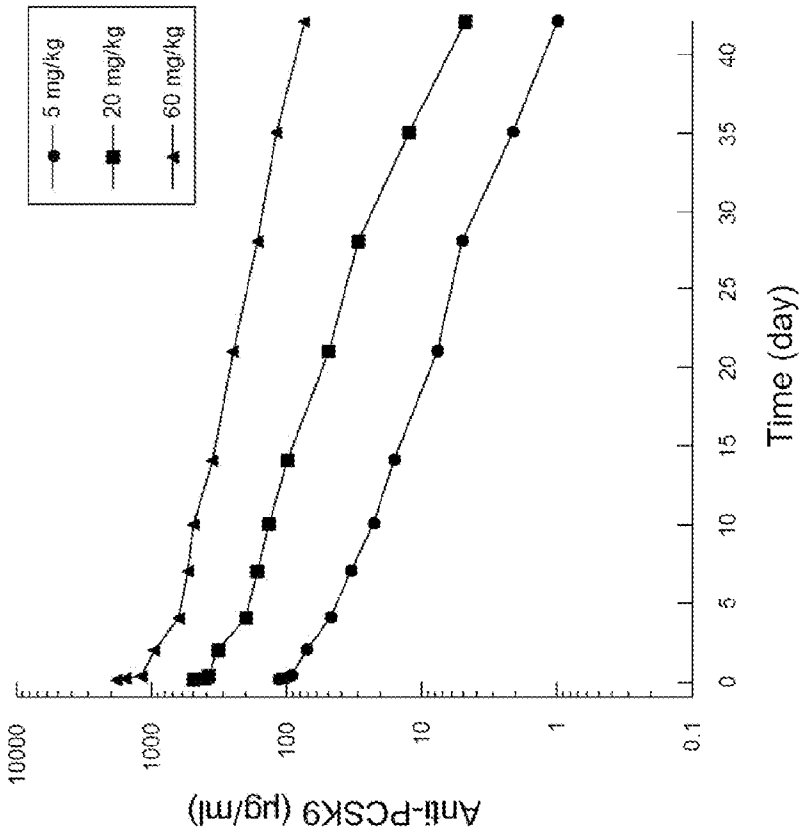

5 mg/kg
20 mg/kg
60 mg/kg
Anti-PCSK9 (μg/ml)
10000
1000
100
10
1
0.1
0
5
10
15
20
25
30
35
40
Time (day)

FIGURE 11

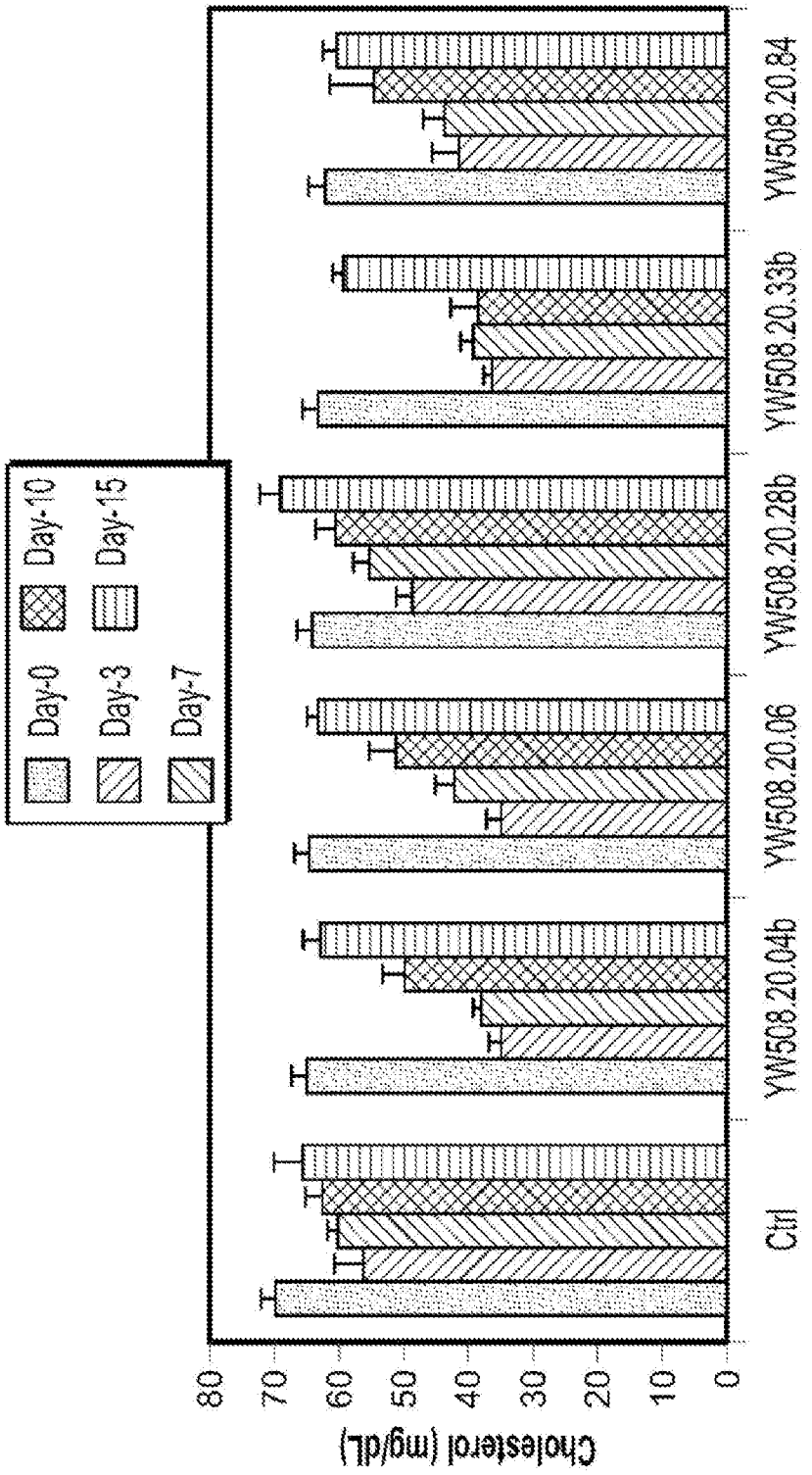

Day-0
Day-3
Day-7
Day-10
Day-15
Cholesterol (mg/dL)
0
10
20
30
40
50
60
70
80
Ctrl
YW508.20.04b
YW508.20.06
YW508.20.28b
YW508.20.33b
YW508.20.84

FIGURE 12

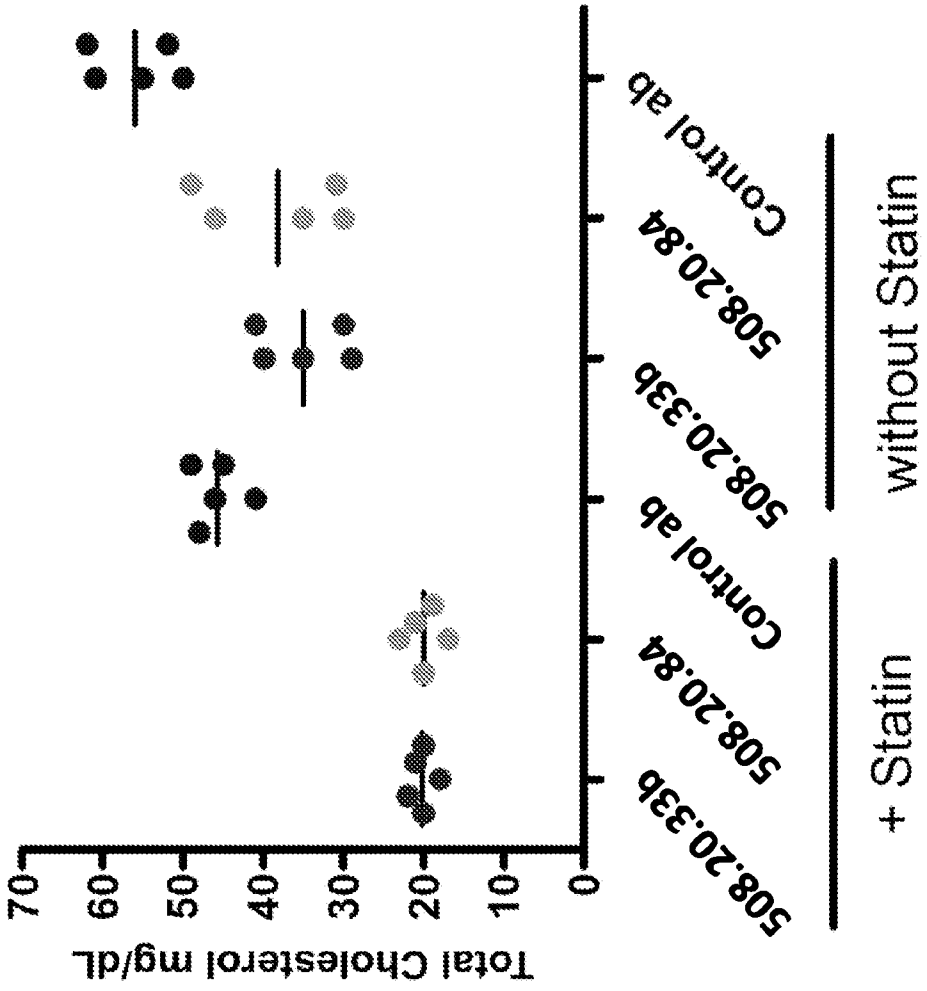

Total Cholesterol mg/dL
70
60
50
40
30
20
10
0
508.20.33b
508.20.84
Control ab
508.20.33b
508.20.84
Control ab
+ Statin
without Statin

FIGURE 13

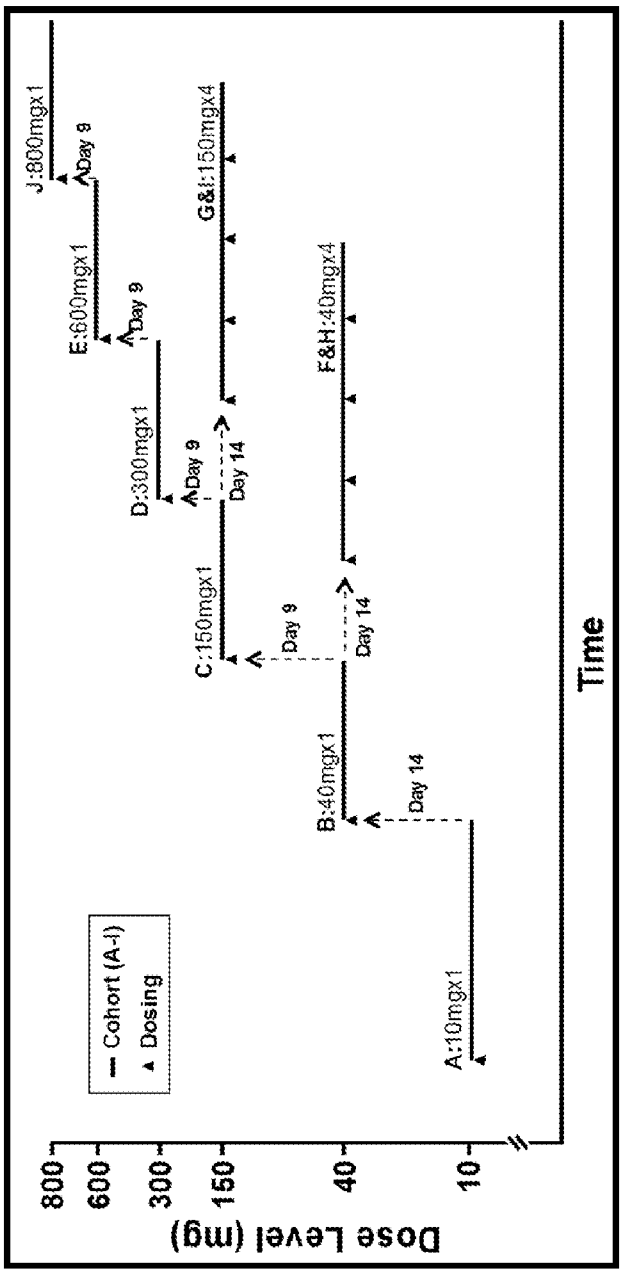

Dose Level (mg)
800
600
300
150
40
10
Time
Cohort (A-I)
Dosing
A:10mgx1
B:40mgx1
Day 14
C:150mgx1
Day 9
Day 14
D:300mgx1
Day 9
E:600mgx1
Day 9
J:800mgx1
Day 9
F&H:40mgx4
G&I:150mgx4

FIGURE 14

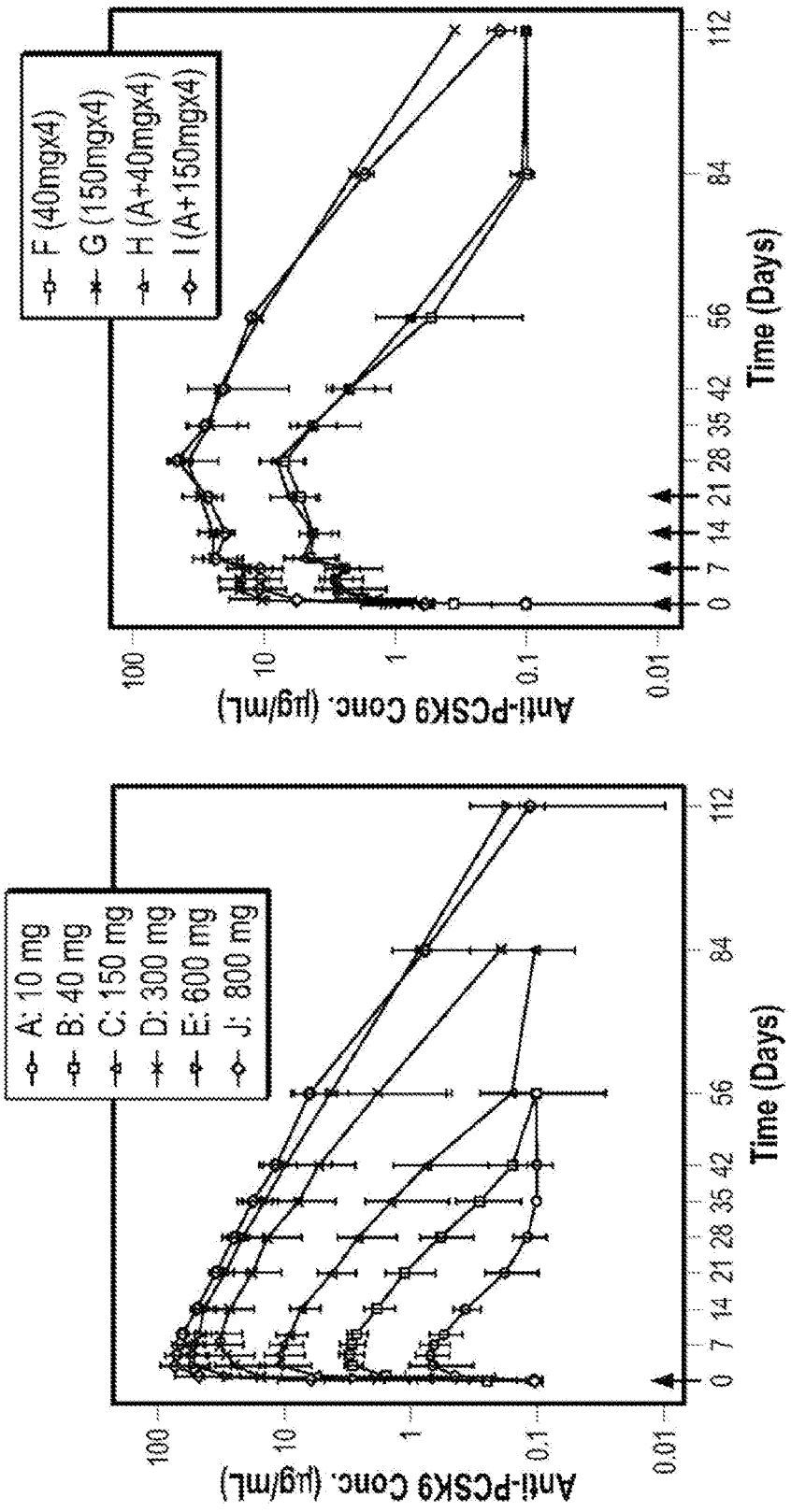

A: 10 mg
B: 40 mg
C: 150 mg
D: 300 mg
E: 600 mg
J: 800 mg
Anti-PCSK9 Conc. (µg/mL)
100
10
1
0.1
0.01
0 7 14 21 28 35 42 56 84 112
Time (Days)
F (40mgx4)
G (150mgx4)
H (A+40mgx4)
I (A+150mgx4)
Anti-PCSK9 Conc. (µg/mL)
100
10
1
0.1
0.01
0 7 14 21 28 35 42 56 84 112
Time (Days)

FIGURE 15

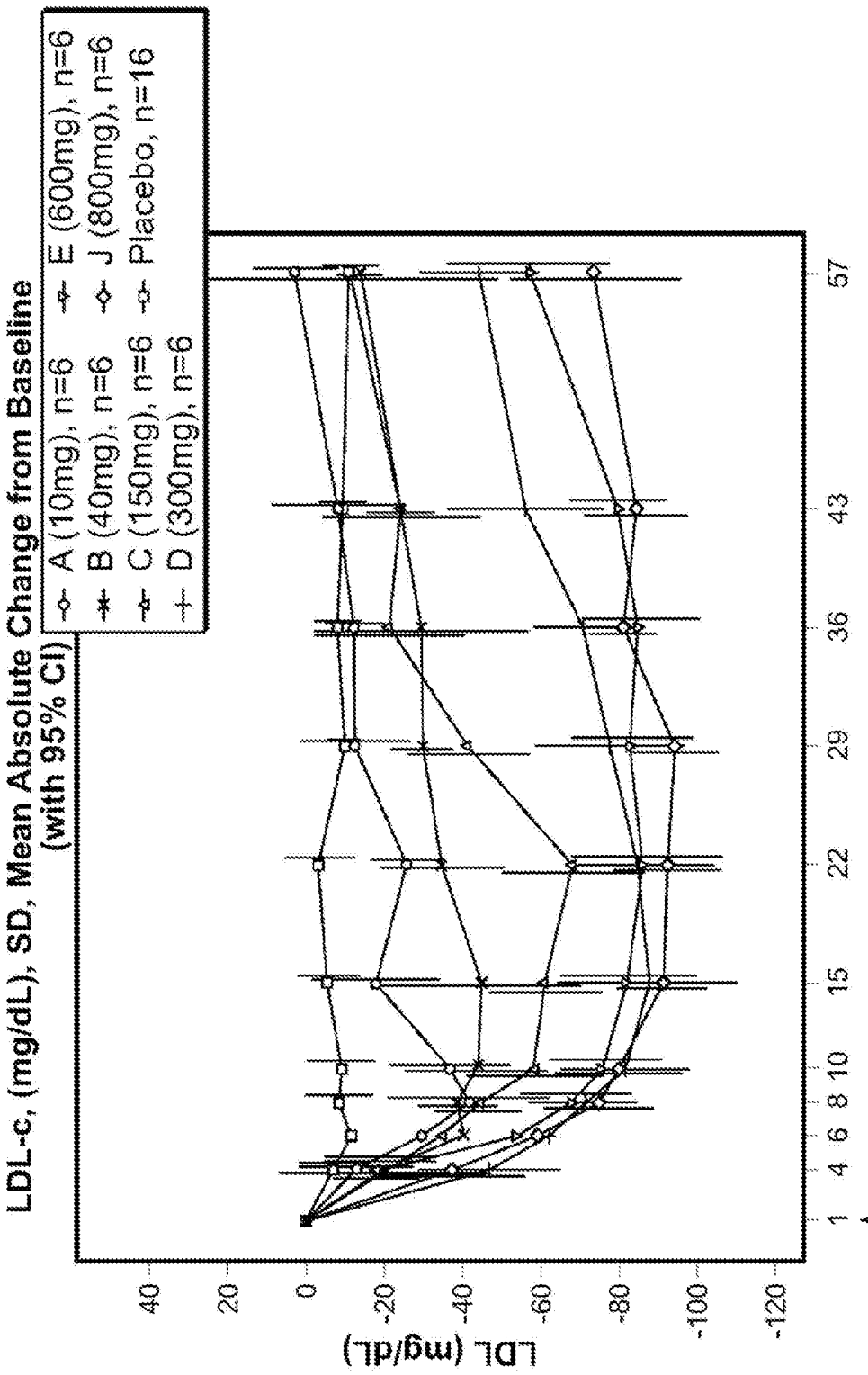

LDL-c, (mg/dL), SD, Mean Absolute Change from Baseline
(with 95% CI)
A (10mg), n=6
B (40mg), n=6
C (150mg), n=6
D (300mg), n=6
E (600mg), n=6
J (800mg), n=6
Placebo, n=16
LDL (mg/dL)
40
20
0
-20
-40
-60
-80
-100
-120
1
4
6
8
10
15
22
29
36
43
57
Single SC Dose
Study Day

FIGURE 16

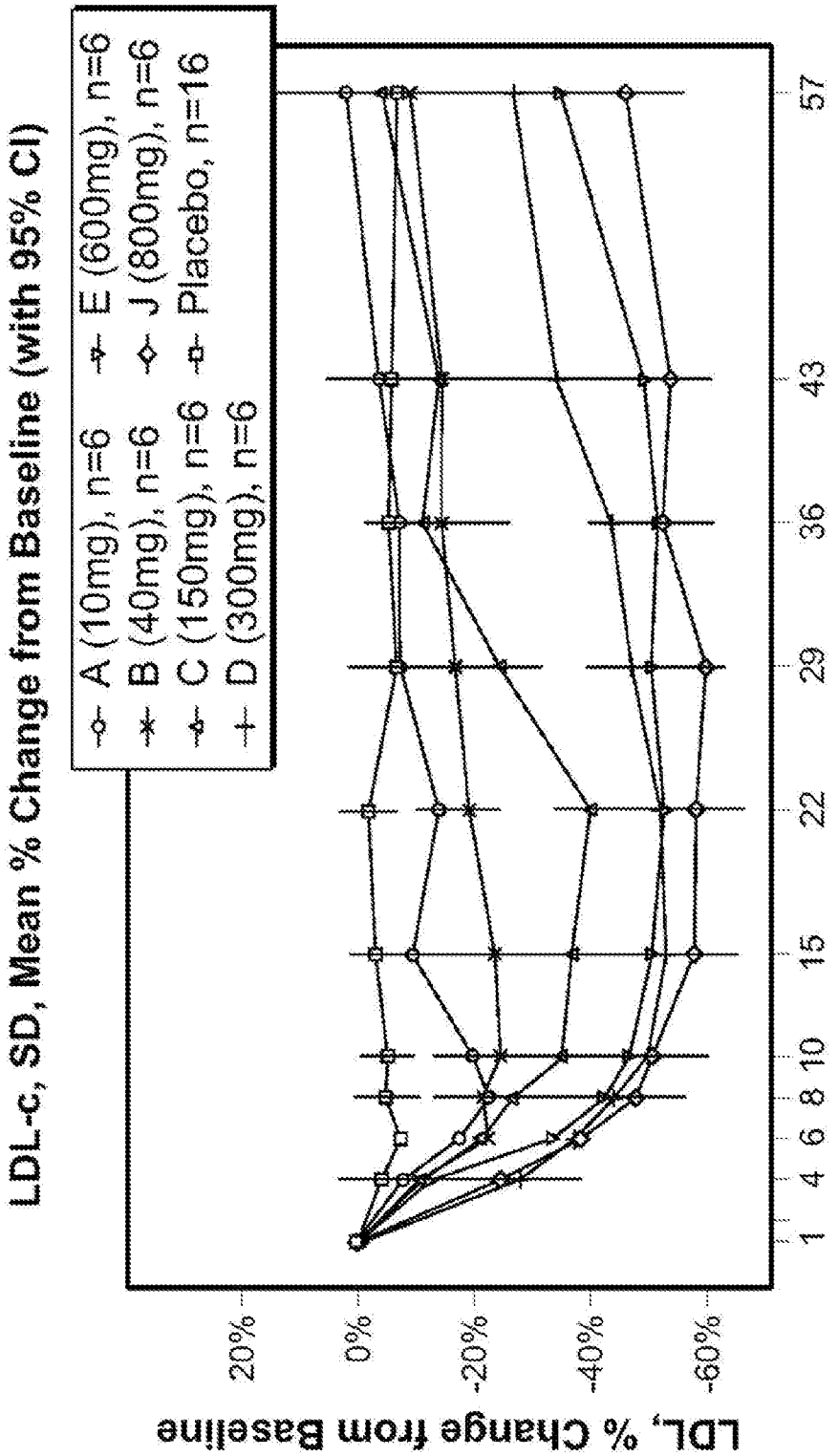

LDL-c, SD, Mean % Change from Baseline (with 95% CI)
A (10mg), n=6
B (40mg), n=6
C (150mg), n=6
D (300mg), n=6
E (600mg), n=6
J (800mg), n=6
Placebo, n=16
LDL, % Change from Baseline
20%
0%
-20%
-40%
-60%
1
4
6
8
10
15
22
29
36
43
57
Study Day
SINGLE SC DOSE

FIGURE 17

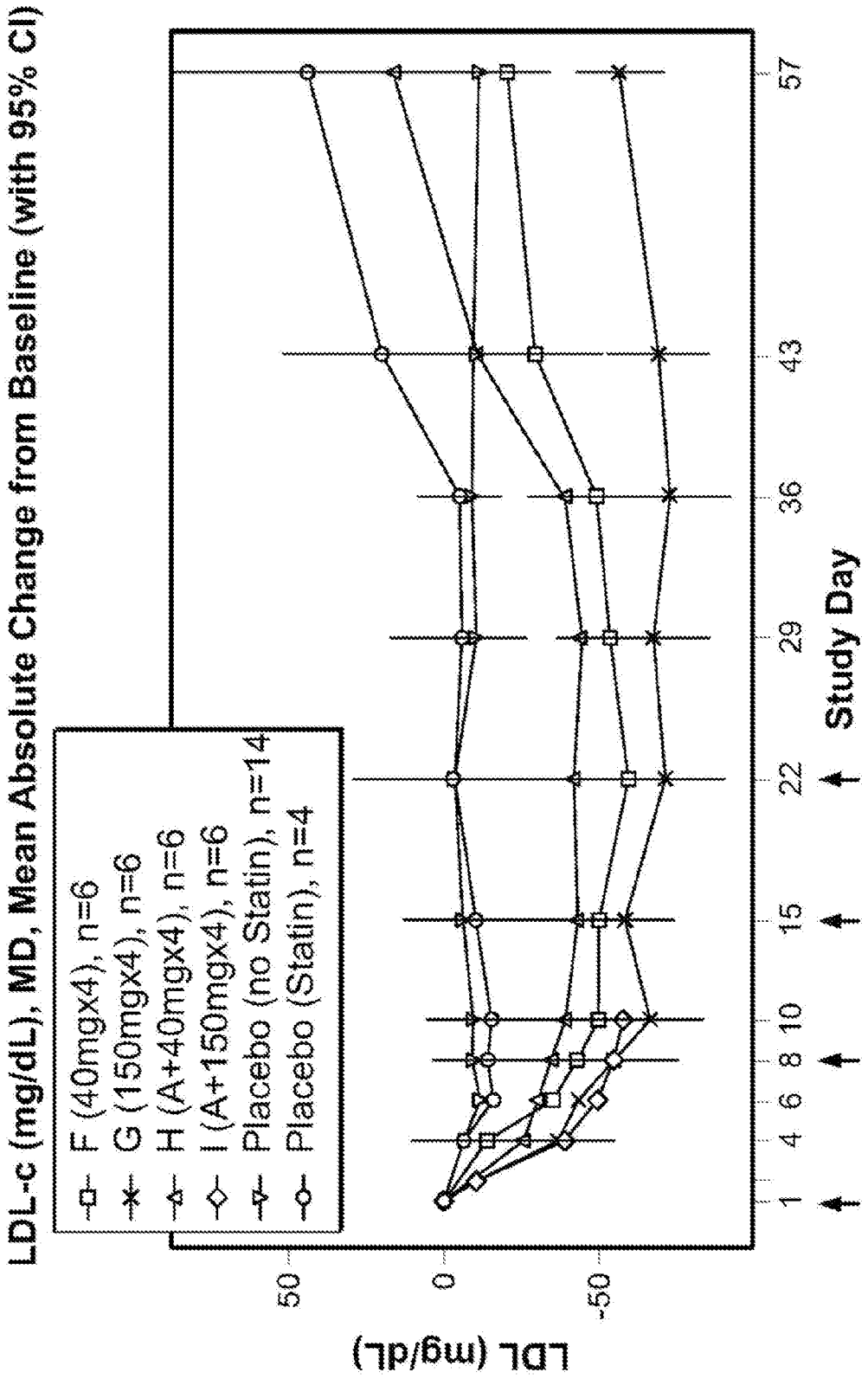

LDL-c (mg/dL), MD, Mean Absolute Change from Baseline (with 95% CI)
F (40mgx4), n=6
G (150mgx4), n=6
H (A+40mgx4), n=6
I (A+150mgx4), n=6
Placebo (no Statin), n=14
Placebo (Statin), n=4
LDL (mg/dL)
50
0
-50
1
4
6
8
10
15
22
29
36
43
57
Study Day
FOUR WEEKLY SC DOSES

FIGURE 18

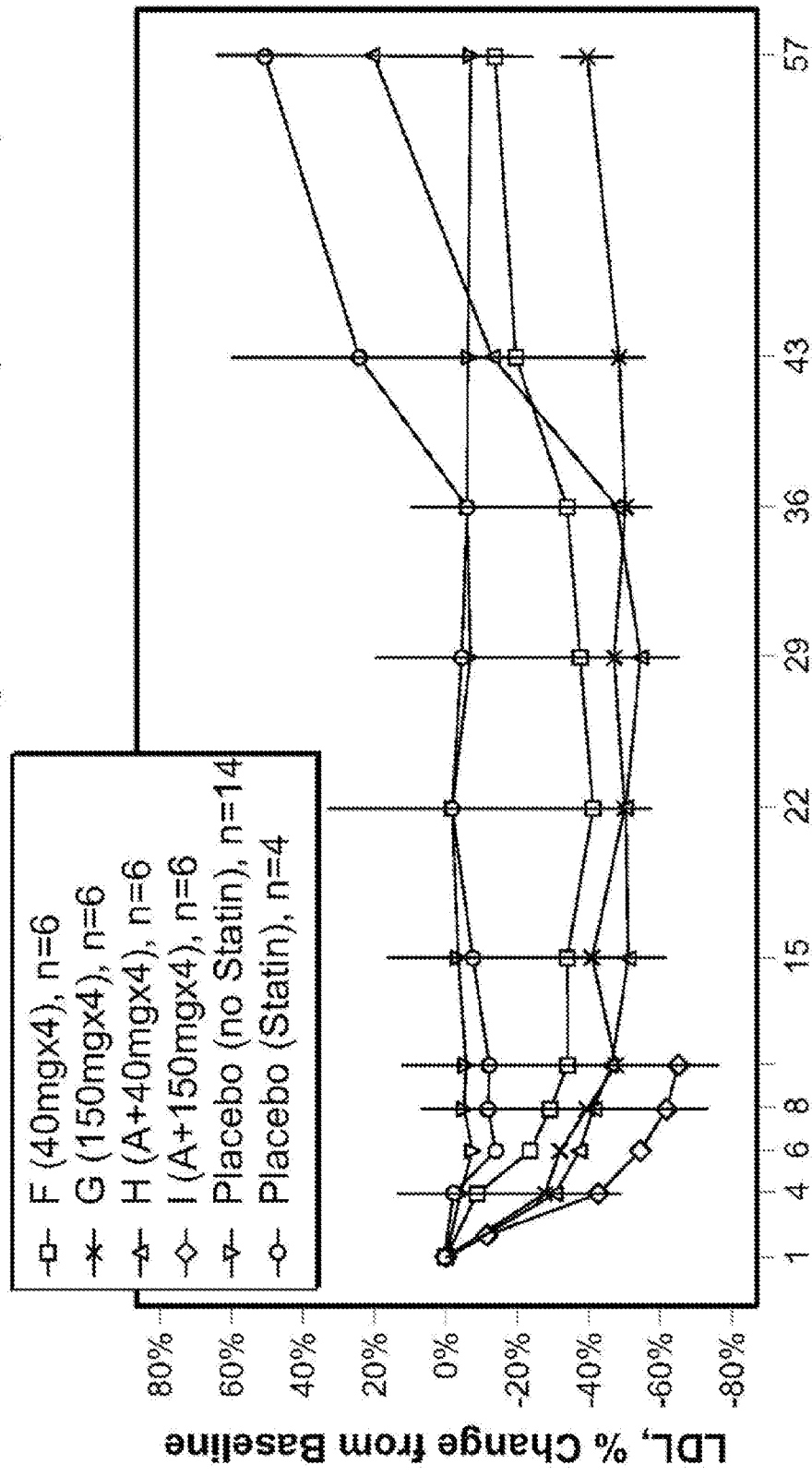

LDL-c, MD, Mean % Change from Baseline (with 95% CI)
F (40mgx4), n=6
G (150mgx4), n=6
H (A+40mgx4), n=6
I (A+150mgx4), n=6
Placebo (no Statin), n=14
Placebo (Statin), n=4
LDL, % Change from Baseline
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
1
4
6
8
15
22
29
36
43
57
Study Day
FOUR WEEKLY SC DOSES

FIGURE 19

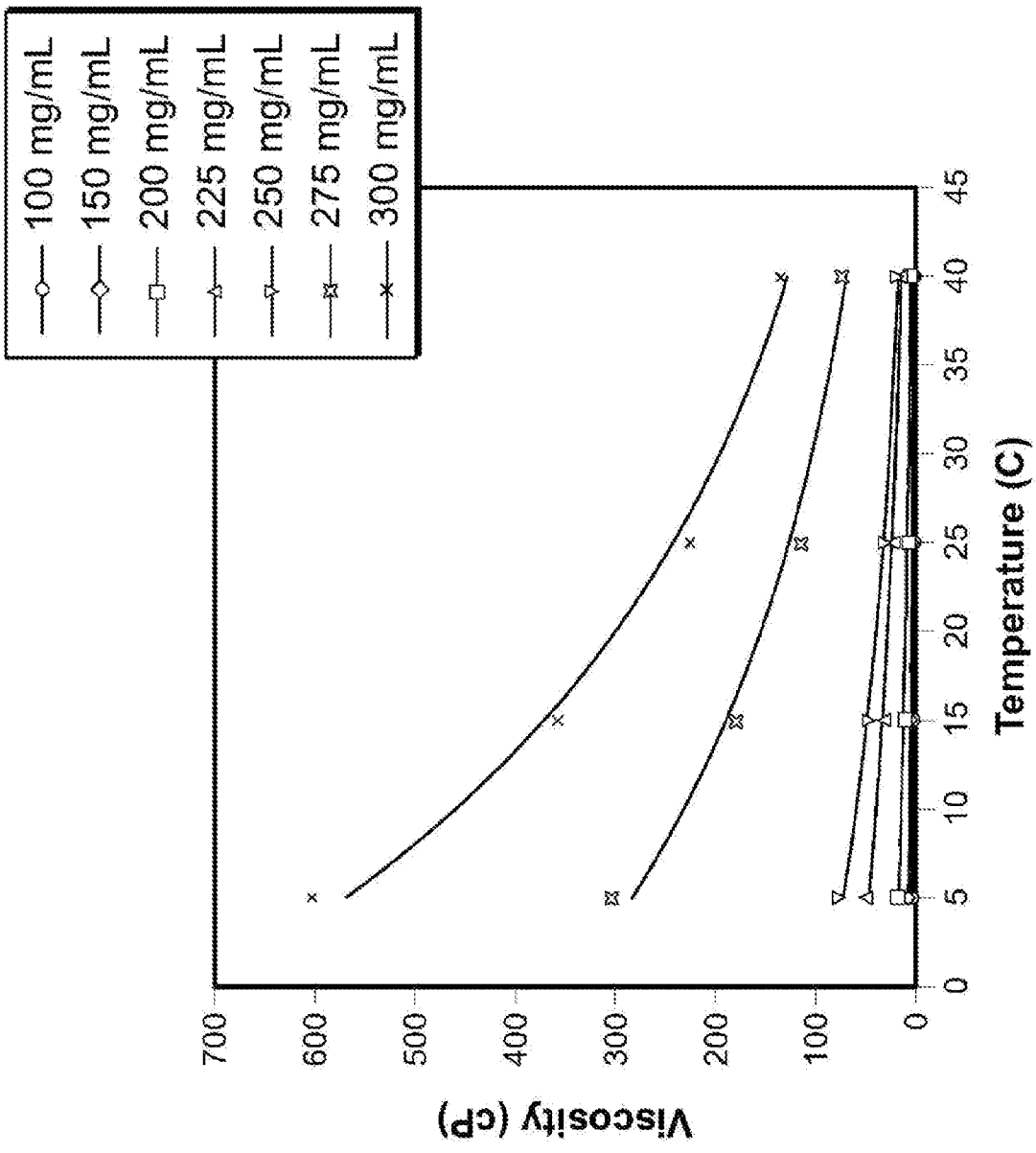

100 mg/mL
150 mg/mL
200 mg/mL
225 mg/mL
250 mg/mL
275 mg/mL
300 mg/mL
Viscosity (cP)
0
100
200
300
400
500
600
700
Temperature (C)
0
5
10
15
20
25
30
35
40
45

FIGURE 20

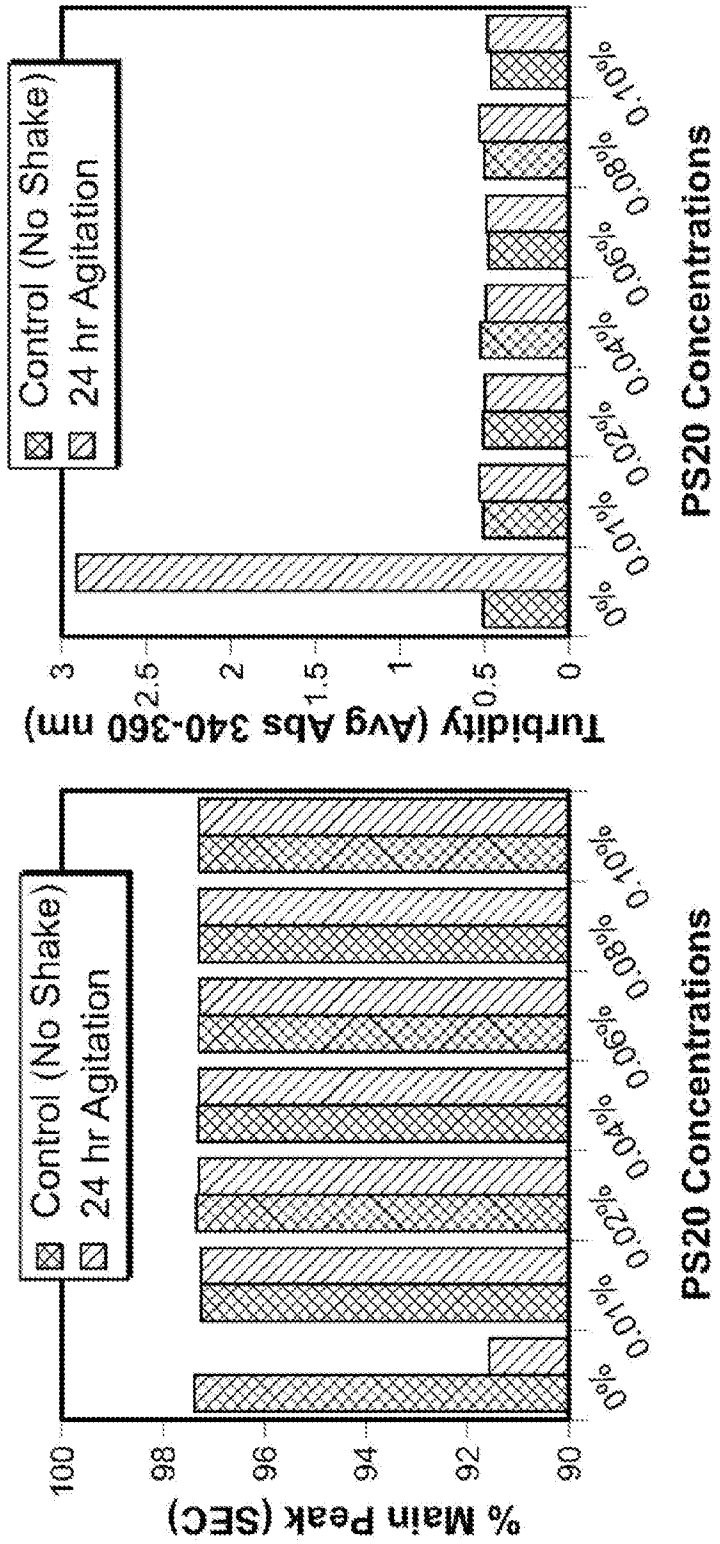

Control (No Shake)
24 hr Agitation
Turbidity (Avg Abs 340-360 nm)
3
2.5
2
1.5
1
0.5
0
0%
0.01%
0.02%
0.04%
0.06%
0.08%
0.10%
PS20 Concentrations
Control (No Shake)
24 hr Agitation
% Main Peak (SEC)
100
98
96
94
92
90
0%
0.01%
0.02%
0.04%
0.06%
0.08%
0.10%
PS20 Concentrations

FIGURE 21

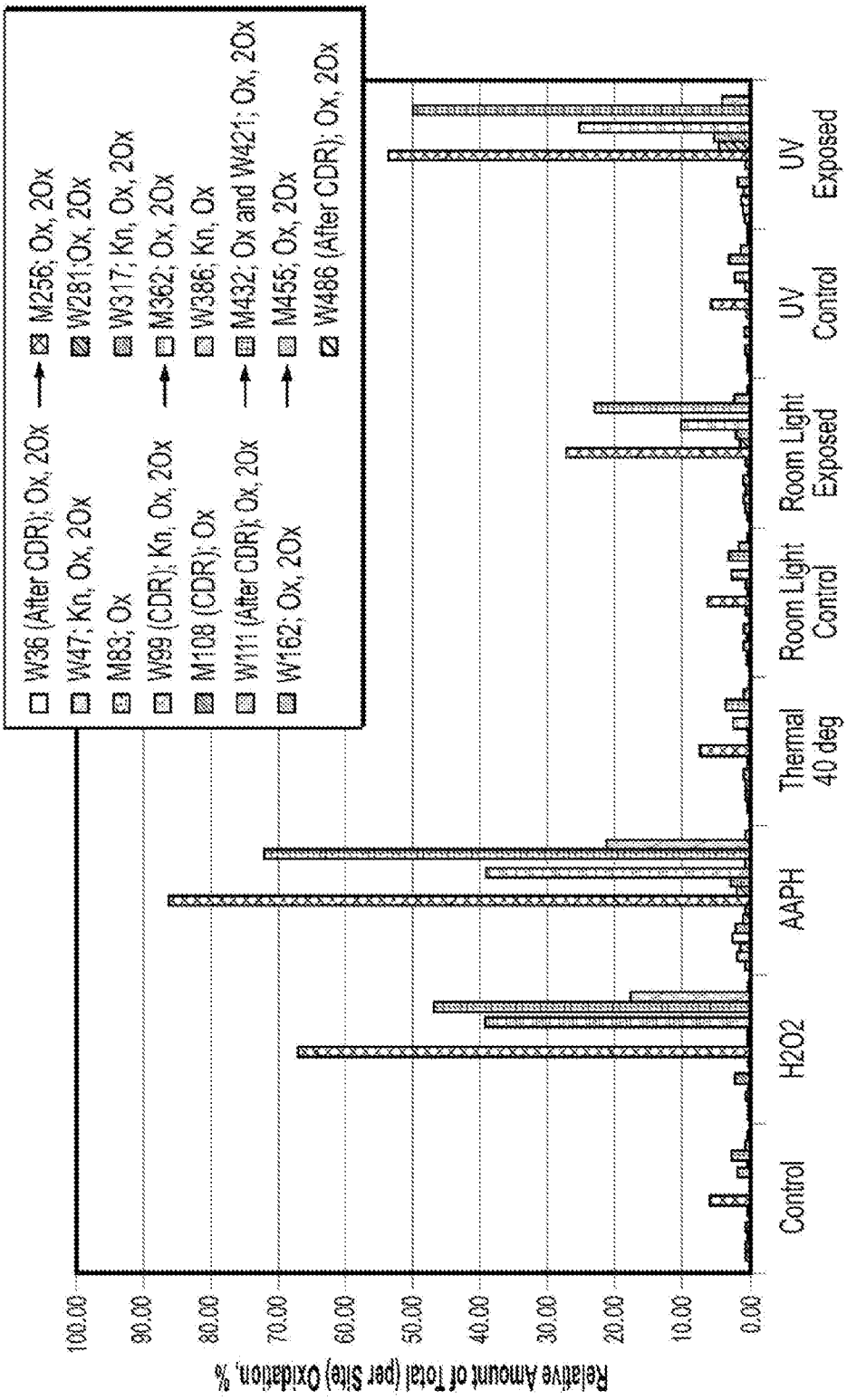

W36 (After CDR); Ox, 2Ox
W47; Kn, Ox, 2Ox
M83; Ox
W99 (CDR); Kn, Ox, 2Ox
M108 (CDR); Ox
W111 (After CDR); Ox, 2Ox
W162; Ox, 2Ox
M256; Ox, 2Ox
W281;Ox, 2Ox
W317; Kn, Ox, 2Ox
M362; Ox, 2Ox
W386; Kn, Ox
M432; Ox and W421; Ox, 2Ox
M455; Ox, 2Ox
W486 (After CDR); Ox, 2Ox
Relative Amount of Total (per Site) Oxidation, %
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Control
H2O2
AAPH
Thermal 40 deg
Room Light Control
Room Light Exposed
UV Control
UV Exposed

FIGURE 22

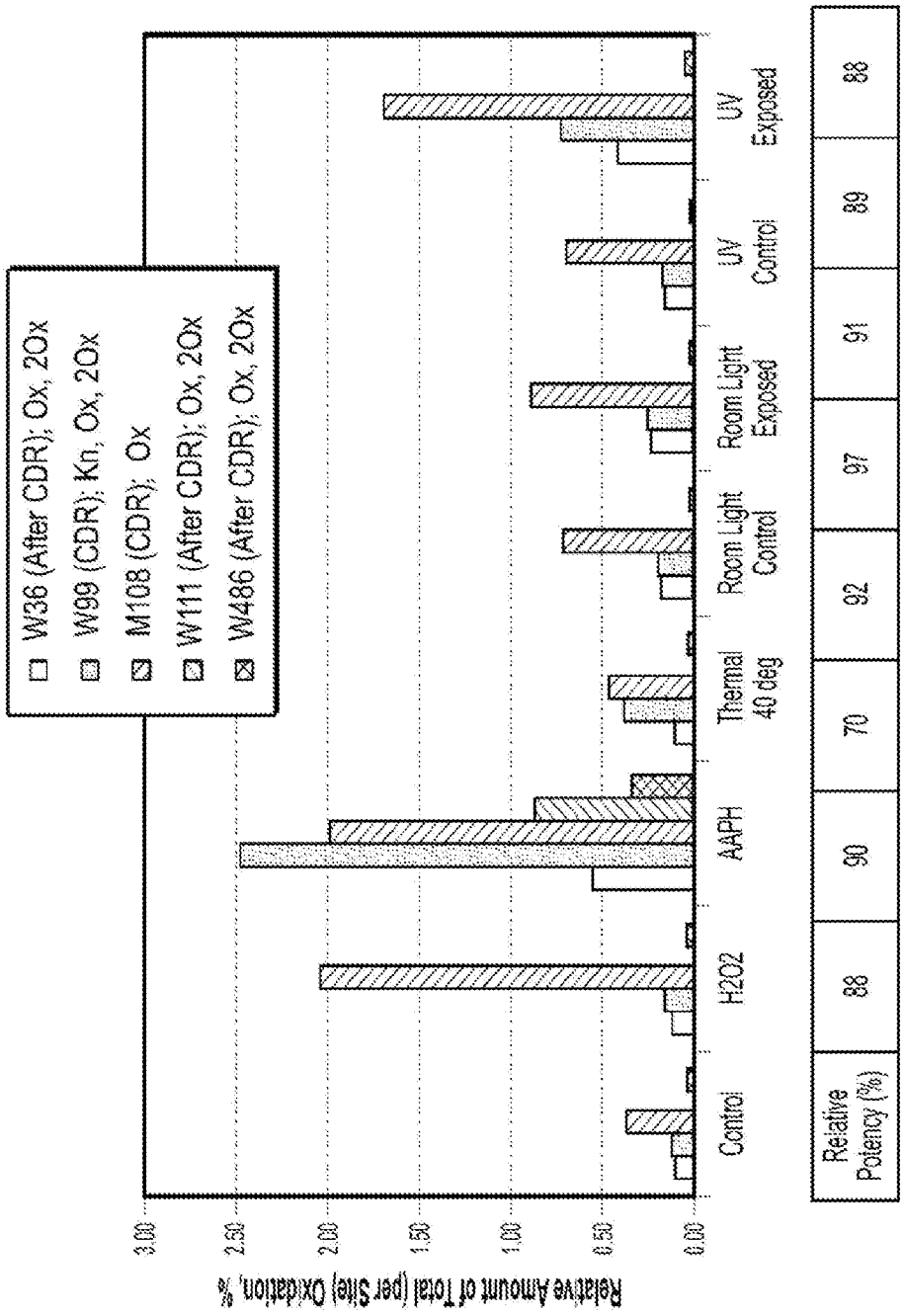

W36 (After CDR); Ox, 2Ox
W99 (CDR); Kn, Ox, 2Ox
M108 (CDR); Ox
W111 (After CDR); Ox, 2Ox
W486 (After CDR); Ox, 2Ox
Relative Amount of Total (per Site) Oxidation, %
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Control
H2O2
AAPH
Thermal 40 deg
Room Light Control
Room Light Exposed
UV Control
UV Exposed
Relative Potency (%)
88
90
70
92
97
91
89
88

FIGURE 23

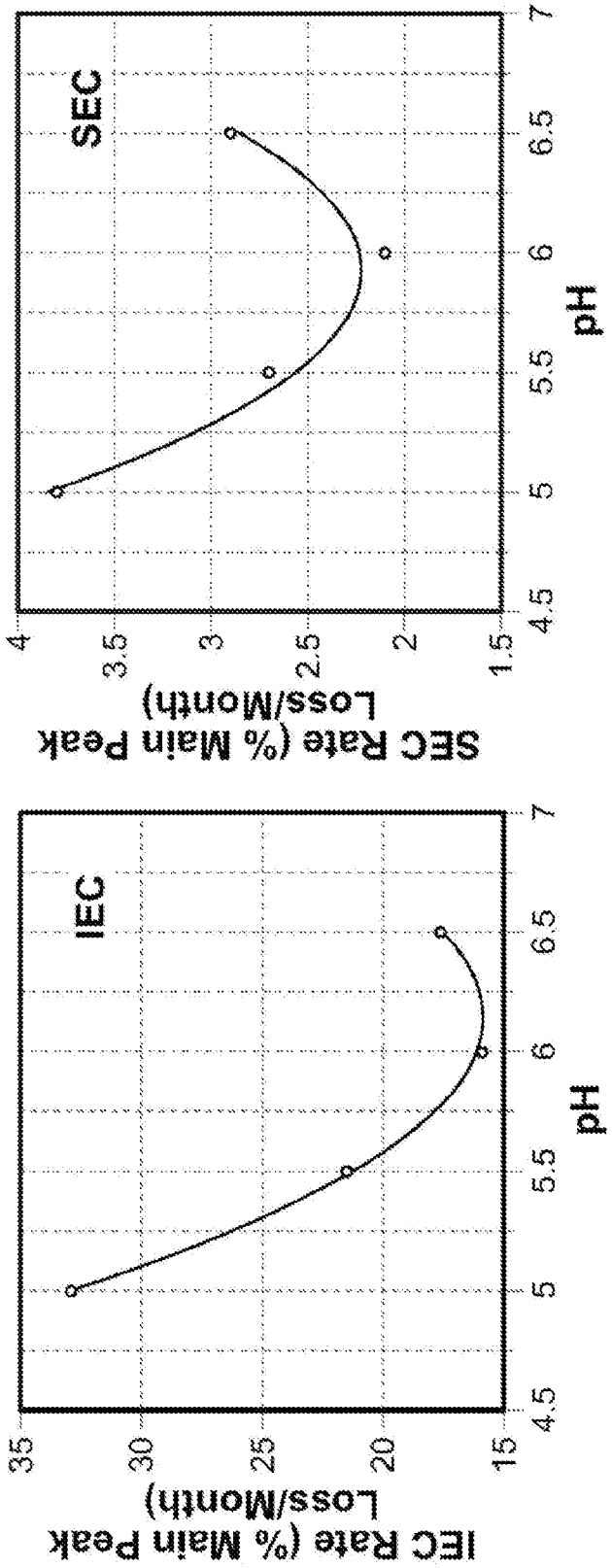

SEC
SEC Rate (% Main Peak Loss/Month)
pH
IEC
IEC Rate (% Main Peak Loss/Month)
pH

FIGURE 24

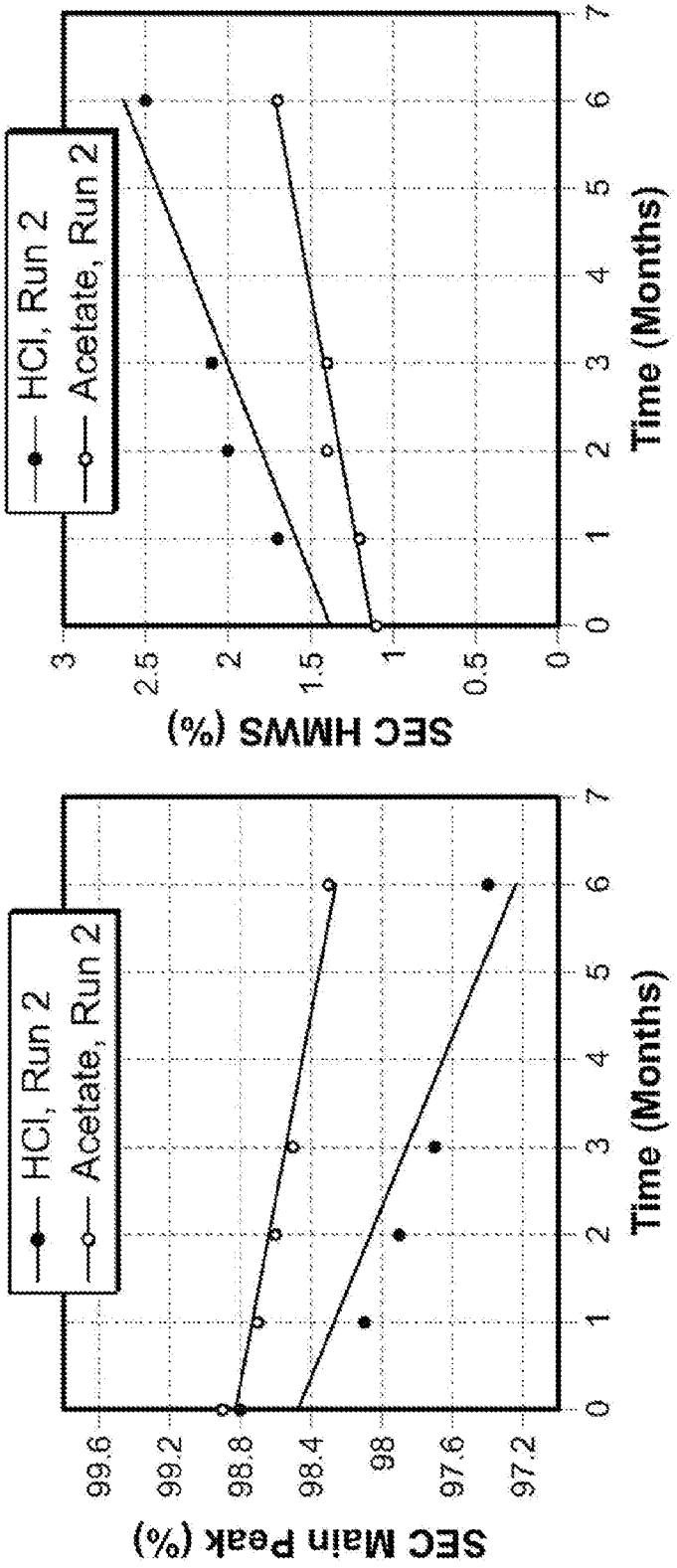

SEC HMWS (%)
3
2.5
2
1.5
1
0.5
0
HCl, Run 2
Acetate, Run 2
0
1
2
3
4
5
6
7
Time (Months)
SEC Main Peak (%)
99.6
99.2
98.8
98.4
98
97.6
97.2
HCl, Run 2
Acetate, Run 2
0
1
2
3
4
5
6
7
Time (Months)

FIGURE 25

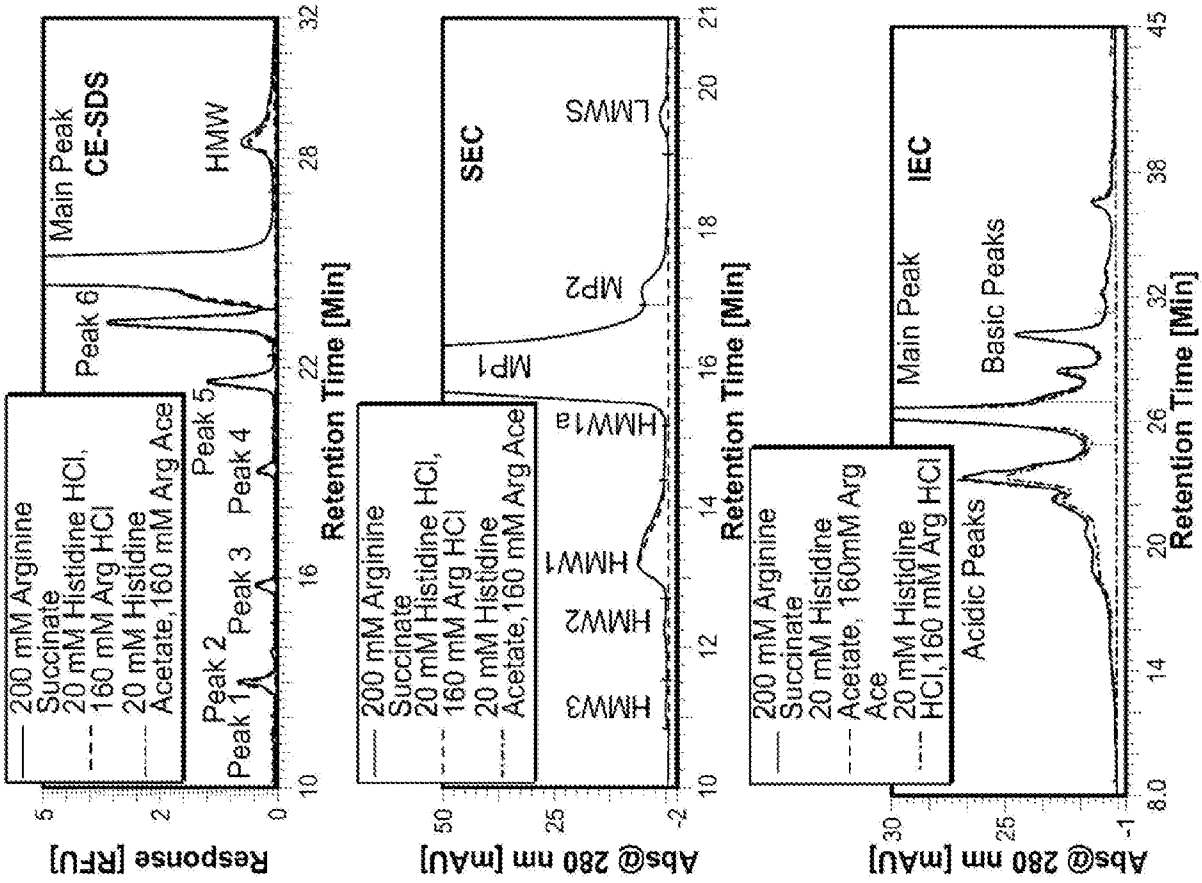
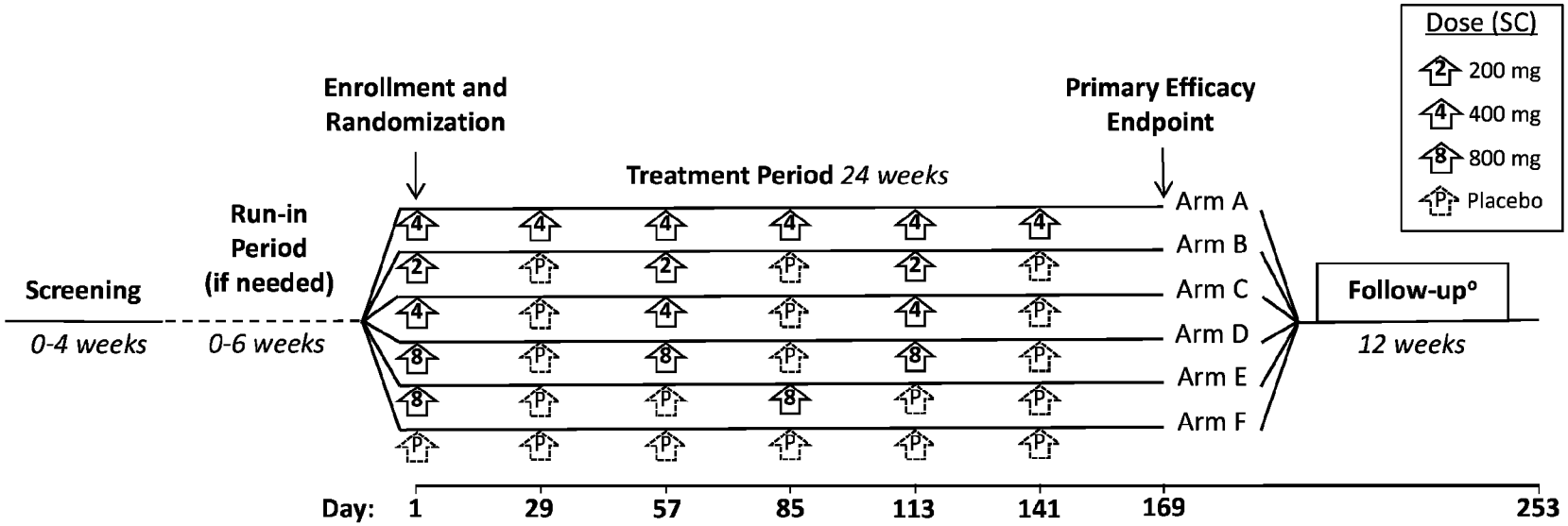

200 mM Arginine
Succinate
20 mM Histidine HCl,
160 mM Arg HCl
20 mM Histidine
Acetate,160 mM Arg Ace
Peak 1
Peak 2
Peak 3
Peak 4
Peak 5
Peak 6
Main Peak
CE-SDS
HMW
Response [RFU]
Retention Time [Min]
200 mM Arginine
Succinate
20 mM Histidine HCl,
160 mM Arg HCl
20 mM Histidine
Acetate,160 mM Arg Ace
HMW3
HMW2
HMW1
HMW1a
MP1
MP2
LMWS
SEC
Abs@ 280 nm [mAU]
Retention Time [Min]
200 mM Arginine
Succinate
20 mM Histidine
Acetate, 160mM Arg
Ace
20 mM Histidine
HCl,160 mM Arg HCl
Acidic Peaks
Main Peak
Basic Peaks
IEC
Abs@ 280 nm [mAU]
Retention Time [Min]

FIGURE 26



Arm	Dose	Frequency	# Patients
A	400 mg	Q4 weeks	57
B	200 mg	Q8 weeks	23
C	400 mg	Q8 weeks	30
D	800 mg	Q8 weeks	51
E	800 mg	Q12 weeks	23
F	Placebo	N/A	64

FIGURE 27

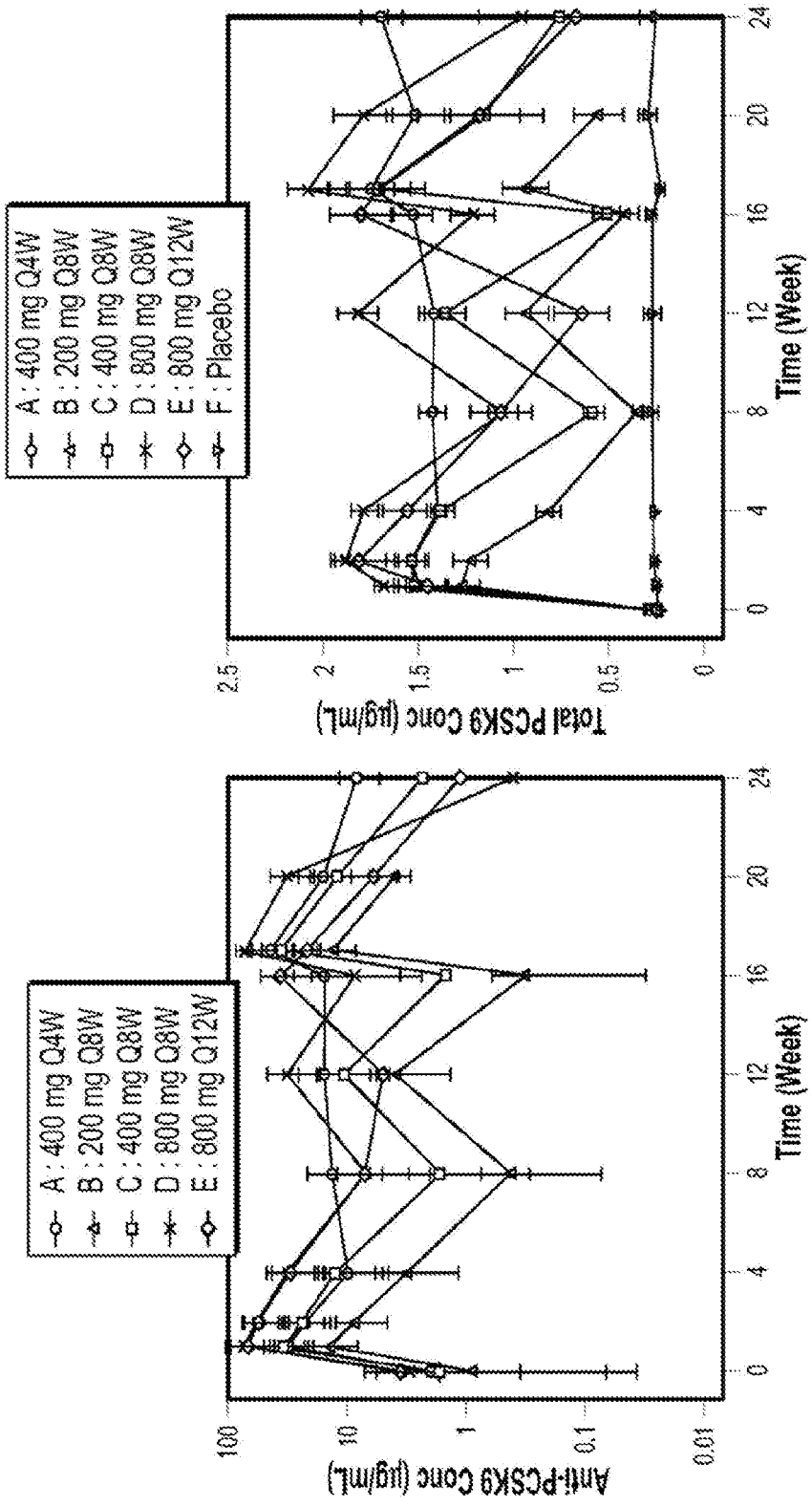
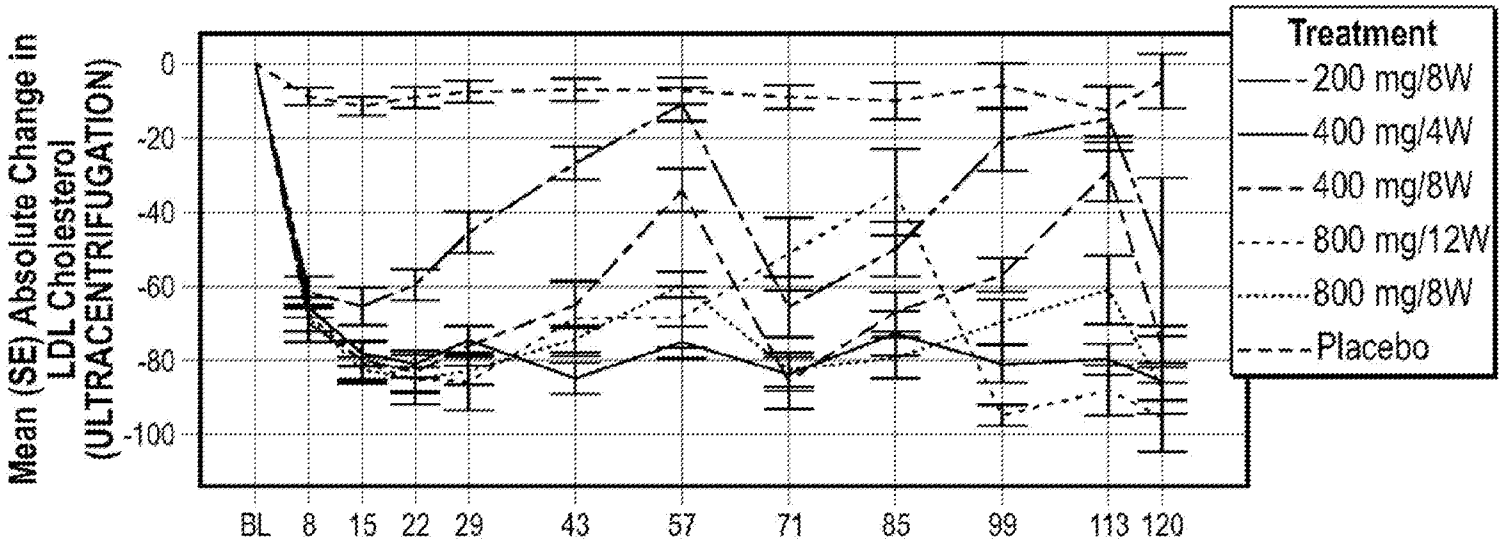

A : 400 mg Q4W
B : 200 mg Q8W
C : 400 mg Q8W
D : 800 mg Q8W
E : 800 mg Q12W
F : Placebo
Total PCSK9 Conc (μg/mL)
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
24
Time (Week)
A : 400 mg Q4W
B : 200 mg Q8W
C : 400 mg Q8W
D : 800 mg Q8W
E : 800 mg Q12W
Anti-PCSK9 Conc (μg/mL)
100
10
1
0.1
0.01
0
4
8
12
16
20
24
Time (Week)

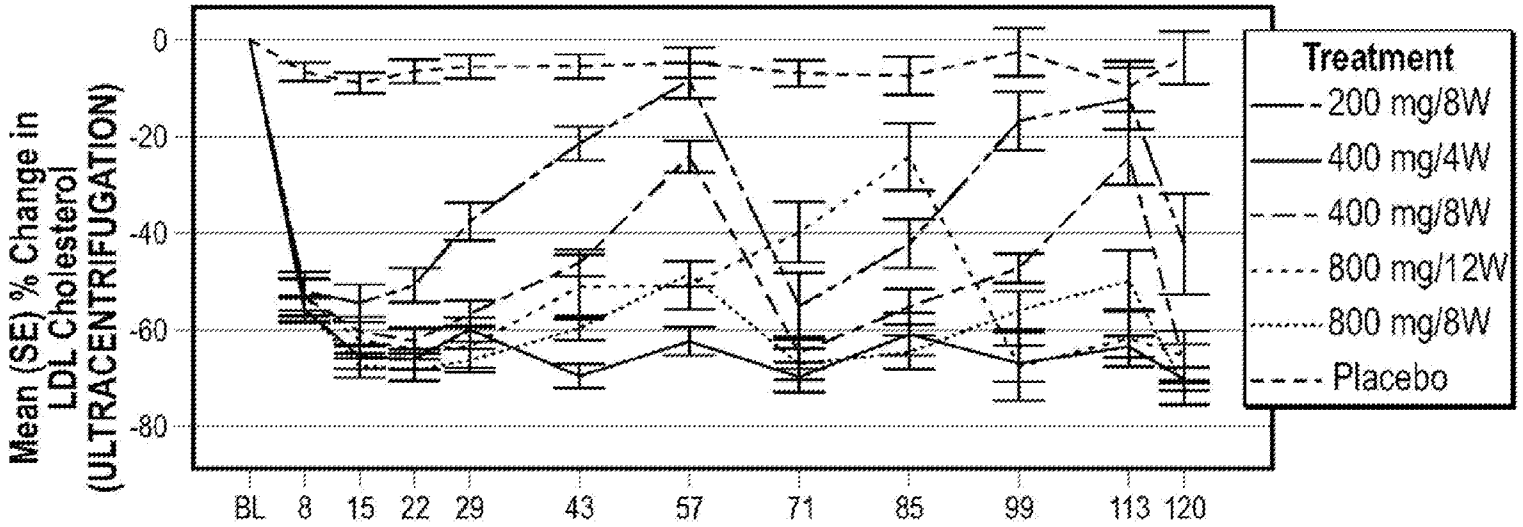
FIGURE 28



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (mg/dL)	-72.7	-50.0	-66.9	-79.3	-34.6	-10.0
Reduction from Placebo	**65.9**	**42.3**	**56.0**	**67.7**	**25.1**	
95% Confidence Interval	52.8, 78.9	23.7, 60.8	40.4, 71.7	54.1, 81.2	4.7, 45.5	
P-value	<.0001	<.0001	<.0001	<.0001	0.0166	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

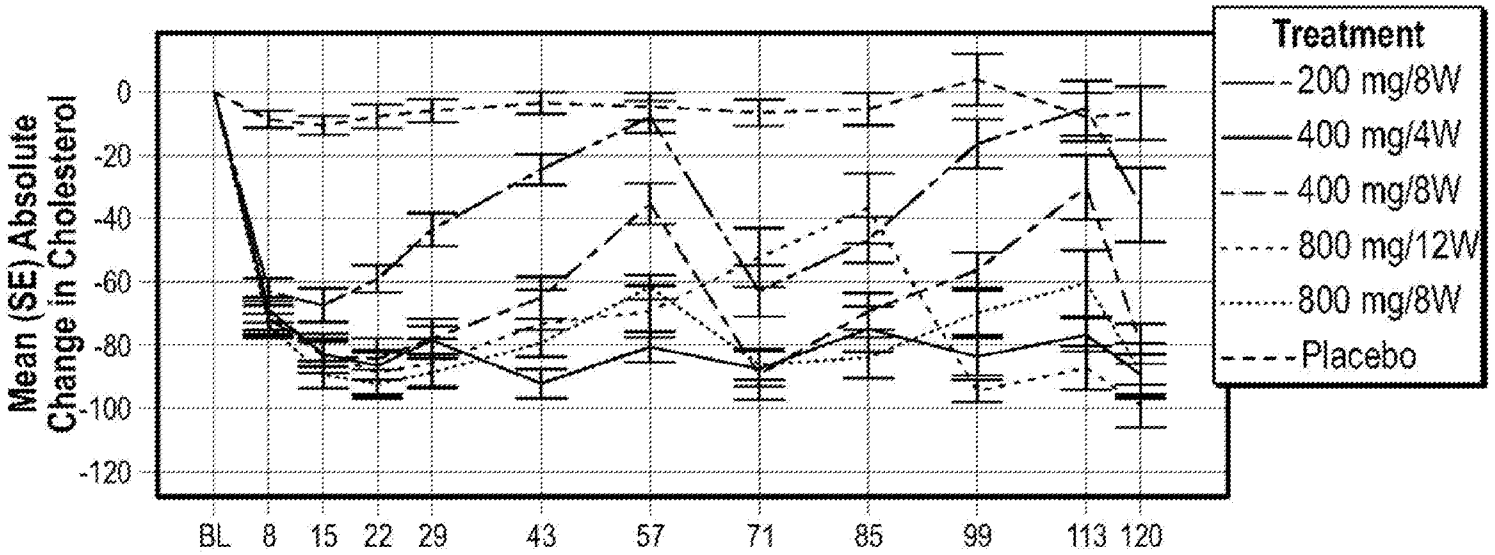
FIGURE 29



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (%)	-61.0	-42.2	-55.3	-64.7	-24.3	-7.5
Reduction from Placebo	**54.1**	**35.2**	**48.0**	**56.9**	**17.5**	
95% Confidence Interval	43.7, 64.5	20.4, 50.0	35.5, 60.5	46.1, 67.7	1.2, 33.8	
P-value	<.0001	<.0001	<.0001	<.0001	0.0356	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

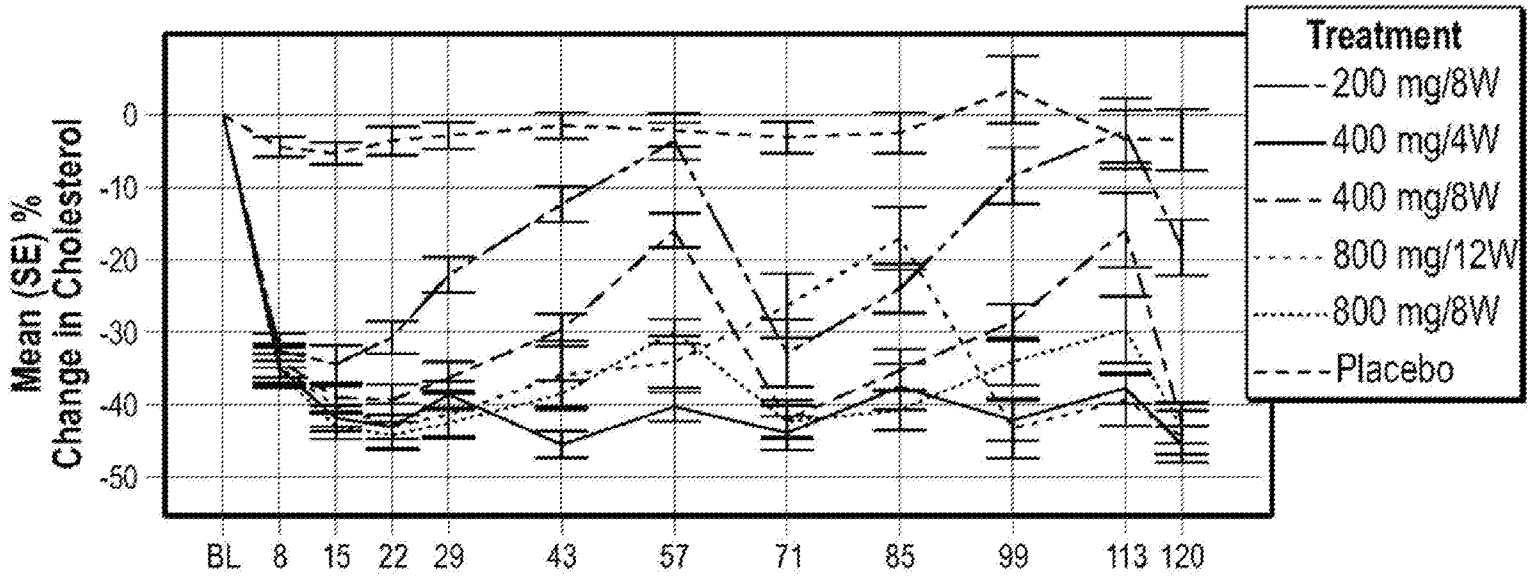
FIGURE 30



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (mg/dL)	-74.9	-46.7	-69.3	-84.0	-36.8	-5.4
Reduction from Placebo	**72.9**	**43.8**	**63.7**	**77.5**	**32.5**	
95% Confidence Interval	57.6, 88.1	22.0, 65.7	45.4, 82.0	61.6, 93.3	8.5, 56.6	
P-value	<.0001	0.0001	<.0001	<.0001	0.0084	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

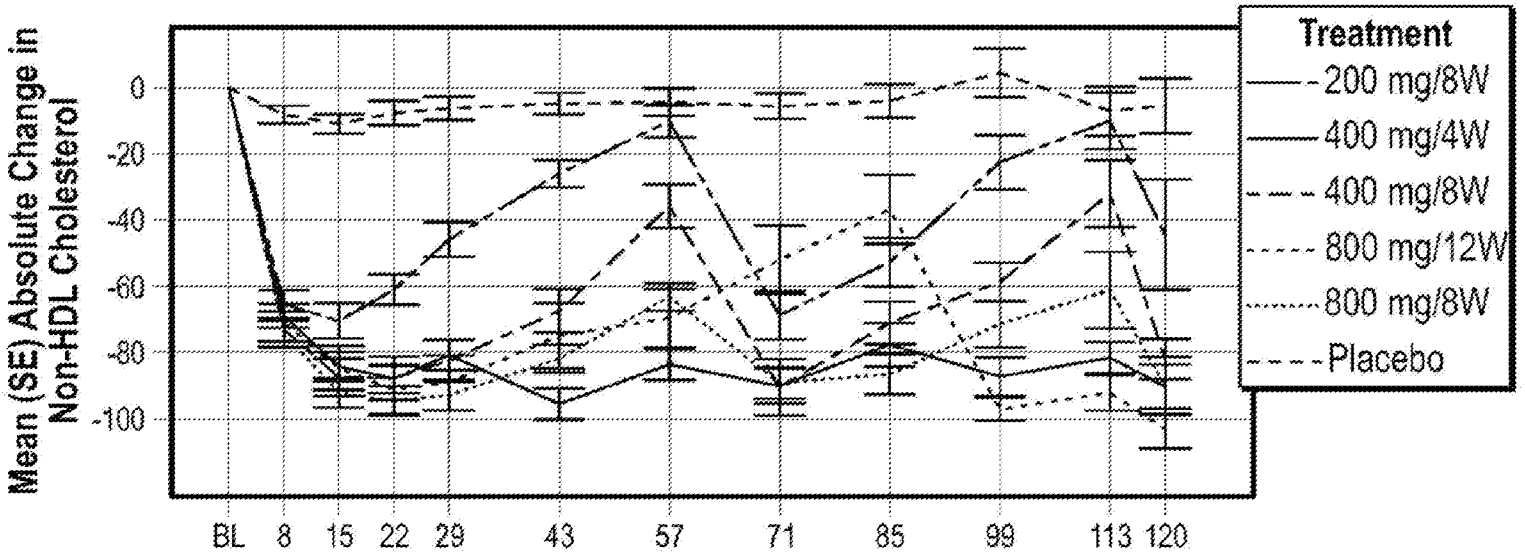
FIGURE 31



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (%)	-37.5	-23.9	-35.2	-40.7	-17.0	-2.4
Reduction from Placebo	**35.9**	**22.1**	**32.9**	**38.0**	**15.1**	
95% Confidence Interval	28.4, 43.4	11.4, 32.8	23.9, 41.8	30.2, 45.7	3.4, 26.9	
P-value	<.0001	<.0001	<.0001	<.0001	0.0122	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

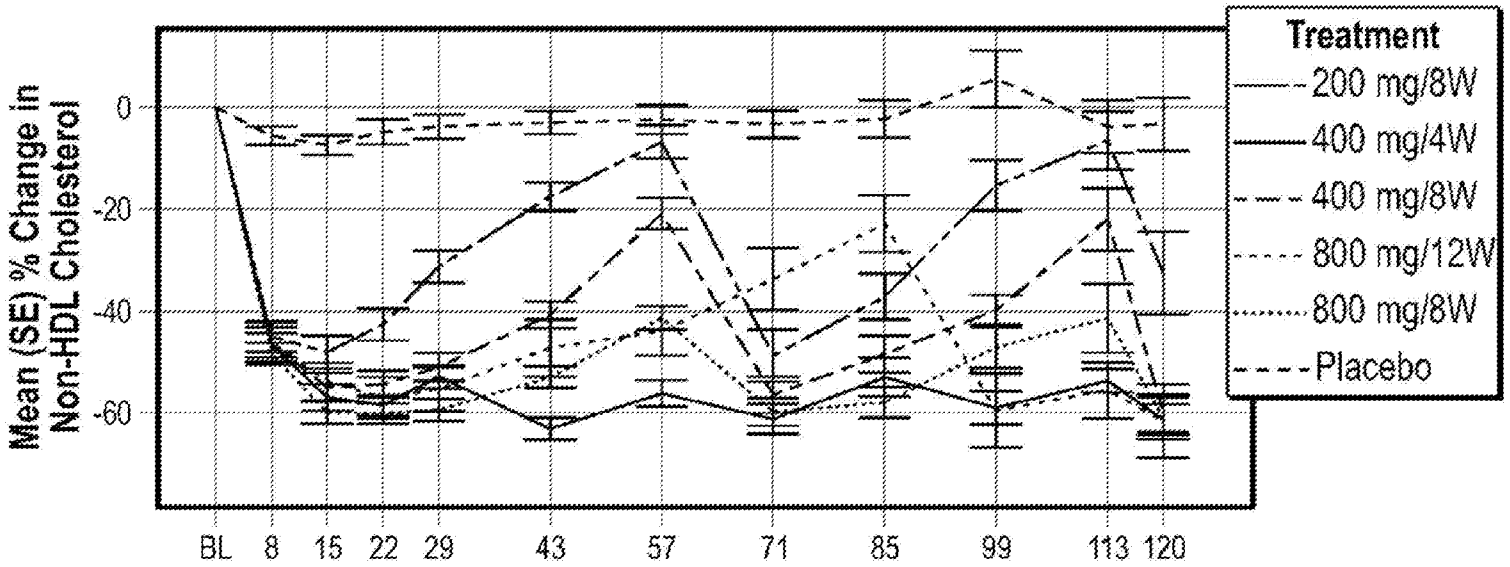
FIGURE 32



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (mg/dL)	-77.7	-52.7	-71.1	-86.6	-36.8	-3.9
Reduction from Placebo	**77.0**	**51.2**	**66.8**	**81.6**	**33.6**	
95% Confidence Interval	62.2, 91.7	30.1, 72.3	49.1, 84.5	66.3, 97.0	10.5, 56.8	
P-value	<.0001	<.0001	<.0001	<.0001	0.0049	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

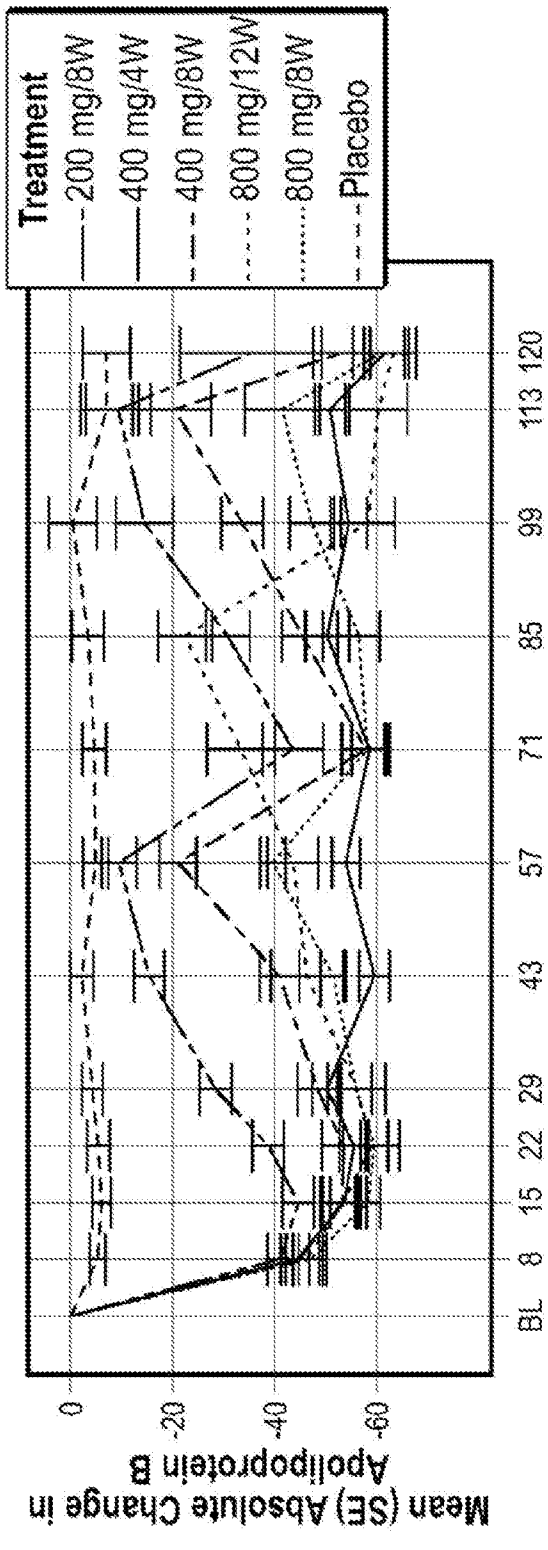
FIGURE 33



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (%)	-53.0	-37.2	-48.4	-57.9	-22.9	-2.4
Reduction from Placebo	**51.0**	**35.1**	**46.2**	**55.4**	**21.0**	
95% Confidence Interval	41.8, 60.3	21.9, 48.4	35.1, 57.3	45.8, 65.0	6.5, 35.6	
P-value	<.0001	<.0001	<.0001	<.0001	0.0050	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

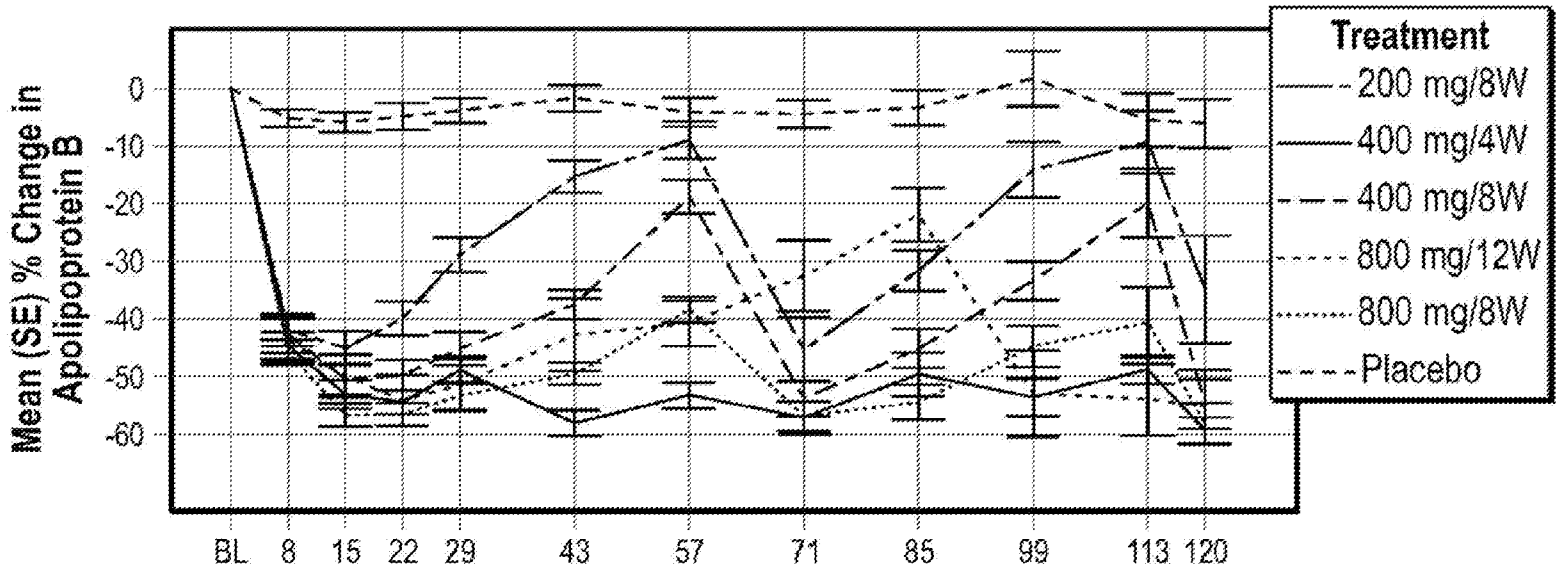
FIGURE 34



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (mg/dL)	-50.3	-30.8	-45.4	-56.5	-22.4	-3.4
Reduction from Placebo	**48.7**	**28.7**	**41.8**	**52.5**	**19.5**	
95% Confidence Interval	39.0, 58.4	14.8, 42.6	30.2, 53.5	42.4, 62.6	4.3, 34.8	
P-value	<.0001	<.0001	<.0001	<.0001	0.0127	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

FIGURE 35



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	Placebo (n=24)
Mean Change on D85 (%)	-49.6	-31.6	-45.1	-54.4	-21.9	-3.3
Reduction from Placebo	**46.5**	**28.5**	**41.9**	**51.0**	**19.0**	
95% Confidence Interval	37.8, 55.3	15.9, 41.0	31.4, 52.4	41.8, 60.1	5.2, 32.8	
P-value	<.0001	<.0001	<.0001	<.0001	0.0073	

Note: Differences from Placebo, 95% CIs and P-values from ANCOVA Model Adjusted for Baseline LDL-c (<120, >=120) and Diabetes Status (Yes, No). P-values are not Adjusted for Multiple Testing and Should be Interpreted with Caution.

FIGURE 36

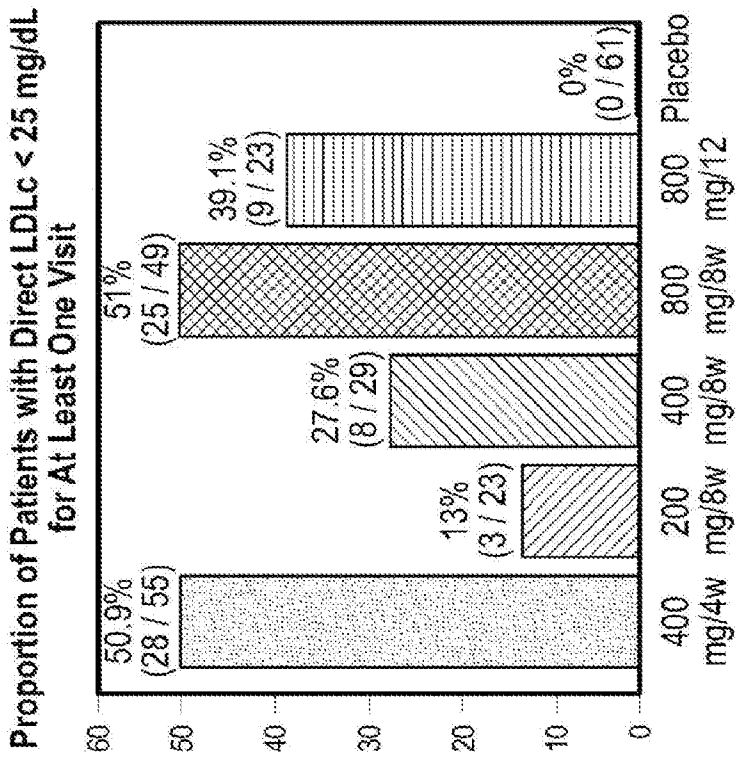


FIGURE 37B

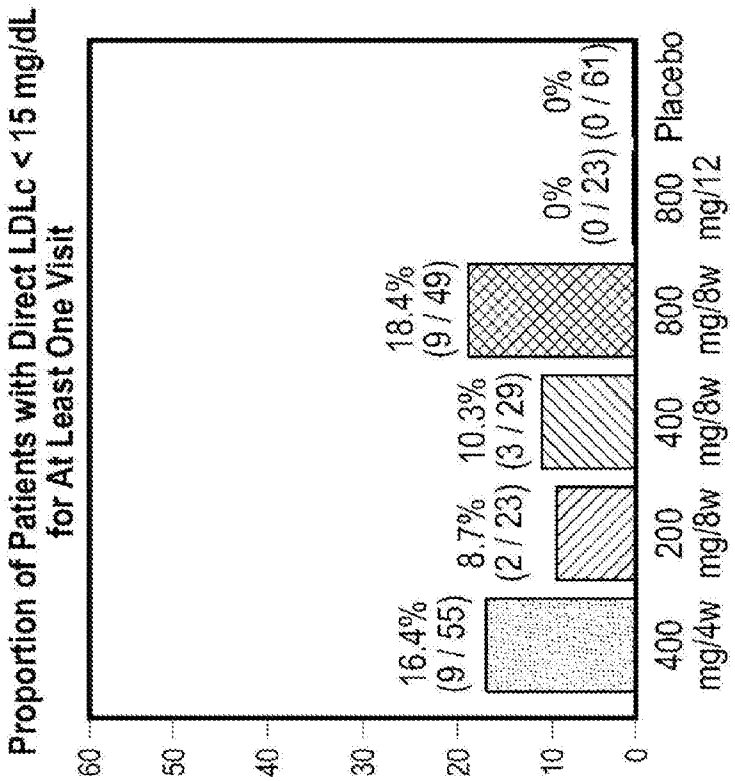


FIGURE 37A

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046032

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/40 A61K39/395
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)
C07K A61K

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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/072263 A1 (IRM LLC; NOVARTIS AG [CH]; RUE SARAH [US]; COHEN STEVEN B [US]; LI JUN) 16 June 2011 (2011-06-16) paragraph [0005] paragraphs [0006] - [0012] paragraphs [0034] - [0036] ----- -/--	1-26, 51-101

[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
17 September 2013	01/10/2013

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	Irion, Andrea

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046032

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NI YAN G ET AL: "A proprotein convertase subtilisin-like/kexin type 9 (PCSK9) C-terminal domain antibody antigen-binding fragment inhibits PCSK9 internalization and restores low density lipoprotein uptake", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, INC, BETHESDA, MD, USA, vol. 285, no. 17, 23 April 2010 (2010-04-23), pages 12882-12891, XP002619048, ISSN: 1083-351X, DOI: 10.1074/JBC.M110.113035 [retrieved on 2010-02-19] abstract	1-101

Y	YAN G NI ET AL: "A PCSK9-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces LDL cholesterol in vivo", JOURNAL OF LIPID RESEARCH, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, INC, US, vol. 52, no. 1, 1 January 2011 (2011-01-01), pages 78-86, XP002686538, ISSN: 0022-2275, DOI: 10.1194/JLR.M011445 [retrieved on 2010-10-19] abstract	1-101

Y	LIWEN ZHANG: "An Anti-PCSK9 Antibody Reduces LDL-Cholesterol On Top Of A Statin And Suppresses Hepatocyte SREBP-Regulated Genes", INTERNATIONAL JOURNAL OF BIOLOGICAL SCIENCES, 1 January 2012 (2012-01-01), pages 310-327, XP055079135, ISSN: 1449-2288, DOI: 10.7150/ijbs.3524 abstract	1-101

X	WO 2009/100297 A1 (MERCK & CO INC [US]; ANGELETTI P IST RICHERCHE BIO [IT]; CONDRA JON H) 13 August 2009 (2009-08-13)	1-26, 51-101
Y	pages 67-68; examples 14,15 pages 68-69; example 16 page 69; example 17 page 45, line 36 - page 48, line 8 claims 27-30	27-50

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046032

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/053759 A1 (MERCK SHARP & DOHME [US]; LUO PETER PEIZHI [US]; NI YAN [US]; WANG KEV) 5 May 2011 (2011-05-05)	1-26, 51-101
Y	page 5, line 9 - page 7, line 21 Examples	27-50

X	WO 2011/053783 A2 (MERCK SHARP & DOHME [US]; LUO PETER PEIZHI [US]; NI YAN [US]; WANG KEV) 5 May 2011 (2011-05-05) examples 5-7; table 2 page 81 - page 82; example 20	1-26, 51-101

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Y	CHAN JOYCE C Y ET AL: "A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES - PNAS, NATIONAL ACADEMY OF SCIENCES, US, vol. 106, no. 24, 16 June 2009 (2009-06-16), pages 9820-9825, XP002570200, ISSN: 0027-8424, DOI: 10.1073/PNAS.0903849106 abstract page 9824, left-hand column, paragraph 4	1-101

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/046032

C(Continuation). 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) page 68, lines 16-23 page 69; table 4 page 76; table 7 page 77, paragraph 10 - page 78, paragraph 22 page 80, line 19 - page 81, line 14	1-26, 51-101
X	WO 2011/111007 A2 (RINAT NEUROSCIENCE CORP [US]; PFIZER [US]; PONS JAUME [US]; CHABOT JEF) 15 September 2011 (2011-09-15) page 64, lines 10-15 claims 30,31	1-26, 51-101
Y	J. CHAPARRO-RIGGERS ET AL: "Increasing Serum Half-life and Extending Cholesterol Lowering in Vivo by Engineering Antibody with pH-sensitive Binding to PCSK9", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 14, 30 March 2012 (2012-03-30), pages 11090-11097, XP055041205, ISSN: 0021-9258, DOI: 10.1074/jbc.M111.319764 abstract	1-101
Y	LIANG H ET AL: "Proprotein convertase substilisin/kexin type 9 antagonism reduces low-density lipoprotein cholesterol in statin-treated hypercholesterolemic nonhuman primates", JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, US, vol. 340, no. 2, 1 February 2012 (2012-02-01), pages 228-236, XP009166142, ISSN: 0022-3565 abstract	1-101
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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EVAN A. STEIN ET AL: "Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol", NEW ENGLAND JOURNAL OF MEDICINE, vol. 366, no. 12, 22 March 2012 (2012-03-22), pages 1108-1118, XP55049842, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1105803 abstract --- EVAN A STEIN ET AL: "Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial", THE LANCET, vol. 380, no. 9836, 26 May 2012 (2012-05-26), pages 29-36, XP55041201, ISSN: 0140-6736, DOI: 10.1016/S0140-6736(12)60771-5 abstract	1-101
Y	EVAN A STEIN ET AL: "Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial", THE LANCET, vol. 380, no. 9836, 26 May 2012 (2012-05-26), pages 29-36, XP55041201, ISSN: 0140-6736, DOI: 10.1016/S0140-6736(12)60771-5 abstract --- WO 2012/088313 A1 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]; WU YAN [US]; CHIU CECILIA) 28 June 2012 (2012-06-28) the whole document	1-101
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Y	WANG W ET AL: "ANTIBODY STRUCTURE, INSTABILITY, AND FORMULATION", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN PHARMACEUTICAL ASSOCIATION, WASHINGTON, US, vol. 96, no. 1, 1 January 2007 (2007-01-01), pages 1-26, XP009084505, ISSN: 0022-3549, DOI: 10.1002/JPS.20727 the whole document ---	27-50

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(19)中华人民共和国国家知识产权局

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李蔚 克森·拉姆 林·路易斯
许令攸 惠特莫尔·廷利
约翰·道格拉斯·大卫
纳格什沃·R·布达
(74)专利代理机构 中科专利商标代理有限责任公司 11021
代理人 吴小明 王旭
(51)Int.Cl.
C07K 16/40(2006.01)
A61K 39/395(2006.01)

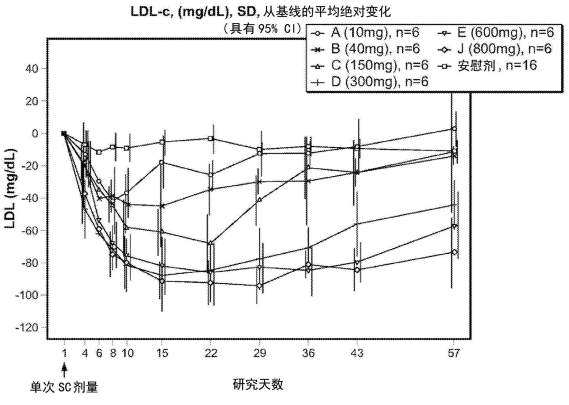
权利要求书7页 说明书82页
序列表15页 附图38页

(54)发明名称

抗-PCSK9抗体，制剂，剂量给药，和使用方法

(57)摘要

本发明提供抗-PCSK9抗体，制剂，剂量给药方案，及其使用方法。



CN 104364266 A

1. 一种包含重链和轻链可变结构域的抗 -PCSK9 抗体，所述重链和轻链可变结构域包含六个高变区 (HVR) 序列：

(i)HVR-H1，所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IH(SEQ ID NO:28)，其中 X$_1$ 是 S 或 T；X$_2$ 是 G,R 或 S；X$_3$ 是 H,T 或 Y；X$_4$ 是 A 或 T；

(ii)HVR-H2，所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ IDNO:4)；

(iii)HVR-H3，所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5)；

(iv)HVR-L1，所述 HVR-L1 包含 RASQDVSX$_1$AVA(SEQ ID NO:29)，其中 X$_1$ 是 S 或 T；

(v)HVR-L2，所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30)，其中 X$_1$ 是 F 或 S；和

(vi)HVR-L3，所述 HVR-L3 包含 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37)，其中 X$_1$ 是 P,R 或 T；X$_2$ 是 A,I,S 或 T；X$_3$ 是 L,P 或 Q；X$_4$ 是 A,H,P 或 S。

2. 权利要求 1 所述的抗体，其中所述抗体包含 (a)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1,SEQ ID NO:2 或 SEQ ID NO:3,(b)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4，和 (c)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

3. 权利要求 2 所述的抗体，所述抗体进一步包含 (a)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7；(b)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26；和 (c)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

4. 权利要求 1 所述的抗体，所述抗体包含 (a)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7；(b)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26；和 (c)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

5. 权利要求 1 所述的抗体，其中所述抗体包含：

(1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3；

(2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

(3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

(4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

(5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

(6)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

6. 权利要求 1 所述的抗体，所述抗体包含 VH 序列 SEQ ID NO:17。

7. 权利要求 1 所述的抗体，所述抗体包含 VL 序列 SEQ ID NO:34。

8. 权利要求 1 所述的抗体，所述抗体包含 VH 序列 SEQ ID NO:17 和 VL 序列 SEQ ID NO:34。

9. 权利要求 1 至 8 任一项所述的抗体，其中所述抗体是单克隆抗体。

10. 权利要求 1 至 8 任一项所述的抗体，其中所述抗体是人源化的。

11. 权利要求 1 至 8 任一项所述的抗体，其中所述抗体是人抗体。

12. 权利要求 1 至 8 任一项所述的抗体，其中所述抗体是选自 Fab,Fab'-SH,Fv,scFv 或 (Fab')$_2$ 片段的抗体片段。

13. 权利要求 1 至 8 任一项所述的抗体，其中框架序列的至少一部分是人共有框架序列。

14. 权利要求 1 所述的抗体，包含：(i) 包含氨基酸序列 SEQ ID NO:35 的重链和包含氨基酸序列 SEQ ID NO:36 的轻链，(ii) 包含 SEQ ID NO:35 的氨基酸 1-450 的重链和包含

氨基酸序列 SEQ ID NO:36 的轻链，(iii) 包含 SEQ ID NO:35 的氨基酸 1-449 的重链和包含氨基酸序列 SEQ ID NO:36 的轻链，或 (iv)(i)、(ii) 或 (iii) 中任一项的重链和轻链，其中 SEQ ID NO:35 的 P449 被酰胺化。

15. 一种抗 -PCSK9 抗体，所述抗体包含 (a)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5，(b)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33，和 (c)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4。

16. 一种抗 -PCSK9 抗体，所述抗体包含 ：包含氨基酸序列 SEQ ID NO:34 的轻链可变结构域。

17. 一种分离的核酸，所述核酸编码权利要求 1 至 16 中任一项所述的抗 -PCSK9 抗体。

18. 一种载体，所述载体包含权利要求 17 所述的核酸。

19. 权利要求 18 所述的载体，其中所述载体是表达载体。

20. 一种宿主细胞，所述宿主细胞包含权利要求 18 或 19 所述的载体。

21. 权利要求 20 所述的宿主细胞，其中所述宿主细胞是原核的。

22. 权利要求 20 所述的宿主细胞，其中所述宿主细胞是真核的。

23. 一种制备抗 -PCSK9 抗体的方法，所述方法包括 ：在适于表达编码所述抗 -PCSK9 抗体的核酸的条件下培养权利要求 20 所述的宿主细胞。

24. 权利要求 23 所述的方法，进一步包括回收由所述宿主细胞产生的所述抗 -PCSK9 抗体。

25. 一种抗 -PCSK9 抗体，所述抗体通过下述方法产生，所述方法包括 ：在适于表达编码所述抗 -PCSK9 抗体的核酸的条件下培养权利要求 20 所述的宿主细胞，和回收由所述宿主细胞产生的所述抗 -PCSK9 抗体。

26. 一种药物组合物，所述药物组合物包含权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体和药用载体。

27. 一种药物组合物，所述药物组合物包含 150 至 225mg/mL 的抗 -PCSK9 抗体，10 至 30mM 的组氨酸乙酸盐，150 至 170mM 的精氨酸乙酸盐，0.01％至 0.03％的聚山梨酯，并且 pH 为 5.8 至 6.2。

28. 权利要求 27 所述的组合物，其中所述组合物中的抗 -PCSK9 抗体或抗体片段为 200mg/mL，所述组合物中的组氨酸乙酸盐为 20mM，所述组合物中的精氨酸乙酸盐为 160mM，和所述组合物中的聚山梨酯 20 为 0.02％，并且 pH 为 6.0。

29. 权利要求 27 所述的组合物，其中所述组合物适于皮下给药。

30. 权利要求 27-29 任一项所述的组合物，其中所述组合物的粘度在 25℃小于 10cP。

31. 权利要求 27-30 任一项所述的组合物，其中所述抗 -PCSK9 抗体包含可变结构域，所述可变结构域包含选自由以下组成的组的一个、两个、三个、四个、五个、或六个高变区 (HVR) 序列 ：

(i)HVR-H1，所述 HVR-H1 包含 $GFTFX_1X_2X_3X_4IH$(SEQ ID NO:28)，其中 X_1 是 S 或 T ；X_2 是 G，R 或 S ；X_3 是 H，T 或 Y ；X_4 是 A 或 T ；

(ii)HVR-H2，所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ；

(iii)HVR-H3，所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ；

(iv)HVR-L1，所述 HVR-L1 包含 $RASQDVSX_1AVA$(SEQ ID NO:29)，其中 X_1 是 S 或 T ；

(v)HVR-L2,所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30),其中 X$_1$ 是 F 或 S ;和

(vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37),其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

32. 权利要求 27-30 任一项所述的组合物,其中所述抗 -PCSK9 抗体包含重链和轻链可变结构域,所述重链和轻链可变结构域包含以下六个高变区 (HVR) 序列 :

(i)HVR-H1,所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IH(SEQ ID NO:28),其中 X$_1$ 是 S 或 T ;X$_2$ 是 G,R 或 S ;X$_3$ 是 H,T 或 Y ;X$_4$ 是 A 或 T ;

(ii)HVR-H2,所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ;

(iii)HVR-H3,所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ;

(iv)HVR-L1,所述 HVR-L1 包含 RASQDVSX$_1$AVA(SEQ ID NO:29),其中 X$_1$ 是 S 或 T ;

(v)HVR-L2,所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30),其中 X$_1$ 是 F 或 S ;和

(vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37),其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

33. 权利要求 32 所述的组合物,其中所述抗体包含 :(a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1,SEQ ID NO:2 或 SEQ ID NO:3,(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4, 和 (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

34. 权利要求 33 所述的组合物,其中所述抗体进一步包含 :(a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9,SEQ ID NO:10,SEQ ID NO:11,SEQ ID NO:12,SEQ ID NO:13,SEQ ID NO:14, 或 SEQ ID NO:33。

35. 权利要求 32 所述的组合物,其中所述抗体包含 :(a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9,SEQ ID NO:10,SEQ ID NO:11,SEQ ID NO:12,SEQ ID NO:13,SEQ ID NO:14, 或 SEQ ID NO:33。

36. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:10。

37. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:11。

38. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:2 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:12。

39. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:13。

40. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:14。

41. 权利要求 32 所述的组合物,其中所述抗体包含 :

(1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3 ;

(2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

(3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

(4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

(5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

(6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

42. 权利要求 32 所述的组合物,其中所述抗体包含 VH 序列 SEQ ID NO:15,SEQ ID NO:27,SEQ ID NO:16, 或 SEQ ID NO:17。

43. 权利要求 32 所述的组合物,其中所述抗体包含 VL 序列 SEQ ID NO:18,SEQ ID NO:19,SEQ ID NO:20,SEQ ID NO:21,SEQ ID NO:22,SEQ ID NO:23, 或 SEQ ID NO:34。

44. 权利要求 32 所述的组合物,其中所述抗体包含 VH 序列 SEQ ID NO:15 和 VL 序列 SEQ ID NO:18。

45. 权利要求 32 所述的组合物,其中所述抗体包含 VH 序列 SEQ ID NO:15 和 VL 序列 SEQ ID NO:19。

46. 权利要求 32 所述的组合物,其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:20。

47. 权利要求 32 所述的组合物,其中所述抗体包含 VH 序列 SEQ ID NO:16 和 VL 序列 SEQ ID NO:21。

48. 权利要求 32 所述的组合物，其中所述抗体包含 VH 序列 SEQ ID NO:17 和 VL 序列 SEQ ID NO:22。

49. 权利要求 32 所述的组合物，其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:23。

50. 权利要求 32 所述的组合物，其中所述抗体包含 VH 序列 SEQ ID NO:17 和 VL 序列 SEQ ID NO:34。

51. 一种皮下给药装置，所述装置含有权利要求 26-50 任一项所述的组合物，用于向个体递送 200 至 1200mg 范围内的抗体的平稳剂量。

52. 权利要求 51 所述的装置，其中所述装置是预填充注射器。

53. 权利要求 51 所述的装置，其中所述装置是 1-mL 预填充注射器并且所述预填充注射器中的抗体浓度是 200mg/mL。

54. 权利要求 51 所述的装置，其中所述装置是 2.25-mL 预填充注射器并且所述预填充注射器中的抗体浓度是 200mg/mL。

55. 一种降低受试者中 LDL- 胆固醇水平的方法，所述方法包括向所述受试者施用有效量的权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物。

56. 一种治疗受试者中胆固醇相关病症的方法，所述方法包括向所述受试者施用有效量的权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物。

57. 一种治疗受试者中高胆固醇血症的方法，所述方法包括向所述受试者施用有效量的权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物。

58. 权利要求 55-57 任一项所述的方法，其中将抗 -PCSK9 抗体以每剂量 200mg, 380mg, 400mg, 600mg, 760mg, 或 800mg 每 4 周，每 6 周，每 8 周，每 10 周，或每 12 周皮下给药。

59. 权利要求 58 所述的方法，其中以 200mg 皮下给药所述抗 -PCSK9 抗体。

60. 权利要求 58 所述的方法，其中以 380mg 皮下给药所述抗 -PCSK9 抗体。

61. 权利要求 58 所述的方法，其中以 400mg 皮下给药所述抗 -PCSK9 抗体。

62. 权利要求 58 所述的方法，其中以 600mg 皮下给药所述抗 -PCSK9 抗体。

63. 权利要求 58 所述的方法，其中以 760mg 皮下给药所述抗 -PCSK9 抗体。

64. 权利要求 58 所述的方法，其中以 800mg 皮下给药所述抗 -PCSK9 抗体。

65. 权利要求 59-64 任一项所述的方法，其中每 4 周施用所述抗 -PCSK9 抗体。

66. 权利要求 59-64 任一项所述的方法，其中每 6 周施用所述抗 -PCSK9 抗体。

67. 权利要求 59-64 任一项所述的方法，其中每 8 周施用所述抗 -PCSK9 抗体。

68. 权利要求 59-64 任一项所述的方法，其中每 10 周施用所述抗 -PCSK9 抗体。

69. 权利要求 59-64 任一项所述的方法，其中每 12 周施用所述抗 -PCSK9 抗体。

70. 权利要求 55-69 任一项所述的方法，所述方法进一步包括向所述受试者施用有效量的第二药物，其中所述抗 -PCSK9 抗体是第一药物。

71. 权利要求 70 所述的方法，其中所述第二药物提高 LDLR 的水平。

72. 权利要求 70 所述的方法，其中所述第二药物降低 LDL- 胆固醇的水平。

73. 权利要求 70 所述的方法，其中所述第二药物包含他汀类。

74. 权利要求 73 所述的方法，其中所述他汀类选自由以下组成的组 :阿托伐他汀，氟伐他汀，洛伐他汀，美伐他汀，匹伐他汀，普伐他汀，罗舒伐他汀，辛伐他汀，及其任何组合。

75. 权利要求 70 所述的方法，其中所述第二药物提高 HDL- 胆固醇的水平。

76. 一种抑制受试者中 PCSK9 结合 LDLR 的方法，所述方法包括向所述受试者施用有效量的权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物。

77. 一种降低受试者中 LDL- 胆固醇水平的方法，所述方法包括以每剂量 400mg 至 1000mg、每 4 周到每 12 周或每个月到每 3 个月向所述受试者皮下给药有效量的抗 -PCSK9 抗体。

78. 权利要求 67 所述的方法，其中所述 LDL- 胆固醇水平从基线降低至少 45％并在最后一次剂量给药之后维持在降低的水平至少一个月。

79. 一种治疗受试者中胆固醇相关病症的方法，所述方法包括以每剂量 400mg 至 1000mg、每 4 周至每 12 周或每个月至每 3 个月向所述受试者皮下给药有效量的抗 -PCSK9 抗体。

80. 一种治疗受试者中高胆固醇血症的方法，所述方法包括以每剂量 400mg 至 1000mg、每 4 周或每 12 周或每个月至每 3 个月向所述受试者皮下给药有效量的抗 -PCSK9 抗体。

81. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物，其用于降低受试者中 LDL- 胆固醇水平。

82. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物，其用于治疗受试者中胆固醇相关病症。

83. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物，其用于治疗受试者中高胆固醇血症。

84. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体，或权利要求 26-50 任一项所述的组合物，其用于抑制受试者中 PCSK9 结合 LDLR。

85. 皮下剂量的抗 -PCSK9 抗体，其用于每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用以降低受试者中 LDL- 胆固醇水平。

86. 皮下剂量的抗 -PCSK9 抗体，其用于每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用以治疗受试者中胆固醇相关病症。

87. 皮下剂量的抗 -PCSK9 抗体，其用于每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用以治疗受试者中高胆固醇血症。

88. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体、或权利要求 26-50 任一项所述的组合物用于降低受试者中 LDL- 胆固醇水平的用途。

89. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体、或权利要求 26-50 任一项所述的组合物用于治疗受试者中胆固醇相关病症的用途。

90. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体、或权利要求 26-50 任一项所述

的组合物用于治疗受试者中高胆固醇血症的用途。

91. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体、或权利要求 26-50 任一项所述的组合物用于抑制受试者中 PCSK9 结合 LDLR 的用途。

92. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于降低受试者中 LDL- 胆固醇水平的用途。

93. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于治疗受试者中胆固醇相关病症的用途。

94. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于治疗受试者中高胆固醇血症的用途。

95. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体或权利要求 26-50 任一项所述的组合物用于制造降低受试者中 LDL- 胆固醇水平的药物的用途。

96. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体或权利要求 26-50 任一项所述的组合物用于制造治疗受试者中胆固醇相关病症的药物的用途。

97. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体或权利要求 26-50 任一项所述的组合物用于制造治疗受试者中高胆固醇血症的药物的用途。

98. 权利要求 1-16 和 25 任一项所述的抗 -PCSK9 抗体或权利要求 26-50 任一项所述的组合物用于制造抑制受试者中 PCSK9 结合 LDLR 的药物的用途。

99. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于制造降低受试者中 LDL- 胆固醇水平的药物的用途。

100. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于制造治疗受试者中胆固醇相关病症的药物的用途。

101. 每 4 周至每 12 周或每个月至每 3 个月以每剂量 400mg 至 1000mg 施用的皮下剂量的抗 -PCSK9 抗体用于制造治疗受试者中高胆固醇血症的药物的用途。

抗 -PCSK9 抗体，制剂，剂量给药，和使用方法

[0001] 对相关专利申请的交叉引用

[0002] 本申请要求 2012 年 6 月 15 日提交的美国临时申请号 61/660,605 和 2013 年 3 月 14 日提交的美国临时申请号 61/786,280 的优先权权益，所述美国临时申请以其整体通过引用并入本文。

发明领域

[0003] 本发明涉及抗 -PCSK9 抗体，抗体制剂，剂量给药方案，及其使用方法。

[0004] 发明背景

[0005] 前蛋白转化酶枯草溶菌素 /kexin 型 9(Proprotein convertase subtilisin/kexin type 9)(PCSK9) 是哺乳动物前蛋白转化酶枯草溶菌素家族的成员。PCSK9 通过控制在血流中循环的低密度脂蛋白 (LDL) 颗粒的水平在胆固醇代谢中起关键作用。升高的 PCSK9 水平已经显示降低肝中 LDL- 受体水平，导致血浆中高水平的 LDL- 胆固醇，并且导致增加的对冠状动脉疾病的易感性。(Peterson 等，J Lipid Res.49(7):1595-9(2008))。因此，高度有利的是制备抑制或拮抗 PCSK9 的活性和 PCSK9 在不同治疗条件下所起的相应作用的基于治疗剂的 PCSK9 的拮抗剂。

[0006] 发明概述

[0007] 本发明部分基于多种针对 PCSK9 的抗体。PCSK9 呈现为重要和有利的治疗靶标，并且本发明提供作为用于靶向与 PCSK9 的表达和 / 或活性相关的病理学状态的治疗和诊断剂的抗体。因此，本发明提供与 PCSK9 相关的方法、组合物、试剂盒和制品。

[0008] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含可变结构域，所述可变结构域包含至少一个、两个、三个、四个、五个或六个选自由以下组成的组的高变区 (HVR) 序列：

[0009] (i)HVR-H1，所述 HVR-H1 包含 $GFTFX_1X_2X_3X_4IH$(SEQ ID NO:28)，其中 X_1 是 S 或 T；X_2 是 G、R 或 S；X_3 是 H、T 或 Y；X_4 是 A 或 T；

[0010] (ii)HVR-H2，所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4)；

[0011] (iii)HVR-H3，所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5)；

[0012] (iv)HVR-L1，所述 HVR-L1 包含 $RASQDVSX_1AVA$(SEQ ID NO:29)，其中 X_1 是 S 或 T；

[0013] (v)HVR-L2，所述 HVR-L2 包含 $SASX_1LYS$(SEQ ID NO:30)，其中 X_1 是 F 或 S；和

[0014] (vi)HVR-L3，所述 HVR-L3 包含 $QQSYX_1X_2X_3X_4T$(SEQ ID NO:31) 或 $QQAYX_1X_2X_3X_4T$(SEQ ID NO:37)，其中 X_1 是 P,R 或 T；X_2 是 A,I,S 或 T；X_3 是 L,P 或 Q；X_4 是 A,H,P 或 S。

[0015] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含可变结构域，所述可变结构域包含以下六种 HVR 序列：

[0016] (i)HVR-H1，所述 HVR-H1 包含 $GFTFX_1X_2X_3X_4IH$(SEQ ID NO:28)，其中 X_1 是 S 或 T；X_2 是 G、R 或 S；X_3 是 H、T 或 Y；X_4 是 A 或 T；

[0017] (ii)HVR-H2，所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4)；

[0018] (iii)HVR-H3，所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5)；

[0019] (iv)HVR-L1,所述 HVR-L1 包含 RASQDVSX$_1$AVA(SEQ ID NO:29),其中 X$_1$ 是 S 或 T ;

[0020] (v)HVR-L2,所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30),其中 X$_1$ 是 F 或 S ;和

[0021] (vi)HVR-L3,所述 HVR-L3 包 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37), 其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

[0022] 在某些实施方案中,提供结合 PCSK9 或其片段的抗体或抗体片段,其中所述抗体包含可变结构域,所述可变结构域包含至少一个、两个、三个、四个、五个或六个选自由以下组成的组的高变区 (HVR) 序列 :

[0023] (i)HVR-H1,所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$(SEQ ID NO:45), 其中 X$_1$ 是 S 或 T ; X$_2$ 是 G,R 或 S ;X$_3$ 是 H,T 或 Y ;X$_4$ 是 A 或 T ;X$_5$ 是 H 或 N ;

[0024] (ii)HVR-H2,所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ;

[0025] (iii)HVR-H3,所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ;

[0026] (iv)HVR-L1,所述 HVR-L1 包含 RASQDVSX$_1$AVA(SEQ ID NO:29), 其中 X$_1$ 是 S 或 T ;

[0027] (v)HVR-L2,所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30), 其中 X$_1$ 是 F 或 S ;和

[0028] (vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37), 其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

[0029] 在某些实施方案中,提供结合 PCSK9 或其片段的抗体或抗体片段,其中所述抗体包含可变结构域,所述可变结构域包含以下六个 HVR 序列 :

[0030] (i)HVR-H1,所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$(SEQ ID NO:45), 其中 X$_1$ 是 S 或 T ; X$_2$ 是 G,R 或 S ;X$_3$ 是 H,T 或 Y ;X$_4$ 是 A 或 T ;X$_5$ 是 H 或 N ;

[0031] (ii)HVR-H2,所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ;

[0032] (iii)HVR-H3,所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ;

[0033] (iv)HVR-L1,所述 HVR-L1 包含 RASQDVSX$_1$AVA(SEQ ID NO:29), 其中 X$_1$ 是 S 或 T ;

[0034] (v)HVR-L2,所述 HVR-L2 包含 SASX$_1$LYS(SEQ ID NO:30), 其中 X$_1$ 是 F 或 S ;和

[0035] (vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37), 其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

[0036] 在某些实施方案中,提供结合 PCSK9 或其片段的抗体或抗体片段,其中所述抗体包含可变结构域,所述可变结构域包含至少一个、两个、三个、四个、五个或六个选自由以下组成的组的高变区 (HVR) 序列 :

[0037] (i)HVR-H1,所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$(SEQ ID NO:45), 其中 X$_1$ 是 S 或 T ; X$_2$ 是 G,R 或 S ;X$_3$ 是 H,T 或 Y ;X$_4$ 是 A 或 T ;X$_5$ 是 H 或 N ;

[0038] (ii)HVR-H2,所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ;

[0039] (iii)HVR-H3,所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ;

[0040] (iv)HVR-L1,所述 HVR-L1 包含 RASQDVSTAVA(SEQ ID NO:7) ;

[0041] (v)HVR-L2,所述 HVR-L2 包含 SASFLYS(SEQ ID NO:8) ;和

[0042] (vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37), 其中 X$_1$ 是 P,R 或 T ;X$_2$ 是 A,I,S 或 T ;X$_3$ 是 L,P 或 Q ;X$_4$ 是 A,H,P 或 S。

[0043] 在某些实施方案中,提供结合 PCSK9 或其片段的抗体或抗体片段,其中所述抗体包含可变结构域,所述可变结构域包含以下六个 HVR 序列 :

[0044] (i)HVR-H1,所述 HVR-H1 包含 GFTFX$_1$X$_2$X$_3$X$_4$IX$_5$(SEQ ID NO:45), 其中 X$_1$ 是 S 或 T ;

X_2 是 G,R 或 S ;X_3 是 H,T 或 Y ;X_4 是 A 或 T ;X_5 是 H 或 N ;

[0045] (ii)HVR-H2,所述 HVR-H2 包含 RISPANGNTNYADSVKG(SEQ ID NO:4) ;

[0046] (iii)HVR-H3,所述 HVR-H3 包含 WIGSRELYIMDY(SEQ ID NO:5) ;

[0047] (iv)HVR-L1,所述 HVR-L1 包含 RASQDVSTAVA(SEQ ID NO:7) ;

[0048] (v)HVR-L2,所述 HVR-L2 包含 SASFLYS(SEQ ID NO:8) ;和

[0049] (vi)HVR-L3,所述 HVR-L3 包含 QQSYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:31) 或 QQAYX$_1$X$_2$X$_3$X$_4$T(SEQ ID NO:37), 其中 X_1 是 P,R 或 T ;X_2 是 A,I,S 或 T ;X_3 是 L,P 或 Q ;X_4 是 A,H,P 或 S。

[0050] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1,SEQ ID NO:2,SEQ ID NO:3, 或 SEQ ID NO:42,(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4, 和 (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。在某些实施方案中，所述抗体进一步包含 (a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9,SEQ ID NO:10,SEQ ID NO:11,SEQ ID NO:12,SEQ ID NO:13,SEQ ID NO:14, 或 SEQ ID NO:33。

[0051] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 (a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 of SEQ ID NO:9,SEQ ID NO:10,SEQ ID NO:11,SEQ ID NO:12,SEQ ID NO:13,SEQ ID NO:14, 或 SEQ ID NO:33。在某些实施方案中，所述抗体进一步包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1,SEQ ID NO:2,SEQ ID NO:3, 或 SEQ ID NO:42,(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4, 和 (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

[0052] 在一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 :

[0053] (1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;

[0054] (2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

[0055] (3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

[0056] (4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 ;

[0057] (5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:26 ;和

[0058] (6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9。

[0059] 在一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 :

[0060] (1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;

[0061] (2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;

[0062] (3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;

[0063] (4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;

[0064] (5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和

[0065] (6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9。

[0066] 在一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0067] (1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1；

[0068] (2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0069] (3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0070] (4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0071] (5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0072] (6)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:10。

[0073] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0074] (1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1；

[0075] (2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0076] (3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0077] (4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0078] (5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0079] (6)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:11。

[0080] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0081] (1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:2；

[0082] (2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0083] (3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0084] (4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0085] (5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0086] (6)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:12。

[0087] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0088] (1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:42；

[0089] (2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0090] (3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0091] (4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0092] (5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0093] (6)HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:12。

[0094] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0095] (1)HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3；

[0096] (2)HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0097] (3)HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0098] (4)HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0099] (5)HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0100] (6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:13。

[0101] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0102] (1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3；

[0103] (2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0104] (3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0105] (4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0106] (5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0107] (6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

[0108] 在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含：

[0109] (1)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1；

[0110] (2)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4；

[0111] (3)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5；

[0112] (4)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7；

[0113] (5)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8；和

[0114] (6)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:14。

[0115] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 (a)VH 序列,所述 VH 序列与氨基酸序列 SEQ ID NO:15,SEQ ID NO:16,SEQ ID NO:17,SEQ ID NO:27, 或 SEQ ID NO:43 具有至少 95％序列同一性；或 (b)VL 序列,所述 VL 序列与氨基酸序列 SEQ ID NO:18,SEQ ID NO:19,SEQ ID NO:20,SEQ ID NO:21,SEQ ID NO:22,SEQ ID NO:23,SEQ ID NO:34, 或 SEQ ID NO:44 具有至少 95％序列同一性。

[0116] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:15,SEQ ID NO:16,SEQ ID NO:17,SEQ ID NO:27, 或 SEQ ID NO:43。在某些实施方案中，所述抗体进一步包含 VL 序列 SEQ ID NO:18,SEQ ID NO:19,SEQ ID NO:20,SEQ ID NO:21,SEQ ID NO:22,SEQ ID NO:23,SEQ ID NO:34, 或 SEQ ID NO:44。

[0117] 在一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:15 和 VL 序列 SEQ ID NO:18。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:44。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:15 和 VL 序列 SEQ ID NO:19。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:19。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:20。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:16 和 VL 序列 SEQ ID NO:21。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:43 和 VL 序列 SEQ ID NO:21。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:17 和 VL 序列 SEQ ID NO:22。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体

或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:27 和 VL 序列 SEQ ID NO:23。在另一个实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体包含 VH 序列 SEQ ID NO:17 和 VL 序列 SEQ ID NO:34。

[0118] 在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体结合 PCSK9 的片段内的表位。在某些实施方案中，提供结合 PCSK9 或其片段的抗体或抗体片段，其中所述抗体结合包含人 PCSK9 氨基酸序列 SEQ ID NO:24 的氨基酸 376 到 379 的 PCSK9 片段内的表位。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D238。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 A239。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D238 和 A239。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D367。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366 和 D367。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 H391。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366,D367 和 H391。根据另一个实施方案，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 A239 和 H391。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 A239,A341,E366,D367 和 H391 中的一个或多个。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括临近人 PCSK9 的 A239,A341,E366,D367 和 H391 的残基中的一个或多个。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包含 (i) 至少一个选自由 R194 和 E195 组成的组的残基，(ii) 至少一个选自由 D238 和 A239 组成的组的残基，(iii) 至少一个选自由 A341 和 Q342 组成的组的残基，和 (iv) 至少一个选自由人 PCSK9 的 E366,D367,I369,S376,T377,C378,F379,S381 和 H391 组成的组的残基。在某些实施方案中，所述功能和 / 或结构表位包含以下残基中的一个、两个、三个、四个、五个、六个、七个、八个、九个、十个、十一个、十二个、十三个、十四个或全部 :人 PCSK9 的 R194,E195,D238,A239,A341,Q342,E366,D367,I369,S376,T377,C378,F379,S381 和 H391。

[0119] 在某些实施方案中，抗 PCSK9 抗体是单克隆抗体。在某些实施方案中，抗 PCSK9 抗体是人源化的。在某些实施方案中，抗 PCSK9 抗体是人抗体。在某些实施方案中，至少一部分的抗 PCSK9 抗体的框架序列是人共有框架序列。在一个实施方案中，抗体是选自以下各项的抗体片段 :Fab，Fab'-SH，Fv，scFv 或 $(Fab')_2$ 片段。

[0120] 在一方面中，提供编码以上抗 PCSK9 抗体中任一个的核酸。在一个实施方案中，提供包含所述核酸的载体。在一个实施方案中，载体是表达载体。在一个实施方案中，提供包含所述载体的宿主细胞。在一个实施方案中，宿主细胞是真核的。在另一个实施方案中，宿主细胞是哺乳动物的。在另一个实施方案中，宿主细胞是原核的。在一个实施方案中，提供制备抗 PCSK9 抗体的方法，其中所述方法包括在适于表达编码所述抗体的核酸的条件下培养所述宿主细胞，以及分离所述抗体。在某个实施方案中，所述方法还包括从宿主细胞回收抗 PCSK9 抗体。在某些实施方案中，提供包含本文所述的任何抗 PCSK9 抗体的组合物。在一个实施方案中，所述组合物还包含药用载体。

[0121] 在一个方面，本文中提供的是一种药物组合物，所述药物组合物包含约 100 至约

225mg/mL 的抗 -PCSK9 抗体，约 180 至约 220mM 的精氨酸琥珀酸盐，约 0.01％至约 0.03％的聚山梨酯，并且 pH 在约 5.2 至约 6.2。在某些实施方案中，组合物中的抗 -PCSK9 抗体或抗体片段为约 150mg/mL，组合物中的精氨酸琥珀酸盐为约 200mM，和组合物中的聚山梨酯 20 为约 0.02％，并且 pH 为约 5.5。在某些实施方案中，所述组合物适于皮下给药。在某些实施方案中，所述组合物的粘度在 25℃小于约 10cP。可以将本领域已知的或本文描述的任何抗 -PCSK9 抗体配制为所述组合物。

[0122] 在一个方面，本文中提供的是药物组合物，所述药物组合物包含约 150 至约 225mg/mL 的抗 -PCSK9 抗体，约 10 至约 30mM 的组氨酸乙酸盐，约 150 至约 170mM 的精氨酸乙酸盐，约 0.01％至约 0.03％的聚山梨酯，并且 pH 为约 5.8 至约 6.2。在某些实施方案中，组合物中的抗 -PCSK9 抗体或抗体片段为约 200mg/mL，组合物中的组氨酸乙酸盐为约 20mM，组合物中的精氨酸乙酸盐为约 160mM，和组合物中的聚山梨酯 20 为约 0.02％，并且 pH 为约 6.0。在某些实施方案中，所述组合物适于皮下给药。在某些实施方案中，所述组合物的粘度在 25℃小于约 10cP。可以将本领域已知的或本文中描述的任何抗 -PCSK9 抗体配制为所述组合物。

[0123] 在一个方面，本文中提供的是一种皮下给药装置，所述装置含有抗 -PCSK9 抗体或包含本文中描述的抗 -PCSK9 抗体的组合物。在某些实施方案中，所述装置用于向个体递送约 200 至约 1200mg 范围内的抗体的平稳剂量 (flat dose)。在某些实施方案中，所述装置是预填充注射器 (例如，0.5-mL，1-mL，1.25-mL，1.5-mL，1.75-mL，2-mL，2.25-mL，或 2.5-mL 注射器)。在某些实施方案中，所述装置是 1-mL 预填充注射器并且预填充注射器中抗体浓度为约 200mg/mL。在某些实施方案中，所述装置是 1.5-mL 预填充注射器并且预填充注射器中抗体浓度为约 200mg/mL。在某些实施方案中，所述装置是 2-mL 预填充注射器并且预填充注射器抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 2.25-mL 预填充注射器并且预填充注射器中抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 2.5-mL 预填充注射器并且预填充注射器中抗体浓度是约 200mg/mL。

[0124] 在一方面中，本发明涉及在受试者中抑制 PCSK9 与 LDL- 受体 (LDLR) 结合的方法，所述方法包括向所述受试者施用有效量的本文所述的任何抗 PCSK9 抗体。在另一方面中，本发明涉及降低受试者的胆固醇水平的方法，所述方法包括向所述受试者施用有效量的本文所述的任何抗 PCSK9 抗体。在一个实施方案中，胆固醇是 LDL- 胆固醇。在另一方面中，本发明涉及降低受试者的 LDL- 胆固醇水平的方法，所述方法包括向所述受试者施用有效量的本文所述的任何抗 PCSK9 抗体。在某些实施方案中，本发明涉及降低受试者的血清 LDL- 胆固醇水平的方法，所述方法包括向所述受试者施用有效量的本文所述的任一种抗 PCSK9 抗体。在另一方面中，本发明涉及治疗受试者的与升高的 LDL- 胆固醇水平相关的病症的方法，所述方法包括向所述受试者施用有效量的本文所述的任一种抗 PCSK9 抗体。

[0125] 在一方面中，本发明涉及治疗胆固醇相关疾病的方法。预期的胆固醇相关疾病的示例性和非限制性列表提供在本文中的“组合物和方法”下。在某些实施方案中，胆固醇相关疾病是高胆固醇血症 (hypercholesterolemia)。在某些实施方案中，本发明涉及治疗高胆固醇血症的方法，所述方法包括向所述受试者施用有效量的本文所述的任一种抗 PCSK9 抗体。在某些实施方案中，本发明涉及预防和 / 或治疗动脉粥样硬化 (atherosclerosis) 和 / 或心血管疾病的方法。在某些实施方案中，本发明涉及降低个体中复发的心血管事件

的风险的方法，所述方法包括向所述个体施用有效量的本文所述的任一种抗 PCSK9 抗体。

[0126] 在一方面中，本发明涉及治疗可以通过消除、抑制或降低 PCSK9 活性而被改善、减缓、抑制或预防的任何疾病或病症的方法。在某些实施方案中，可以通过使用他汀类治疗或预防的疾病或病症也可以使用本文所述的任一种抗 PCSK9 抗体来治疗。在某些实施方案中，可以受益于防止胆固醇合成或提高的 LDLR 表达的疾病或病症也可以使用本文所述的任一种抗 PCSK9 抗体来治疗。

[0127] 在本文中描述的方法的某些实施方案中，每两周，每个月，每两个月，或每三个月，以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每两周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每个月以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每两个月以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每三个月以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。

[0128] 在本文中描述的方法的某些实施方案中，每两周，每个月，每两个月，或每三个月以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每两周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每个月以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每两个月以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每三个月以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。

[0129] 在本文中描述的方法的某些实施方案中，每 2 周，每 4 周，每 6 周，每 8 周，每 10 周，或每 12 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每

2 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 4 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 6 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 10 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周以每剂量 200mg, 220mg, 380mg, 400mg, 600mg, 760mg, 800mg, 1000mg, 1140mg, 或 1200mg 皮下给药所述抗 -PCSK9 抗体。

[0130] 在本文中描述的方法的某些实施方案中，每 2 周，每 4 周，每 6 周，每 8 周，每 10 周，或每 12 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 2 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 4 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 6 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 10 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周以每剂量 200-1200mg, 200-1000mg, 200-800mg, 200-600mg, 200-400mg, 400-1200mg, 400-1000mg, 400-800mg, 400-600mg, 600-1200mg, 600-1000mg, 600-800mg, 800-1200mg, 800-1000mg, 800-900mg, 750-850mg, 750-800mg, 775-825mg, 350-450mg, 375-425mg, 或 375-400mg 皮下给药所述抗 -PCSK9 抗体。

[0131] 在本文中描述的方法的某些实施方案中，每 8 周以每剂量 600mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 800mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 10 周以每剂量 800mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周以每剂量 800mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 760mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 10 周以每剂量 760mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周以每剂量 760mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 4 周以每剂量 400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 400mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周每剂量 400mg 以皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 4 周以每剂量 380mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 380mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 12 周以每剂量 380mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 2 周以每剂量 220mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 4 周以每剂量 220mg 皮下给药所述抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，每 8 周以每剂量 220mg 皮下给药所述抗 -PCSK9 抗体。

[0132] 在本文中描述的方法的某些实施方案中，监测接受抗 -PCSK9 抗体的受试者的 LDL-c 水平并且如果其水平降低至低于 25 或 15mg/dL，则通过调节剂量和 / 或施用频率将其剂量下调至起始剂量约 50％或 25％。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 800mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降至低于 25mg/dL，则将剂量调整至每 8 周 200mg 的抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 800mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降至低于 15mg/dL，则将剂量调整至每 8 周 200mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 760mg 抗 -PCSK9 抗体的起始剂量，监测受试者 LDL-c 水平并且如果受试者的 LDL-c 水平降至低于 25mg/dL，则将剂量调整至每 8 周 200mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 760mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将剂量调整至每 8 周 200mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 760mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将剂量调整至每 8 周 190mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 8 周 760mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将剂量调整至每 8 周 190mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 4 周 400mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将剂量调整至每 4 周 100mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 4 周 400mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果

受试者的 LDL-c 水平降低至低于 15mg/dL，则将剂量调整至每 4 周 100mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 4 周 380mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将剂量调整至每 4 周 100mg 抗 -PCSK9 抗体。在本文中描述的方法的某些实施方案中，向受试者施用每 4 周 380mg 抗 -PCSK9 抗体的起始剂量，监测受试者的 LDL-c 水平并且如果受试者的 LDL-c 水平降低至 15mg/dL，将剂量调整至每 4 周 100mg 抗 -PCSK9 抗体。

[0133] 在某些实施方案中，使用皮下给药装置施用在前提到的任何皮下剂量。在某些实施方案中，所述装置是预填充注射器（例如，0.5-mL，1-mL，1.25-mL，1.5-mL，1.75-mL，2-mL，2.25-mL，或 2.5-mL 注射器）。在某些实施方案中，所述装置是 1-mL 预填充注射器并且预填充注射器中的抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 1.5-mL 预填充注射器并且预填充注射器中的抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 2-mL 预填充注射器并且预填充注射器中的抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 2.25-mL 预填充注射器并且预填充注射器中的抗体浓度是约 200mg/mL。在某些实施方案中，所述装置是 2.5-mL 预填充注射器并且预填充注射器中的抗体浓度是约 200mg/mL。在某些实施方案中，可以使用一个以上注射器以获得全部平稳剂量，例如，一个注射器，两个注射器，三个注射器，或四个注射器。在备选的实施方案中，可以使用高容量，一次性，皮下输注装置以获得全部平稳剂量，例如，可以施用 3mL，4mL，5mL，6mL，7mL，8mL，9mL 或 10mL 的剂量。

[0134] 在某些实施方案中，所述剂量是 800mg 并且使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器每 8 周施用。在某些实施方案中，所述剂量是 800mg 并且每 8 周使用三个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 800mg 并且每 10 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 800mg 并且每 10 周使用三个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 800mg 并且每 12 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 800mg 并且每 12 周使用三个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 800mg 并且每 8 周使用含有 4mL 200mg/mL 的抗 -PCSK9 抗体的高容量、一次性、皮下输注装置施用。

[0135] 在某些实施方案中，所述剂量是 760mg 并且每 8 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 760mg 并且每 10 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 760mg 并且每 12 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。

[0136] 在某些实施方案中，所述剂量是 600mg 并且每 8 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案中，所述剂量是 600mg 并且每 12 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。

[0137] 在某些实施方案中，所述剂量是 400mg 并且每 4 周使用一个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.5mL 注射器施用。在某些实施方案中，所述剂量是 400mg 并且每 4 周使用两个含有 200mg/mL 浓度的抗 -PCSK9 抗体的 2.25mL 注射器施用。在某些实施方案

中，所述剂量是380mg并且每4周使用一个含有200mg/mL浓度的抗-PCSK9抗体的2.25mL注射器施用。

[0138] 在某些实施方案中，本文所述的方法还包括向所述受试者施用有效量的第二药物，其中所述抗PCSK9抗体是第一药物。在一个实施方案中，第二药物提升水LDLR蛋白的水平。在另一个实施方案中，第二药物降低LDL-胆固醇的水平。在另一个实施方案中，第二药物包含他汀类(statin)。在另一个实施方案中，他汀类选自由以下组成的组：阿托伐他汀(atorvastatin)，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)，罗舒伐他汀(rosuvastatin)，辛伐他汀(simvastatin)，及其任意组合。在另一个实施方案中，第二药物提升HDL-胆固醇的水平。在某些实施方案中，受试者或个体是人。

[0139] 在一方面中，本发明涉及检测怀疑含有PCSK9蛋白的样品中PCSK9蛋白的方法，所述方法包括(a)将样品与本文所述的抗PCSK9抗体接触；和(b)检测抗PCSK9抗体和PCSK9蛋白间的复合物的形成。在一个实施方案中，抗PCSK9抗体是被可检测地标记的。

[0140] 本文所述的任何实施方案或其任何组合适用于本文所述的发明的任何和所有抗PCSK9抗体、方法和用途。

[0141] 附图简述

[0142] 图1显示抗PCSK9抗体的重链HVR序列，H1(SEQ ID NO 1,1,1,1,1,2,42,3,3,1，分别地，以出现的次序)，H2(都被公开为SEQ ID NO:4)，和H3(都被公开为SEQ ID NO:5)，和轻链HVR序列，L1(SEQ ID NO:6,7,7,7,7,7,7,7,7和7，分别地，以出现的次序)，L2(SEQ ID NO:26,8,8,8,8,8,8,8,8和8，分别地，以出现的次序)和L3(SEQ ID NO:9,9,10,10,11,12,12,13,33和14，分别地，以出现的次序)。

[0143] 图2A-B显示抗PCSK9抗体的以下的氨基酸序列：(A)重链可变结构域(SEQ ID NO:15,27,15,27,27,16,43,17,17和27，分别地，以出现的次序)和(B)轻链可变结构域(SEQ ID NO:18,44,19,19,20,21,21,22,34和23，分别地，以出现的次序)。位置按照Kabat被编号并且高变区被加框。

[0144] 图3A-D显示抗PCSK9抗体(IgG)对(A)人PCSK9，(B)鼠PCSK9，(C)食蟹猴(cyno)PCSK9和大鼠PCSK9，以及(D)恒河猴(rhesus)PCSK9的解离常数。

[0145] 图4.在竞争结合ELISA中抗PCSK9抗体抑制PCSK9与LDLR的结合。空白(无抗体；空心方框)和对照抗体(空心圆)以虚线显示。抗PCSK9抗体以实线显示。抗PCSK9抗体的IC_{50}值显示在表中。

[0146] 图5.将不同浓度的抗PCSK9抗体与15μg/ml PCSK9温育并加入到HepG2细胞达4小时。处理细胞以用于表面LDLR的FACS分析。数据指示抗PCSK9抗体有效地防止LDLR下调。阳性对照是未用PCSK9处理的细胞。

[0147] 图6.使用抗LDLR抗体的蛋白印迹显示30μg的PCSK9处理1小时显著地下调小鼠肝中的LDLR水平。

[0148] 图7.使用抗LDLR抗体的蛋白印迹显示所有五种抗PCSK9抗体防止小鼠肝中的LDLR下调。底部的免疫印迹是每个治疗组4个肝(每个肝10μg蛋白)的组合(pool)。

[0149] 图8显示单次静脉内注射后在C57JBL/6小鼠血清中的抗PCSK9抗体浓度。显示的是剂量给药组0.5mg/kg；5mg/kg；和20mg/kg(n = 3)的平均浓度。

[0150] 图 9 显示单次静脉内注射 5mg/kg 抗 PCSK9 抗体后 C57JBL/6WT 和 $PCSK9^{-/-}$ 小鼠血清中的抗 PCSK9 抗体浓度的比较。显示了每个剂量给药组的平均浓度 (n = 3)。

[0151] 图 10 显示单次静脉内注射后个体食蟹猴的血清中的抗 PCSK9 抗体浓度。包括三个剂量给药组 :5mg/kg ;20mg/kg ;和 60mg/kg。

[0152] 图 11 显示单次静脉内注射后食蟹猴血清中的抗 PCSK9 抗体浓度。显示的是剂量给药组 5mg/kg,20mg/kg 和 60mg/kg(n = 3) 的平均浓度。

[0153] 图 12 显示用单剂量 (10mg/kg 体重) 的对照 (Crtl) 或抗 PCSK9 抗体处理的小鼠的血清中的总胆固醇水平。在图中所示的不同日期测量胆固醇水平。

[0154] 图 13 显示在来自用单剂量 (10mg/kg 体重) 的对照或抗 PCSK9 抗体处理的小鼠的血清中的总胆固醇水平。

[0155] 图 14 显示 I 期临床试验设计的图解,包括群组 A-J。对于总共 8 名患者 / 群组和 80 名总患者,各个群组包括六名用活性剂治疗的患者和两名用安慰剂治疗的患者。

[0156] 图 15 显示对于研究群组 A-J 的药物代谢动力学数据。来自单剂量群组 A-E 和 J 的结果显示于左图并且来自多剂量群组 F-I 的结果显示于右图。红色箭头表示药物施用的时间安排。

[0157] 图 16 显示对于单剂量群组,LDL-c(mg/dL) 水平从基线的平均绝对数值变化。

[0158] 图 17 显示对于单剂量群组,LDL-c 水平基线的平均百分数变化。

[0159] 图 18 显示对于多剂量群组,LDL-c(mg/dL) 水平从基线的平均绝对数值变化。

[0160] 图 19 显示对于多剂量群组,LDL-c 水平基线的平均百分数变化。

[0161] 图 20 显示作为 200mM 精氨酸琥珀酸盐 ,0.02％ PS20,pH 5.5 的制剂中蛋白浓度的函数的抗 -PCSK9 的粘度。

[0162] 图 21 显示对于 2cc 玻璃瓶中的含有不同浓度聚山梨酯 20(PS20) 的对照和搅动的抗 -PCSK9 样品的尺寸排阻层析 (SEC)(左图) 和浊度 (右图) 分析。

[0163] 图 22 显示通过肽制图 (mapping) 在不同条件下抗 -PCSK9 中蛋氨酸和色氨酸残基的氧化。

[0164] 图 23 显示通过肽制图在不同条件下抗 -PCSK9 的 CDR 中和临近抗 -PCSK9 的 CDR 的蛋氨酸和色氨酸残基的氧化。

[0165] 图 24 显示对于 200mg/mL 抗 -PCSK9 从 pH 5.0 至 6.5 的离子交换层析 (IEC)(左图) 和 SEC(右图)pH 率曲线 (200mM 精氨酸琥珀酸盐 ,0.02％ PS20,pH 5.0-6.0 或 20mM 组氨酸 HCL,160mM 精氨酸 HCl,0.02％ PS20,pH 6.5)。

[0166] 图 25 显示冷冻储藏于 HCl(200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸 HCl,160mM 精氨酸 HCl,0.02％ PS20,pH 6.0) 和乙酸盐 (200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸乙酸盐 ,160mM 精氨酸乙酸盐 ,0.02％ PS20,pH 6.0) 制剂中的过程中,通过对抗 -PCSK9 的 SEC 获得的百分数主峰 (左图) 和百分数高分子量种类 (HMWS)(右图) 数据。

[0167] 图 26 显示在 40℃储存 1 个月后通过 CE-SDS(上),SEC(中), 和 IEC(下) 获得的 pH 6.0 时对 200mg/mL 抗 -PCSK9 的反荷离子效应。

[0168] 图 27 显示 II 期临床试验的研究设计 , 包括研究剂量群组、抗 -PCSK9 抗体剂量方案和试验的各组中患者数量的概览。

[0169] 图 28 显示接受抗 -PCSK9 抗体或安慰剂的患者中的平均药物代谢动力学 (+/- 标

准偏差)(左图)和平均总 PCSK9(+/- 标准误差)(右图)。

[0170] 图 29 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的直接 LDL 胆固醇从基线的绝对数值变化。

[0171] 图 30 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的直接 LDL 胆固醇从基线的相对变化。

[0172] 图 31 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的总胆固醇从基线的绝对数值变化。

[0173] 图 32 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的总胆固醇从基线的相对变化。

[0174] 图 33 显示接受抗 -PCSK9 抗体或安慰剂的患者中非 HDL 胆固醇从基线的绝对数值变化。

[0175] 图 34 显示接受抗 -PCSK9 抗体或安慰剂的患者中非 HDL 胆固醇从基线的相对变化。

[0176] 图 35 显示接受抗 -PCSK9 抗体或安慰剂的患者中载脂蛋白 B 从基线的绝对数值变化。

[0177] 图 36 显示接受抗 -PCSK9 抗体或安慰剂的患者中载脂蛋白 B 从基线的相对变化。

[0178] 图 37A 显示对于接受抗 -PCSK9 抗体或安慰剂后的至少一次探访,直接 LDL-c 值小于或等于 15mg/dL 的患者的比例 , 和

[0179] 图 37B 显示确定对于接受抗 -PCSK9 抗体或安慰剂之后的至少一次探访直接 LDL-c 值小于或等于 25mg/dL 的患者的比例所进行的实验的结果。

[0180] 发明实施方案详述

[0181] 本文描述或引用的技术和方法一般被很好的理解,并且通常为本领域技术人员使用常规方法所采用,如,例如在以下文献中所述的被广泛使用的方法 :Sambrook 等 ,Molecular Cloning:A Laboratory Manual(分子克隆 : 实验室手册) 第 3 版 (2001)Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.CURRENT PROTOCOLS IN MOLECULAR BIOLOGY(分子生物学中的最新实验方法)(F.M.Ausubel 等编辑 ,(2003)) ;METHODS IN ENZYMOLOGY 系列 (Academic Press,Inc.) :PCR 2:A PRACTICAL APPROACH(PCR 2 : 实用方法)(M.J.MacPherson,B.D.Hames 和 G.R.Taylor 编辑 (1995)), Harlow 和 Lane 编辑 (1988)ANTIBODIES,A LABORATORY MANUAL(抗体,实验室手册),和 ANIMAL CELL CULTURE(动物细胞培养)(R.I.Freshney 编辑 (1987)) ;Oligonucleotide Synthesis(寡核苷酸合成)(M.J.Gait 编辑 1984) ;Methods in Molecular Biology(分子生物学中的方法),Humana Press ;Cell Biology:A Laboratory Notebook(细胞生物学 : 实验室手册)(J.E.Cellis 编辑 1998)Academic Press ;Animal Cell Culture(动物细胞培养)(R.I.Freshney) 编辑 ,1987) ;Introduction to Cell and Tissue Culture(细胞和组织培养导言)(J.P.Mather 和 P.E.Roberts,1998)Plenum Press ; Cell and Tissue Culture:Laboratory Procedures(细胞和组织培养 : 实验室方法) (A.Doyle,J.B.Griffiths 和 D.G.Newell 编 辑 ,1993-8)J.Wiley 和 Sons ;Handbook of Experimental Immunology(实验免疫学手册)(D.M.Weir 和 C.C.Blackwell 编辑) ; Gene Transfer Vectors for Mammalian Cells(用于哺乳动物细胞的基因转移载体)

(J.M.Miller 和 M.P.Calos 编辑,1987);PCR:The Polymerase Chain Reaction(PCR:聚合酶链反应),(Mullis 等编辑,1994);Current Protocols in Immunology(免疫学最新方法)(J.E.Coligan 等编辑,1991);Short Protocols in Molecular Biology(分子生物学中的小方法)(Wiley 和 Sons,1999);Immunobiology(免疫生物学)(C.A.Janeway 和 P.Travers,1997);Antibodies(抗体)(P.Finch,1997);Antibodies:A Practical Approach(抗体:实用方法)(D.Catty 编辑,IRL Press,1988-1989);Monoclonal Antibodies:A Practical Approach(单克隆抗体:实用方法)(P.Shepherd 和 C.Dean 编辑,Oxford University Press,2000);Using Antibodies:A Laboratory Manual(使用抗体:实验室手册)(E.Harlow 和 D.Lane(Cold Spring Harbor Laboratory Press,1999);The Antibodies(抗体)(M.Zanetti 和 J.D.Capra 编辑,Harwood Academic Publishers,1995);以及 Cancer:Principles and Practice of Oncology(癌症:肿瘤学的原理和实践)(V.T.DeVita 等编辑,J.B.Lippincott Company,1993)。

[0182] I. 定义

[0183] 除非另外限定,本文所用的技术和科学术语具有与本发明所属的技术领域中的普通技术人员所通常理解的相同的含义。Singleton 等,Dictionary of Microbiology and Molecular Biology(微生物学和分子生物学词典)第 2 版,J.Wiley&Sons(New York,N.Y.1994),以及 March,Advanced Organic Chemistry Reactions,Mechanisms and Structure(高等有机化学反应,机制和结构)第 4 版,John Wiley&Sons(New York,N.Y.1992),对于本申请中所用的许多术语,为本领域技术人员提供了一般指导。本文引用的所有文献(包括专利申请和出版物)通过引用以其整体并入。

[0184] 为了解释本说明书,将使用以下定义,并且只要适当,以单数形式使用的术语也可以包括复数,并且反之亦然。要理解,本文所用的术语仅是为了描述具体的实施方案,并且不意欲是限制性的。在以下所述的任何定义与通过引用结合于本文中的任何文献冲突的情况中,以以下所述的定义为准。

[0185] 在本说明书和权利要求中,免疫球蛋白重链中残基的编号是如在 Kabat 等,Sequences of Proteins of Immunological Interest,第 5 版.Public Health Service,National Institutes of Health,Bethesda,Md.(1991)中的 EU 索引(EU index)的编号,上述文献明确通过引用结合于本文中。“如在 Kabat 中的 EU 索引”是指人 IgG_1 EU 抗体的残基编号。

[0186] 用于本文目的的“接纳体人框架”是包含衍生自人免疫球蛋白框架或如下所定义的人共有框架的轻链可变结构域(VL)框架或重链可变结构域(VH)框架的氨基酸序列的框架。“衍生自”人免疫球蛋白框架或人共有框架的接纳体人框架可以包含其相同的氨基酸序列,或其可以包含氨基酸序列的变化。在一些实施方案中,氨基酸变化的数目为 10 以下,9 以下,8 以下,7 以下,6 以下,5 以下,4 以下,3 以下,或 2 以下。在一些实施方案中,VL 接纳体人框架的序列与 VL 人免疫球蛋白框架序列或人共有框架序列相同。

[0187] “亲和力”是指分子(例如抗体)的单一结合位点与其结合配偶体(例如抗原)之间全部非共价相互作用总和的强度。除非另有说明,在用于本文时,“结合亲和力”指反映结合对的成员(例如抗体与抗原)之间 1:1 相互作用的内在结合亲和力。分子 X 对其配偶体 Y 的亲和力通常可用解离常数(Kd)来表述。亲和力可通过本领域知道的常用方法来测量,

包括本文中所描述的那些。用于测量结合亲和力的具体说明性和示例性实施方案在以下描述。

[0188] “亲和力成熟的”抗体指这样的抗体，在该抗体的一个或多个高变区 (HVR) 中具有一处或多处改变，导致该抗体对抗原的亲和力与没有这些改变的亲本抗体相比有提高。

[0189] 术语“抗 PCSK9 抗体”、“抗 PCSK9”、“PCSK9 抗体”或“结合 PCSK9 的抗体”是指这样的抗体，所述抗体能够以足够的亲和力结合 PCSK9 抗体以致所述抗体可以用作靶向 PCSK9 中的诊断剂和 / 或治疗剂。在一个实施方案中，抗 PCSK9 抗体与不相关的、非 PCSK9 蛋白结合的程度低于所述抗体与 PCSK9 结合的约 10％，如例如通过放射性免疫测定 (RIA) 测量的。在某些实施方案中，结合 PCSK9 的抗体的解离常数 (Kd) ≤ 1μM，≤ 100nM，≤ 10nM，≤ 1nM，≤ 0.1nM，≤ 0.01nM，或≤ 0.001nM(例如 10^{-8}M 以下，例如 10^{-8}M 至 10^{-13}M，例如 10^{-9}M 至 10^{-13}M)。在某些实施方案中，抗 PCSK9 抗体结合来自不同物种的 PCSK9 中保守的 PCSK9 表位。

[0190] 术语 ″抗体″在本文中以最广义使用，并且包括不同抗体结构，包括但不限于单克隆抗体，多克隆抗体，多特异性抗体 (例如，双特异性抗体)，和抗体片段，只要它们显示所需的抗原结合活性。

[0191] ″抗体片段″是指不同于完整抗体的分子，其包含完整抗体的部分，所述部分结合完整抗体结合的抗原。抗体片段的实例包括但不限于 Fv，Fab，Fab’，Fab’-SH，$F(ab')_2$；双抗体；线性抗体；单链抗体分子 (例如 scFv)；和由抗体片段形成的多特异性抗体。木瓜蛋白酶消化抗体产生两个相同的抗原结合片段，称为“Fab”片段，各自具有单一抗原结合位点，和残留的“Fc”片段，其名称反映了它易于结晶的能力。胃蛋白酶处理产生 $F(ab')_2$ 片段，其具有两个抗原结合位点并且仍然能够交联抗原。

[0192] 与参照抗体“结合相同表位的抗体”是指这样的抗体，其在竞争测定中阻断 50％以上的所述参照抗体与其抗原的结合，反之，参照抗体在竞争测定中阻断 50％以上的该抗体与其抗原的结合。本文中提供一个示例性竞争测定。在某些实施方案中，基于与 PCSK9 结合的抗 PCSK9 抗体 Fab 片段的晶体结构来确定表位。

[0193] 术语“嵌合”抗体是指这样的抗体，其中一部分重链和 / 或轻链来源于特定来源或物种，而剩余的重链和 / 或轻链来源于不同来源或物种。

[0194] 抗体的“类别”是指其重链具有的恒定结构域或恒定区的类型。有五个主要类别的抗体：IgA，IgD，IgE，IgG 和 IgM，并且这些中的数个可以进一步被划分为亚类 (同种型)，例如，IgG_1，IgG_2，IgG_3，IgG_4，IgA_1 和 IgA_2。对应于不同类别的免疫球蛋白的重链恒定结构域分别被称为 α，δ，ε，γ 和 μ。

[0195] 术语“细胞毒性剂”用在本发明中指抑制或防止细胞功能和 / 或引起细胞死亡或破坏的物质。细胞毒性剂包括但不限于：放射性同位素 (例如，At^{211}，I^{131}，I^{125}，Y^{90}，Re^{186}，Re^{188}，Sm^{153}，Bi^{212}，P^{32}，Pb^{212} 和 Lu 的放射性同位素)；化疗剂或药物 (例如，甲氨蝶呤 (methotrexate)，阿霉素 (adriamicin)，长春花生物碱 (vinca alkaloids)(长春新碱 (vincristine)，长春碱 (vinblastine)，依托泊苷 (etoposide))，多柔比星 (doxorubicin)，美法仑 (melphalan)，丝裂霉素 (mitomycin)C，苯丁酸氮芥 (chlorambucil)，柔红霉素 (daunorubicin) 或其它嵌入剂)；生长抑制剂；酶及其片段如核酸水解酶；抗生素；毒素如小分子毒素或细菌、真菌、植物或动物起源的酶促活性毒素，包

括其片段和 / 或变体 ;和下面公开的各种抗肿瘤或抗癌剂。

[0196] 术语“双抗体 (diabodies)”指具有两个抗原结合位点的抗体片段,所述片段在相同的多肽链 (VH-VL) 中包含与轻链可变结构域 (VL) 连接的重链可变结构域 (VH)。通过使用太短所以不能在相同链上的两个结构域之间配对的接头,迫使所述结构域与另一条链的互补结构域配对从而产生两个抗原结合位点。双抗体可以是二价的或双特异性的。双抗体更充分地描述于例如 EP 404,097 ;WO 1993/01161 ;Hudson 等,Nat.Med.9:129-134(2003) ;和 Hollinger 等 , 美国国家科学院学报 (Proc.Natl.Acad.Sci.USA)90:6444-6448(1993) 中。三抗体和四抗体同样描述于 Hudson 等,Nat.Med.9:129-134(2003) 中。

[0197] “效应子功能”指那些可归于抗体 Fc 区且随抗体同种型而变化的生物学活性。抗体效应子功能的实例包括 :C1q 结合和补体依赖性细胞毒性 (CDC) ;Fc 受体结合 ;抗体依赖性细胞介导的细胞毒性 (ADCC) ;吞噬作用 ;细胞表面受体 (例如 B 细胞受体) 下调 ;和 B 细胞活化。

[0198] 试剂例如药物制剂的″有效量″是指以需要的剂量和在需要的时间阶段有效获得所需的治疗或预防结果的量。

[0199] “Fab”片段包括重链可变结构域和轻链可变结构域,并且还包括轻链的恒定结构域以及重链的第一恒定结构域 (CH1)。Fab’片段因在重链 CH1 结构域的羧基末端增加了一些残基 (包括来自抗体铰链区的一个或多个半胱氨酸) 而与 Fab 片段不同。Fab’-SH 是本文中对其中恒定结构域的半胱氨酸残基携带一个游离硫醇基的 Fab’ 的称谓。$F(ab')_2$ 抗体片段最初是作为成对 Fab’ 片段生成的,在 Fab’ 片段之间具有铰链半胱氨酸。抗体片段的其它化学偶联也是已知的。

[0200] 术语“Fc 区”在本文中用于定义免疫球蛋白重链的 C 端区域,所述区域包含至少一部分的恒定区。该术语包括天然序列 Fc 区和变体 Fc 区。在某些实施方案中,人 IgG 重链 Fc 区从 Cys226 或 Pro230 延伸至重链的羰基端。然而,Fc 区的 C 端赖氨酸 (Lys447) 可以存在或者可以不存在。除非另外说明,Fc 区或恒定区中的氨基酸残基的编号是根据 EU 编号系统,其也被称为 EU 索引,如在 Kabat 等,Sequences of Proteins of Immunological Interest,第 5 版 Public Health Service,National Institutes of Health,Bethesda,MD,1991 中所述。

[0201] ″构架″或″FR″是指除高变区 (HVR) 残基之外的可变结构域残基。可变结构域的 FR 通常由四个 FR 结构域组成 :FR1,FR2,FR3 和 FR4。因此,HVR 和 FR 序列通常出现在 VH(或 VL) 的以下序列中 :FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4。

[0202] 术语“全长抗体”、“完整的抗体”和“完整抗体”在本文被可交换地用于指结构与天然抗体结构基本相似或具有包含如本文所定义的 Fc 区的重链的抗体。

[0203] “Fv”是包含完整抗原结合位点的最小抗体片段。在一个实施方案中,双链 Fv 种类由一个重链可变结构域和一个轻链可变结构域以紧密的,非共价缔合的二聚体组成。在单链 Fv(scFv) 种类中,一个重链可变结构域和一个轻链可变结构域可以通过柔性肽接头共价连接从而使轻链和重链可以以类似于双链 Fv 种类的“二聚体”结构缔合。在这种构型中,每个可变结构域的三个 HVRs 相互作用从而限定在 VH-VL 二聚体的表面上的抗原结合位点。总而言之,六个 HVRs 将抗原结合特异性赋予抗体。然而,即使是单个可变结构域 (或只包含对抗原特异的三个 HVRs 的 Fv 的一半) 也具有识别和结合抗原的能力,尽管亲和性

低于完整结合位点。

[0204] 术语″宿主细胞″、″宿主细胞系″和″宿主细胞培养物″被可交换地使用并且是指其中引入外源核酸的细胞，包括这种细胞的后代。宿主细胞包括″转化体″和″转化的细胞″，其包括初级转化的细胞和来源于其的后代，而不考虑传代的数目。后代在核酸含量上可能与亲本细胞不完全相同，而是可以包含突变。本文中包括与在最初转化的细胞中筛选或选择的具有相同功能或生物学活性的突变体后代。

[0205] “人抗体”指具有这样的氨基酸序列的抗体，所述氨基酸序列对应于这样抗体的氨基酸序列，所述抗体由人或人细胞生成或来源于非人来源，其利用人抗体库或其它人抗体编码序列。人抗体的这种定义明确排除包含非人抗原结合残基的人源化抗体。

[0206] “人共有构架”是指这样的构架，即在选择人免疫球蛋白 VL 或 VH 构架序列中，其代表最常出现的氨基酸残基。一般而言，对人免疫球蛋白 VL 或 VH 序列的选择是从可变结构域序列的亚型中选择。一般而言，该序列的亚型是如 Kabat 等，Sequences of Proteins of Immunological Interest，第五版，NIH Publication 91-3242，Bethesda MD(1991)，1-3 卷中的亚型。在一个实施方案中，对于 VL，该亚型是如 Kabat 等（见上文）中的亚型 κ I。在一个实施方案中，对于 VH，该亚型是如 Kabat 等（见上文）中的亚型 III。

[0207] “人源化”抗体是指包含来自非人 HVR 的氨基酸残基和来自人 FR 的氨基酸残基的嵌合抗体。在某些实施方案中，人源化抗体将包含基本上所有的至少一个、通常两个可变结构域，其中所有或基本上所有的 HVR（例如，CDR）对应于非人抗体的那些，并且所有或基本上所有的 FR 对应于人抗体的那些。人源化抗体任选可以包含至少一部分的来源于人抗体的抗体恒定区。抗体（例如非人抗体）的“人源化形式”是指已经进行了人源化的抗体。

[0208] 术语“高胆固醇血症”当用于本文中时是指其中胆固醇水平升高到理想水平以上的病症。在某些实施方案中，LDL- 胆固醇水平升高到理想水平以上。在某些实施方案中，血清 LDL- 胆固醇水平升高到理想水平以上。

[0209] 术语“高变区”或“HVR”当在本文中使用时，是指抗体可变结构域的每个区域，其序列高可变和 / 或形成结构上限定的环（“高变环”）。通常，天然四链抗体包含六个 HVR；三个在 VH(H1，H2，H3) 中，三个在 VL(L1，L2，L3) 中。HVR 通常包含来自高变环和 / 或“互补决定区”(CDR) 的氨基酸残基，后者具有最高序列可变性和 / 或涉及抗原识别。示例性高变环发生在氨基酸残基 26-32(L1)，50-52(L2)，91-96(L3)，26-32(H1)，53-55(H2)，和 96-101(H3)。(Chothia 和 Lesk，J.Mol.Biol.196:901-917(1987))。示例性 CDR(CDR-L1，CDR-L2，CDR-L3，CDR-H1，CDR-H2，和 CDR-H3) 发生在氨基酸残基 L1 的 24-34，L2 的 50-56，L3 的 89-97，H1 的 31-35B，H2 的 50-65，和 H3 的 95-102。(Kabat 等，Sequences of Proteins of Immunological Interest，第 5 版 Public Health Service，National Institutes of Health，Bethesda，MD(1991))。除了 VH 中的 CDR1，CDR 通常包含形成高变环的氨基酸残基。CDR 还包含“特异性决定残基”或“SDR”，其是与抗原接触的残基。SDR 包含在被称为缩短的 (abbreviated)-CDR 或 a-CDR 的 CDR 区域中。示例性 a-CDR(a-CDR-L1，a-CDR-L2，a-CDR-L3，a-CDR-H1，a-CDR-H2，和 a-CDR-H3) 发生在氨基酸残基 L1 的 31-34，L2 的 50-55，L3 的 89-96，H1 的 31-35B，H2 的 50-58，和 H3 的 95-102。（见 Almagro 和 Fransson，Front.Biosci.13:1619-1633(2008))。除非另外说明，可变结构域中的 HVR 残基和其他残基（例如，FR 残基）在本文中根据 Kabat 等（见上文）编号。

[0210] “免疫缀合物”是与一个或多个异源分子（包括但不限于细胞毒性剂）缀合的抗体。

[0211] “个体”或“受试者”是哺乳动物。哺乳动物包括但不限于，家养动物（例如，牛，羊，猫，狗和马），灵长类动物（例如，人和非人灵长类动物如猴），兔，以及啮齿类动物（例如，小鼠和大鼠）。在某些实施方案中，个体或受试者是人。

[0212] ″分离的″抗体是这样的抗体，其已经与其天然环境的组分分离。在一些实施方案中，将抗体纯化至超过 95％或 99％纯度，如通过例如电泳（例如，SDS-PAGE，等电聚焦 (IEF)，毛细管电泳）或层析（例如，离子交换或反相 HPLC）确定的。对于用于评估抗体纯度的方法的综述，参见，例如，Flatman 等，J.Chromatogr.B 848:79-87(2007)。

[0213] ″分离的″核酸是指已经与其天然环境的组分分离的核酸分子。分离的核酸包括包含在通常包含该核酸分子的细胞中的核酸分子，但是该核酸分子存在于染色体外或在不同于其天然染色体位置的染色体位置处。

[0214] “分离的编码抗 PCSK9 抗体的核酸”是指一个或多个核酸分子，其编码抗体重和轻链（或其片段），包括在单一载体或分开的载体中的这样的核酸分子，以及存在于宿主细胞中的一个或多个位置处的这样的核酸分子。

[0215] 术语“单克隆抗体”在用于本文时指从一群基本上同质的抗体中获得的抗体，即除了可能的变体抗体（该变体抗体例如含有天然存在的突变或在产生单克隆抗体制剂的过程中出现，此类变体通常少量存在）外，构成群体的个体抗体是相同的和 / 或结合相同的表位。相比于通常包括针对不同决定簇（表位）的不同抗体的多克隆抗体制剂，单克隆抗体制剂中的每个单克隆抗体针对抗原上的单一决定簇。因此，修饰语“单克隆”表明抗体从基本上同质的抗体群获得的特征，不应解释为要求通过任何特定方法来产生抗体。例如，将根据本发明使用的单克隆抗体可通过多种技术来生成，包括但不限于杂交瘤法，重组 DNA 法，噬菌体展示法，和利用包含所有或部分的人免疫球蛋白基因座的转基因动物的方法，这样的方法和用于制备单克隆抗体的其他示例性方法描述于本文中。

[0216] “裸抗体”是指没有缀合异源部分（如细胞毒性部分）或放射性标记的抗体。裸抗体可以存在于药物制剂中。

[0217] ″天然抗体″是指天然存在的具有变化的结构的免疫球蛋白分子。例如，天然 IgG 抗体是约 150,000 道尔顿的异源四聚体糖蛋白，其由两个相同的轻链和两个相同的重链组成，所述链通过二硫键结合。从 N 末端至 C 末端，每个重链具有可变区 (VH)，其也被称为可变重结构域或重链可变结构域，其后是三个恒定结构域 (CH1，CH2 和 CH3)。类似地，从 N 末端到 C 末端，每个轻链具有可变区 (VL)，其也被称为可变轻结构域或轻链可变结构域，其后是恒定轻 (CL) 结构域。基于其恒定结构域的氨基酸序列，抗体的轻链可以被分配给被称为 kappa(κ) 和 lambda(λ) 的两个类型中的一个。

[0218] 术语“包装说明书”用于指通常包括在治疗产品的商品包装中的使用说明，其包含关于适应征、用法、剂量、给药、组合疗法、禁忌症的信息和 / 或关于使用这样的治疗产品的警告。

[0219] 相对于参比多肽序列的“百分比（％）氨基酸序列同一性”定义为在将所述序列进行比对（并在必要时导入空位）以获取最大百分比序列同一性，且不将任何保守置换视为序列同一性的部分之后，候选序列中的氨基酸残基与参比多肽序列中的氨基酸残基相同的

百分数。可使用本领域各种方法进行序列比对以便测定百分比氨基酸序列同一性，例如，使用公众可得到的计算机软件如 BLAST、BLAST-2、ALIGN 或 MEGALIGN(DNASTAR) 软件。本领域技术人员可以决定测量比对的适宜参数，包括对所比较的序列全长获得最大比对所需的任何算法。然而，为此目的，％氨基酸序列同一性值使用序列比较计算机程序 ALIGN-2 产生。ALIGN-2 序列比较计算机程序的作者是 Genentech, Inc，并且源代码已经随用户文档提交至美国版权局 (Washington D.C., 20559)，其美国版权注册登记号为 TXU510087。公众可通过 Genentech, Inc. (South San Francisco, California) 得到 ALIGN-2 程序，或者可以从源代码编译。ALIGN-2 程序应当为在 UNIX 操作系统、包括数字 UNIXV4.0D 上使用而进行编译。ALIGN-2 程序设定了所有序列比对参数并且不变。

[0220] 在 ALIGN-2 应用于氨基酸序列比较的情况中，给定氨基酸序列 A 相对于 (to)、与 (with)、或针对 (against) 给定氨基酸序列 B 的％氨基酸序列同一性 (或者这样说 :给定氨基酸序列 A 具有或含有相对于、与或针对给定氨基酸序列 B 的某一％氨基酸序列同一性) 如下计算 :

[0221] 100 乘以 X/Y 比值

[0222] 其中 X 是用序列比对程序 ALIGN-2 在该程序的 A 和 B 比对中评分为相同匹配的氨基酸残基数，且其中 Y 是 B 中的氨基酸残基总数。可以理解，当氨基酸序列 A 与氨基酸序列 B 的长度不相等时，A 相对于 B 的％氨基酸序列同一性将不等于 B 相对于 A 的％氨基酸序列同一性。除非另外具体说明，在本文用的所有％氨基酸序列同一性的值都是用 ALIGN-2 计算机程序如前段所描述的那样得到的。

[0223] 术语“药物制剂”或“药物组合物”指这样的制剂，其以允许包含在其中的活性成分的生物学活性有效的形式存在，并且不包含对施用所述制剂的受试者具有不可接受的毒性的另外的成分。

[0224] “药用载体”是指药物制剂中不同于活性成分的成分，其对受试者是无毒的。药用载体包括但不限于缓冲剂、赋形剂、稳定剂或防腐剂。

[0225] 除非另外说明，术语“前蛋白转化酶枯草溶菌素 /kexin 型 9(Proprotein convertase subtilisin/kexin type 9)(PCSK9)”、“PCSK9”或“NARC-1”当用于本文中时是指来自任何脊椎动物来源 (包括哺乳动物如灵长类动物 (例如人) 和啮齿类动物 (例如，小鼠和大鼠)) 的任何天然 PCSK9，除非另有说明。该术语涵盖“全长”未加工的 PCSK9 以及由细胞内加工产生的任何形式的 PCSK9 或其任何片段。该术语还包括天然存在的 PCSK9 的变体，例如，剪接变体或等位变体。

[0226] 术语“PCSK9 活性”或 PCSK9 的“生物学活性”当用于本文中时包括 PCSK9 的任何生物学作用。在某些实施方案中，PCSK9 活性包括 PCSK9 与底物或受体相互作用或结合的能力。在某些实施方案中，PCSK9 的生物学活性是 PCSK9 与 LDL- 受体 (LDLR) 结合的能力。在某些实施方案中，PCSK9 结合并催化涉及 LDLR 的反应。在某些实施方案中，PCSK9 活性包括 PCSK9 降低或减少 LDLR 的利用度的能力。在某些实施方案中，PCSK9 的生物学活性包括 PCSK9 提高受试者的 LDL 的量的能力。在某些实施方案中，PCSK9 的生物学活性包括 PCSK9 降低受试者的可以用于与 LDL 结合的 LDLR 的量的能力。在某些实施方案中，PCSK9 的生物学活性包括 PCSK9 降低可以用于与 LDL 结合的 LDLR 的量的能力。在某些实施方案中，PCSK9 的生物学活性包括由 PCSK9 信号传导 (signaling) 所致的任何生物学活性。

[0227] “单链 Fv”或“scFv”抗体片段包含抗体的 VH 和 VL 结构域，其中这些结构域以单多肽链存在。一般地，所述 scFv 多肽在 VH 和 VL 结构域之间还包含多肽接头，所述接头使 scFv 形成对于抗原结合需要的结构。关于 scFv 的综述参见例如 Pluckthün 于 The Pharmacology of Monoclonal Antibodies（单克隆抗体药理学），卷 113，Rosenburg 和 Moore 编辑，(Springer-Verlag, New York, 1994), pp. 269-315 中。

[0228] 用于本文时，“治疗 (treatment)”（及其语法变化如“治疗 (treat)”或“治疗 (treating)”）指在尝试改变待治疗的个体的天然进程中的临床干预，并且可以为了预防或在临床病理学的进程中进行。治疗的理想效果包括但不限于防止疾病发生或复发，缓和症状，消除疾病的任何直接或间接病理学后果，减少疾病进展速率，改善或减轻疾病状态，和症状缓解或改善的预后。在一些实施方案中，将本发明的抗体用于延缓疾病的发生或减缓疾病的进展。

[0229] 术语“可变区”或“可变结构域”是指参与抗体与抗原结合的抗体重或轻链的结构域。天然抗体的重链和轻链 (VH 和 VL，分别地）的可变结构域通常具有相似的结构，其中每个结构域包含四个保守的构架区 (FR) 和三个高变区 (HVR)。（参见，例如，Kindt 等 Kuby Immunology，第六版，W.H. Freeman&Co. 91 页 (2007)）。单个 VH 或 VL 结构域可以足以给予抗原结合特异性。此外，可以使用来自与特定抗原结合的抗体的 VH 或 VL 结构域来分离结合所述抗原的抗体，以分别筛选互补 VL 或 VH 结构域的文库。参见，例如，Portolano 等，J. Immunol. 150:880-887(1993) ;Clarkson 等，Nature 352:624-628(1991)。

[0230] 术语 ″ 载体 ″ 当在本文中使用时是指能够增殖与其相连的另一个核酸的核酸分子。该术语包括作为自我复制核酸结构的载体以及结合到已经引入其的宿主细胞的基因组中的载体。一些载体能够指导与其可操作相连的核酸的表达。这样的载体在本文中被称为 ″ 表达载体 ″。

[0231] 如本文使用的，除非另有说明，单数形式“一个 (a)”，“一个 (an)”，和“那个 (the)”包括复数参考。

[0232] 如本文使用的“约”是指在本技术领域本领域技术人员容易已知的对于各个值的通常误差范围。本文对“约”某值或参数的引用包括（和描述）指向那个值或参数本身的实施方案 .

[0233] 要理解本本文中描述的发明的方面和实施方案包括“包含”方面和实施方案，“由”方面和实施方案“组成，”和“基本上由”方面和实施方案“组成”。

[0234] II 组合物和方法

[0235] 在一方面中，本发明部分基于利用抗 PCSK9 抗体获得的实验和临床结果。所得的结果指示利用抗 PCSK9 抗体阻断 PCSK9 的生物学活性导致防止 LDLR 减少。此外，结果证实施用抗 PCSK9 抗体降低受试者的总 LDL- 胆固醇水平。因此，如本文所述的本发明的 PCSK9 抗体提供重要的治疗和诊断剂，所述治疗和诊断剂用于靶向与 PCSK9 相关的病理学状况，例如胆固醇相关疾病。

[0236] 在某些实施方案中，“胆固醇相关疾病”包括以下中的任一种或多种 ：高胆固醇血症、心脏病、代谢综合征 (metabolic syndrome)、糖尿病、冠状动脉心脏病 (coronary heart disease)、卒中 (stroke)、心血管疾病 (cardiovascular diseases)、阿尔茨海默病 (Alzheimers disease) 和一般性的异常脂血症 (dyslipidemia)（其显示为例如升高

的总血清胆固醇、升高的 LDL、升高的甘油三酯、升高的 VLDL 和 / 或低的 HDL)。可以使用抗 PCSK9 抗体 (单独地或与一种或多种其他药剂组合地) 治疗的原发性和继发性异常脂血症的一些非限制性实例包括代谢综合征、糖尿病 (diabetes mellitus)、家族性混合性高脂血症 (familial combined hyperlipidemia)、家族性高甘油三酯血症 (familial hypertriglyceridemia)、家族性高胆固醇血症 (familial hypercholesterolemias), 包括杂合性高胆固醇血症 (heterozygous hypercholesterolemia)、纯合性高胆固醇血症 (homozygous hypercholesterolemia)、家族性缺陷性载脂蛋白 (familial defective apoplipoprotein)B-100 ;多基因性高胆固醇血症 (polygenic hypercholesterolemia) ; 残粒移去障碍病 (remnant removal disease)、肝脂肪酶缺失 (hepatic lipase deficiency) ;继发于以下任何的异常脂血症 :饮食不慎 (dietary indiscretion)、甲状腺机能障碍 (hypothyroidism)、药物 (包括雌激素和孕酮疗法、β 阻断剂和噻嗪类利尿剂 (thiazide diuretics)) ;肾病综合征 (nephrotic syndrome)、慢性肾衰竭 (chronic renal failure)、库欣综合征 (Cushing's syndrome)、原发性胆汁性肝硬变 (primary biliary cirrhosis)、糖原沉积病 (glycogen storage diseases)、肝细胞瘤 (hepatoma)、胆汁淤积 (cholestasis)、肢端肥大症 (acromegaly)、胰岛素瘤 (insulinoma)、单纯性生长素缺乏症 (isolated growth hormone deficiency) 和酒精所致高甘油三酯血症 (alcohol-induced hypertriglyceridemia)。本文所述的抗 PCSK9 抗体可以用于预防或治疗动脉粥样硬化疾病,如例如,冠状动脉心脏病、冠脉疾病、周围动脉疾病 (peripheral arterial disease)、卒中 (缺血性 (ischaemic) 和出血性 (hemorrhagic))、心绞痛 (angina pectoris) 或脑血管疾病和急性冠脉综合征 (acute coronary syndrome)、心肌梗死 (myocardial infarction)。在某些实施方案中,本文所述的抗 PCSK9 抗体可以用于降低以下的风险 :非致死性心脏病发作 (nonfatal heart attacks)、致死性和非致死性卒中、某些类型的心脏手术、心力衰竭的住院治疗、患有心脏病的患者的胸痛、和 / 或心血管事件,其由于确定的心脏病导致,如在前的心脏病发作、在前的心脏手术、和 / 或有阻塞的动脉的证据的胸痛。在某些实施方案中,本文所述的抗 PCSK9 抗体和方法可以用于降低复发的心血管事件的风险。

[0237] A. 示例性抗 -PCSK9 抗体

[0238] 在一方面中,本发明提供结合 PCSK9 的分离的抗体。在某些实施方案中,抗 PCSK9 抗体调节 PCSK9 活性。

[0239] 在一方面中,本发明提供抗 PCSK9 抗体,其包含至少一个、两个、三个、四个、五个或六个选自以下的 HVR :(a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ; (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。

[0240] 在一方面中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体包含六个包括以下的 HVR :(a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所

述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。

[0241] 在一方面中,本发明提供抗体,所述抗体包含至少一个、至少两个或全部三个选自以下的 VH HVR 序列 :(a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;和 (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。在一个实施方案中,抗体包含 HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。在另一个实施方案中,抗体包含 HVR-H3 和 HVR-L3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。在另一个实施方案中,抗体包含 :HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33 ;和 HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4。在另一个实施方案中,抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;和 (c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

[0242] 在另一方面中,本发明提供抗体,所述抗体包含至少一个、至少两个或全部三个选自以下的 VL HVR 序列 :(a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。在一个实施方案中,抗体包含 (a)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7 ;(b)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26 ;和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。

[0243] 在另一方面中,本发明的抗体包含 (a)VH 结构域,所述 VH 结构域包含至少一个、至少两个或全部三个选自以下的 VH HVR 序列 :(i)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42,(ii)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4,和 (iii)HVR-H3,所述 HVR-H3 包含选自 SEQ ID NO:5 的氨基酸序列 ;和 (b)VL 结构域,所述 VL 结构域包含至少一个、至少两个或全部三个选自以下的 VL HVR 序列 :(i)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7,(ii)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26,和 (c)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。

[0244] 在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序

列 SEQ ID NO:6 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:26 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:10。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:11。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:2 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:12。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:42 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:12。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:13。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:14。在另一个方面中 , 本发明提供抗体,所述抗体包含 (a)HVR-H1,所述 HVR-H1 包含氨基酸序列 SEQ ID NO:3 ;(b)HVR-H2,所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4 ;(c)HVR-H3,所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5 ;(d)HVR-L1,所述 HVR-L1 包含氨基酸序列 SEQ ID NO:7 ;(e)HVR-L2,所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 ;和 (f)HVR-L3,所述 HVR-L3 包含氨基酸序列 SEQ ID NO:33。

[0245] 在某些实施方案中,抗 PCSK9 抗体是人源化的。在一个实施方案中,抗 PCSK9 抗体包含如在任意以上实施方案中的 HVR,并且还包含接纳体人框架,例如,人免疫球蛋白框架

或人共有框架。

[0246] 在另一方面中，抗 PCSK9 抗体包含重链可变结构域 (VH) 序列，所述序列与氨基酸序列 SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:27 或 SEQ ID NO:43 具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或 100％序列同一性。在某些实施方案中，具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％或 99％同一性的 VH 序列相对于参考序列包含置换（例如，保守性置换）、插入或缺失，但是包含所述序列的抗 PCSK9 抗体保持结合 PCSK9 的能力。在某些实施方案中，在 SEQ ID NO:15，SEQ ID NO:16，SEQ NO:17、SEQ ID NO:27 或 SEQ ID NO:43 中，总计 1 至 10 个氨基酸被置换、插入和 / 或缺失。在某些实施方案中，置换、插入或缺失发生在 HVR 外的区域（即，在 FR 中）。任选地，抗 PCSK9 抗体包含 SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:27 或 SEQ ID NO:43 中的 VH 序列，包括所述序列的翻译后修饰。在一个特别的实施方案中，VH 包含一个、两个或三个选自以下的 HVR：(a) HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3 或 SEQ ID NO:42，(b) HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4，和 (c) HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

[0247] 在另一方面中，提供抗 PCSK9 抗体，其中所述抗体包含轻链可变结构域 (VL)，所述轻链可变结构域 (VL) 与氨基酸序列 SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:34、或 SEQ ID NO:44 具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或 100％序列同一性。在某些实施方案中，具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％或 99％同一性的 VL 序列相对于参考序列包含置换（例如，保守性置换）、插入或缺失，但是包含所述序列的抗 PCSK9 抗体保持结合 PCSK9 的能力。在某些实施方案中，在 SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:34 或 SEQ ID NO:44 中，总计 1 至 10 个氨基酸被置换、插入和 / 或缺失。在某些实施方案中，置换，插入或缺失发生在 HVR 外的区域（即，在 FR 中）。任选地，抗 PCSK9 抗体包含 SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:34 或 SEQ ID NO:44 中的 VL 序列，包括所述序列的翻译后修饰。在一个特别的实施方案中，VL 包含一个、两个或三个选自以下的 HVR：(a) HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7；(b) HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26；和 (c) HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14 或 SEQ ID NO:33。

[0248] 在另一个方面中，提供抗 -PCSK9 抗体，其中所述抗体包含如上文提供的任意实施方案中 VH，和如上文提供的任意实施方案中的 VL。在一个实施方案中，所述抗体分别包含 SEQ ID NO:15 和 SEQ ID NO:18 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:27 和 SEQ ID NO:44 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:15 和 SEQ ID NO:19 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:27 和 SEQ ID NO:19 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:27 和 SEQ ID NO:20 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:16

和 SEQ ID NO:21 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:43 和 SEQ ID NO:21 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:17 和 SEQ ID NO:22 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:27 和 SEQ ID NO:23 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。在一个实施方案中，所述抗体分别包含 SEQ ID NO:17 和 SEQ ID NO:34 中的 VH 和 VL 序列，包括那些序列的翻译后修饰。

[0249] 在另一个方面中，抗 -PCSK9 抗体包含重链序列，所述重链序列与氨基酸序列 SEQ ID NO:35 具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，99％，或 100％序列同一性。在某些实施方案中，具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，或 99％同一性的重链序列含有相对于参考序列的置换（例如，保守置换），插入，或缺失，但是包含该序列的抗 -PCSK9 抗体保留了结合 PCSK9 的能力。在某些实施方案中，SEQ ID NO:35 中总共 1 至 10 个氨基酸被置换，插入和 / 或缺失。在某些实施方案中，置换，插入，或缺失发生在 HVR 以外的区域（即，FR 中）。任选地，抗 -PCSK9 抗体重链包含 SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:27, 或 SEQ ID NO:43 中的 VH 序列，包括该序列的翻译后修饰。在特定实施方案中，所述重链包含选自以下各项的一个，两个或三个 HVR：(a) HVR-H1，所述 HVR-H1 包含氨基酸序列 SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, 或 SEQ ID NO:42, (b) HVR-H2，所述 HVR-H2 包含氨基酸序列 SEQ ID NO:4, 和 (c) HVR-H3，所述 HVR-H3 包含氨基酸序列 SEQ ID NO:5。

[0250] 在另一个方面中，提供抗 -PCSK9 抗体，其中所述抗体包含轻链，所述轻链与氨基酸序列 SEQ ID NO:36 具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，99％，或 100％序列同一性。在某些实施方案中，具有至少 90％，91％，92％，93％，94％，95％，96％，97％，98％，或 99％同一性的轻链序列含有相对于参考序列的置换（例如，保守置换）插入，或缺失，但包含该序列的抗 -PCSK9 抗体保留了结合 PCSK9 的能力。在某些实施方案中，SEQ ID NO:36 中总共 1 至 10 个氨基酸被置换，插入和 / 或缺失。在某些实施方案中，置换，插入，或缺失发生在 HVR 以外的区域（即，FR 中）。任选地，抗 -PCSK9 抗体轻链包含 SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, 或 SEQ ID NO:44 中的 VL 序列，包括该序列的翻译后修饰。在特定实施方案中，所述轻链包含选自以下各项的一个，两个或三个 HVR：(a) HVR-L1，所述 HVR-L1 包含氨基酸序列 SEQ ID NO:6 或 SEQ ID NO:7；(b) HVR-L2，所述 HVR-L2 包含氨基酸序列 SEQ ID NO:8 或 SEQ ID NO:26；和 (c) HVR-L3，所述 HVR-L3 包含氨基酸序列 SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, 或 SEQ ID NO:33。

[0251] 在另一个方面中，提供抗 -PCSK9 抗体，其中所述抗体包含如上文提供的任意实施方案中的重链，和如上文提供的任意实施方案中的轻链。在一个实施方案中，所述抗体包含重链，所述重链包含氨基酸序列 SEQ ID NO:35, 和轻链，所述轻链包含氨基酸序列 SEQ ID NO:36。在某些实施方案中，SEQ ID NO:35 在 C 末端被截短一个或两个氨基酸，例如，其不含有 K451, 或 G450 和 K451。在某些实施方案中，SEQ ID NO:35 中的 P449 是酰胺化的 (amidated)。

[0252] 抗体 508.20.33b 重链氨基酸序列 (SEQ ID NO:35)：

[0253] EVQLVESGGGLVQPGGSLRLSCAASGFTFSSTAIHWVRQAPGKGLEWVARISPANGNTNYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCARWIGSRELYIMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0254] 抗体 508.20.33b 轻链氨基酸序列 (SEQ ID NO:36)：

[0255] DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGT DFTLTUSSLQPEDFATYYCQQAYPALHTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0256] 在某些实施方案中，SEQ ID NO:35 在 C 末端被截短一个或两个氨基酸，例如，其不含有 K451，或 G450 和 K451（例如，所述重链包含 SEQ ID NO:35 的氨基酸 1-449 或 SEQ ID NO:35 的氨基酸 1-450）。在某些实施方案中，SEQ ID NO:35 中 P449 是酰胺化的。

[0257] 在某些实施方案中，可以通过组合丙氨酸扫描 (combinatorial alanine scanning) 来定位功能表位。在该方法中，组合丙氨酸扫描策略可以用于鉴别与抗 PCSK9 抗体的相互作用所需的 PCSK9 蛋白中的氨基酸。在某些实施方案中，表位是构象性的 (conformational) 并且与 PCSK9 结合的抗 PCSK9 抗体 Fab 片段的晶体结构可以用于鉴定表位。在一方面中，本发明提供抗体，所述抗体与任何本文提供的抗 PCSK9 抗体结合相同的表位。例如，在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:15 的 VH 序列和 SEQ ID NO:19 的 VL 序列的抗 PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:19 的 VL 序列的抗 PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:20 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:16 的 VH 序列和 SEQ ID NO:21 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:43 的 VH 序列和 SEQ ID NO:21 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:17 的 VH 序列和 SEQ ID NO:22 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:23 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:17 的 VH 序列和 SEQ ID NO:34 的 VL 序列的抗 -PCSK9 抗体结合相同的表位。

[0258] 在一方面中，本发明提供抗 PCSK9 抗体或其抗原结合片段，所述抗 PCSK9 抗体或其抗原结合片段与任一种本文所述的抗体竞争地结合人 PCSK9。在某些实施方案中，竞争性结合可以使用 ELISA 测定来确定。例如，在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:15 的 VH 序列和 SEQ ID NO:19 的 VL 序列的抗 PCSK9 抗体竞争地结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:19 的 VL 序列的抗 PCSK9 抗体竞争地结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:20 的 VL 序列的抗 -PCSK9 抗

体竞争结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:16 的 VH 序列和 SEQ ID NO:21 的 VL 序列的抗 -PCSK9 抗体竞争结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:43 的 VH 序列和 SEQ ID NO:21 的 VL 序列的抗 -PCSK9 抗体竞争结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:17 的 VH 序列和 SEQ ID NO:22 的 VL 序列的抗 -PCSK9 抗体竞争结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:27 的 VH 序列和 SEQ ID NO:23 的 VL 序列的抗 -PCSK9 抗体竞争结合 PCSK9。在某些实施方案中，提供这样的抗体，所述抗体与包含 SEQ ID NO:17 的 VH 序列和 SEQ ID NO:34 的 VL 序列的抗 -PCSK9 抗体竞争结合 PCSK9。

[0259] 在某些实施方案中，提供这样的抗体，所述抗体结合如本文所述的 PCSK9 的片段内的表位。在某些实施方案中，提供这样的抗体，所述抗体结合在包含 SEQ ID NO:24 的人 PCSK9 氨基酸序列的氨基酸 376 至 379 的 PCSK9 的片段内的表位。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D238。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 A239。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D238 和 A239。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 D367。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366 和 D367。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 H391。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 E366、D367 和 H391。根据另一个实施方案，根据本发明的抗体的功能和 / 或结构表位包括人 PCSK9 的残基 A239 和 H391。在某些实施方案中，功能和 / 或结构表位包括人 PCSK9 的残基 A239，A341，E366、D367 和 H391 中的一个或多个。在某些实施方案中，功能和 / 或结构表位包括邻近人 PCSK9 的 A239，A341，E366、D367 和 H391 的残基中的一个或多个。在某些实施方案中，根据本发明的抗体的功能和 / 或结构表位包含 (i) 至少一个选自由以下组成的组的残基 ：人 PCSK9 的 R194 和 E195，(ii) 至少一个选自由以下组成的组的残基 ：人 PCSK9 的 D238 和 A239，(iii) 至少一个选自由以下组成的组的残基 ：人 PCSK9 的 A341 和 Q342，和 (iv) 至少一个选自由以下组成的组的残基 ：人 PCSK9 的 E366、D367、I369、S376、T377、C378、F379、S381 和 H391。在某些实施方案中，功能和 / 或结构表位包含以下残基中的一个、两个、三个、四个、五个、六个、七个、八个、九个、十个、十一个、十二个、十三个、十四个或全部 ：人 PCSK9 的 R194，E195，D238，A239，A341，Q342，E366、D367、I369、S376、T377、C378、F379、S381 和 H391。

[0260] 在本发明的另一个方面中，根据任何以上实施方案的抗 PCSK9 抗体是单克隆抗体，包括嵌合抗体、人源化抗体或人抗体。在一个实施方案中，抗 PCSK9 抗体是抗体片段，例如，Fv，Fab，Fab’，scFv，双抗体或 $F(ab')_2$ 片段。在另一个实施方案中，抗体是全长抗体，例如，完整 IgG_1 抗体或如本文所定义的其他抗体类别或同种型。

[0261] 在另一方面中，根据任何以上实施方案的抗 PCSK9 抗体可以结合如在以下部分 1-7 中所述的任何特征（单独地或组合地）：

[0262] 1. 抗体亲和力

[0263] 在某些实施方案中，本文提供的抗体具有的解离常数 (Kd) ≤ 1μM，≤ 100nM，≤ 10nM，≤ 1nM，≤ 0.1nM，≤ 0.01nM，或≤ 0.001nM（例如 10^{-8}M 以下，例如 10^{-8}M 至 10^{-13}M，例如，10^{-9}M 至 10^{-13}M）。

[0264] 在一个实施方案中，Kd 通过用目的抗体的 Fab 形式及其抗原进行的放射性标记的抗原结合测定法 (RIA) 测量，如由以下测定所述的。Fab 对抗原的溶液结合亲和力通过在存在未标记抗原的滴定系列的情况下，用最小浓度的 (^{125}I)- 标记的抗原平衡 Fab，接着用抗 -Fab 抗体 - 包被的板捕获结合的抗原来测量（参见，例如，Chen 等，J.Mol.Biol.293:865-881(1999)）。为了确定测定的条件，将 MICROTITER® 多孔板 (Thermo Scientific) 用 5μg/ml 的在 50mM 碳酸钠 (pH 9.6) 中的捕获抗 -Fab 抗体 (Cappel Labs) 包被过夜，并随后用 PBS 中的 2% (w/v) 牛血清白蛋白在室温（约 23℃）封闭 2-5 小时。在非吸附板 (Nunc#269620) 中，将 100pM 或 26pM[^{125}I]- 抗原与目的 Fab 的系列稀释物混合（与在 Presta 等，癌症研究 (Cancer Res).57:4593-4599(1997) 中的抗 -VEGF 抗体，Fab-12 的评估一致）。接着，将目的 Fab 温育过夜；然而温育可以持续更长的阶段（例如 65 小时）从而确保达到平衡。随后，将混合物转移到捕获板中以在室温进行温育（例如 1 小时）。接着，去除溶液，并将所述板用在 PBS 中的 0.1% 聚山梨酯 20（TWEEN-20®）洗涤 8 次。当所述板已经干燥时，加入 150μl/ 孔的闪烁剂 (MICROSCINT-20™；Packard)，并将所述板在 TOPCOUNT ™γ 计数器 (Packard) 上计数 10 分钟。选择提供少于或等于 20% 的最大结合的每种 Fab 的浓度用在竞争性结合测定中。

[0265] 根据另一个实施方案，Kd 是通过表面等离子共振测定法使用 BIACORE® -2000 或 BIACORE® -3000 仪器 (BIAcore, Inc., Piscataway, NJ) 在 25℃使用固定化抗原 CM5 芯片在～ 10 个应答单位 (RU) 测量的。简而言之，依照供应商的说明书用盐酸 N- 乙基 -N'-(3- 二甲基氨基丙基)- 碳二亚胺 (EDC) 和 N- 羟基 - 琥珀酰亚胺 (NHS) 活化羧甲基化右旋糖苷生物传感器芯片 (CM5, BIACORE Inc.)。用 10mM 乙酸钠 pH4.8 将抗原稀释至 5μg/ml（～ 0.2μM），然后以 5μl/ 分钟的流速注入至获得约 10 个应答单位 (RU) 的偶联蛋白质。注入抗原后，注入 1M 乙醇胺以封闭未反应基团。为了进行动力学测量，在 25℃以约 25μl/ 分钟的流速注入在含 0.05% 聚山梨酯 20(TWEEN-20™) 表面活性剂的 PBS(PBST) 中的两倍连续稀释的 Fab(0.78nM 至 500nM)。使用简单一对一朗格缪尔 (Langmuir) 结合模型（BIACORE® Evaluation Software version 3.2）通过同时拟合结合和解离传感图，计算结合速率 (k_{on}) 和解离速率 (k_{off})。平衡解离常数 (Kd) 以比率 k_{off}/k_{on} 计算。参见例如 Chen 等，J.Mol.Biol.（分子生物学杂志）293:865-881(1999)。如果根据上文表面等离子共振测定法，结合速率超过 $10^6M^{-1}s^{-1}$，那么结合速率可使用荧光淬灭技术来测定，即根据分光计诸如配备了断流装置的分光光度计 (Aviv Instruments) 或 8000 系列 SLM-AMINCO™ 分光光度计 (ThermoSpectronic) 中用搅拌比色杯 (stirred cuvette) 的测量，在存在浓度渐增的抗原的条件下，测量 PBS，pH 7.2 中的 20nM 抗 - 抗原抗体 (Fab 形式）在 25℃的荧光发射强度（激发＝ 295nm；发射＝ 340nm，16nm 带通）的升高或降低。

[0266] 2. 抗体片段

[0267] 在某些实施方案中，本文提供的抗体是抗体片段。抗体片段包括但不限于，Fab，

Fab', Fab'-SH, $F(ab')_2$, Fv, 和 scFv 片段, 以及以下描述的其他片段。对于特定抗体片段的综述, 请参见 Hudson 等 Nat.Med.9:129-134(2003)。对于 scFv 片段的综述, 请参见, 例如, Pluckthün, The Pharmacology of Monoclonal Antibodies(单克隆抗体的药理学), 卷 113, Rosenburg 和 Moore 编辑, (Springer-Verlag, New York), 269-315 页 (1994);还请参见 WO 93/16185;和美国专利号 5,571,894 和 5,587,458。对于包含拯救受体 (salvage receptor) 结合表位残基和具有升高的体内半衰期的 Fab 和 $F(ab')_2$ 片段的讨论, 参见美国专利号 5,869,046。

[0268] 双抗体是具有两个抗原结合位点的抗体片段, 其可以是二价或双特异性的。参见, 例如, EP 404,097;WO 1993/01161;Hudson 等, Nat.Med.9:129-134(2003);和 Hollinger 等, Proc.Natl.Acad.Sci.USA 90:6444-6448(1993)。三抗体和四抗体也描述于 Hudson 等, Nat.Med.9:129-134(2003) 中。

[0269] 单一结构域抗体是包含抗体的全部或部分重链可变结构域或全部或部分轻链可变结构域的抗体片段。在某些实施方案中, 单一结构域抗体是人单一结构域抗体 (Domantis, Inc., Waltham, MA;参见, 例如, 美国专利号 6,248,516B1)。

[0270] 抗体片段可以通过不同技术制备, 包括但不限于完整抗体的蛋白水解消化以及通过重组宿主细胞 (例如大肠杆菌或噬菌体) 产生, 如本文所述。3. 嵌合和人源化抗体

[0271] 在某些实施方案中, 本文中所提供的抗体是嵌合抗体。某些嵌合抗体于例如美国专利第 4,816,567 号;及 Morrison 等人, Proc.Natl.Acad.Sci.USA, 81:6851-6855(1984) 中描述。在一个实例中, 嵌合抗体包含非人类可变区 (例如源自小鼠、大鼠、仓鼠、兔或例如猴的非人类灵长类动物的可变区) 及人类恒定区。在另一实例中, 嵌合抗体是种类或亚类已经自亲本抗体的种类或亚类发生变化的"种类转变"抗体。嵌合抗体包括其抗原结合片段。

[0272] 在某些实施方案中, 嵌合抗体是人源化抗体。通常, 非人类抗体经人源化以降低对人类的免疫原性, 同时保持亲本非人类抗体的特异性及亲和力。一般而言, 人源化抗体包含一个或多个可变结构域, 其中 HVRs, 例如 CDRs(或其部分) 源自非人类抗体, 且 FRs(或其部分) 是源自人类抗体序列。人源化抗体任选地也将包含人类恒定区的至少一部分。在一些实施方案中, 人源化抗体中的一些 FR 残基经来自非人类抗体 (例如 HVR 残基所源自的抗体) 的相应残基置换, 例如以恢复或提高抗体特异性或亲和力。

[0273] 人源化抗体及其制备方法于例如 Almagro 和 Fransson, Front.Biosci.13:1619-1633(2008) 中评述, 且进一步于例如 Riechmann 等人, Nature(自然)332:323-329(1988);Queen 等人, Proc.Nat'l.Acad.Sci.USA 86:10029-10033(1989);美国专利第 5,821,337 号、第 7,527,791 号、第 6,982,321 号和第 7,087,409 号;Kashmiri 等人, Methods(方法)36:25-34(2005)(描述 SDR(a-CDR) 移植);Padlan, Mol.Immunol.(分子免疫学)28:489-498(1991)(描述"表面重整");Dall'Acqua 等人, Methods 36:43-60(2005)(描述"FR 重组 (shuffling)");及 Osbourn 等人, Methods(方法)36:61-68(2005) 和 Klimka 等人, Br.J.Cancer(英国癌症杂志), 83:252-260(2000)(描述 FR 重组 (shuffling) 的"导向选择"方法) 中描述。

[0274] 可用于人源化的人类构架区包括, 但不限于, 使用"最佳拟合"法选择的构架区 (参见例如 Sims 等人, J.Immunol.(免疫学杂志)151:2296(1993));源自特定亚群的轻链

或重链可变区的人类抗体共同序列的构架区（参见例如 Carter 等人，Proc.Natl.Acad.Sci.USA,89:4285(1992)；及 Presta 等人，J.Immunol.（免疫学杂志），151:2623(1993)）；人类成熟（体细胞突变）构架区或人类生殖系构架区（参见例如 Almagro 及 Fransson，Front.Biosci.13:1619-1633(2008)）；及源自筛选 FR 文库的构架区（参见例如 Baca 等人，J.Biol.Chem.（生物化学杂志）272:10678-10684(1997) 及 Rosok 等人，J.Biol.Chem.（生物化学杂志）271:22611-22618(1996)）。

[0275] 4. 人抗体

[0276] 在某些实施方案中，本文中所提供的抗体是人抗体。可使用本领域中已知的各种技术来制备人抗体。人抗体一般描述于 van Dijk 和 van de Winkel，Curr.Opin.Pharmacol.（当前药学观点）5:368-74(2001) 以及 Lonberg，Curr.Opin.Immunol.（当前免疫学观点）20:450-459(2008)。

[0277] 可通过向已经经过修饰因而对于抗原攻击刺激产生完整人抗体或具有人类可变区的完整抗体的转基因动物施用免疫原，来制备人抗体。这些动物通常含有全部或一部分人类免疫球蛋白基因座，其替代了内源免疫球蛋白基因座，或存在于染色体外或随机整合于动物染色体内。在这些转基因小鼠中，内源免疫球蛋白基因座一般已经失活。关于从转基因动物获得人抗体的方法的综述，参见 Lonberg，Nat.Biotech.（自然生物技术）23:1117-1125(2005)。也参见例如描述 XENOMOUSE™ 技术的美国专利第 6,075,181 号及第 6,150,584 号；描述 HUMAB® 技术的美国专利第 5,770,429 号；描述 K-M MOUSE® 技术的美国专利第 7,041,870 号，及描述 VELOCIMOUSE® 技术的美国专利申请公开号 US 2007/0061900。这些动物产生的完整抗体的人类可变区可进一步修饰，例如通过与不同人类恒定区组合。

[0278] 人抗体也可通过基于杂交瘤的方法制得。用于产生人单克隆抗体的人骨髓瘤及小鼠 - 人融合骨髓瘤细胞系已经描述。（参见例如 Kozbor J.Immunol.（免疫学杂志），133:3001(1984)；Brodeur 等人，Monoclonal Antibody Production Techniques and Applications（单克隆抗体产生技术及应用），第 51-63 页 (Marcel Dekker，Inc.，New York,1987)；和 Boerner 等人，J.Immunol.（免疫学杂志），147:86(1991)。）通过人类 B 细胞杂交瘤技术产生的人抗体也在 Li 等人，Proc.Natl.Acad.Sci..USA,103:3557-3562(2006) 中描述。其他方法包括例如美国专利第 7,189,826 号（描述由杂交瘤细胞系产生单克隆人类 IgM 抗体）及 Ni，Xiandai Mianyixue,26(4):265-268(2006)（描述人 - 人杂交瘤）中所述的那些方法。人杂交瘤技术 (Trioma 技术）也于 Vollmers 和 Brandlein，Histology and Histopathology（组织学和组织病理学），20(3):927-937(2005)，及 Vollmers 和 Brandlein,Methods and Findings in Experimental and Clinical Pharmacology（实验和临床药学方法和发现），27(3):185-91(2005) 中描述。

[0279] 也可通过分离选自源自人噬菌体展示文库的 Fv 克隆可变结构域序列产生人抗体。随后可将这些可变结构域序列与所需人恒定结构域组合。下文描述自抗体文库选择人抗体的技术。

[0280] 5. 源自文库的抗体

[0281] 可通过在组合文库中筛选具有所需活性的抗体来分离本发明抗体。举例来说，本领域中已知多种用于产生噬菌体展示文库并且在这些文库中筛选具有所需结

合特征的抗体的方法。这些方法于例如Hoogenboom等人，Methods in Molecular Biology(分子生物学方法)178:1-37(O'Brien等人编，Human Press，Totowa，NJ,2001)中评述，并且进一步于例如McCafferty等人，Nature(自然)348:552-554;Clackso等人，Nature(自然)352:624-628(1991);Marks等人，J.Mol.Biol.(分子生物学杂志)222:581-597(1992);Marks及Bradbury，Methods in Molecular Biology(分子生物学方法)248:161-175(Lo编，Human Press，Totowa，NJ,2003);Sidhu等人，J.Mol.Biol.(分子生物学杂志)338(2):299-310(2004);Lee等人，J.Mol.Biol.(分子生物学杂志)340(5):1073-1093(2004);Fellouse，Proc.Natl.Acad.Sci.USA 101(34):12467-12472(2004);和Lee等人，J.Immunol.Methods(免疫学方法杂志)284(1-2):119-132(2004)中描述。

[0282] 在某些噬菌体展示方法中，VH及VL基因库(repertoire)是通过聚合酶链式反应(PCR)分别克隆并且随机重组于噬菌体文库中，随后可如Winter等人，Ann.Rev.Immunol.(免疫学年度综述)，12:433-455(1994)中所述在其中筛选抗原结合噬菌体。噬菌体通常呈现单链Fv(scFv)片段或Fab片段形式的抗体片段。来自免疫来源的文库无需构建杂交瘤即可提供免疫原的高亲和力抗体。或者，天然库可经克隆(例如自人)以在无任何免疫的情况下提供针对多种非自体抗原以及自体抗原的抗体单一来源，如Griffiths等人，EMBO J,12:725-734(1993)所述。最后，也可以通过从干细胞克隆未重排的V基因区段，并且使用含有随机序列的PCR引物来编码高变性CDR3区，并且实现体外重排来合成制得天然文库，如Hoogenboom及Winter，J.Mol.Biol.(分子生物学杂志)，227:381-388(1992)所述。描述人抗体噬菌体文库的专利公开物包括例如:美国专利第5,750,373号，及美国专利公开第2005/0079574号、第2005/0119455号、第2005/0266000号、第2007/0117126号、第2007/0160598号、第2007/0237764号、第2007/0292936号和第2009/0002360号。

[0283] 从人抗体文库分离的抗体或抗体片段被视为本文的人抗体或人抗体片段。

[0284] 6.多特异性抗体

[0285] 在某些实施方案中，本文中所提供的抗体是多特异性抗体，例如双特异性抗体。多特异性抗体是对至少两个不同位点具有结合特异性的单克隆抗体。在某些实施方案中，一种结合特异性是针对PCSK9而另一种是针对任何其他抗原。在某些实施方案中，双特异性抗体可结合至PCSK9的两个不同表位。双特异性抗体也可用于将细胞毒性剂定位于表达PCSK9的细胞。双特异性抗体可制成全长抗体或抗体片段形式。

[0286] 制造多特异性抗体的技术包括，但不限于，具有不同特异性的两个免疫球蛋白重链-轻链对的重组共表达(参见Milstein及Cuello，Nature(自然)305:537(1983);WO 93/08829;及Traunecker等人，EMBO J.10:3655(1991))，及"凸起-入-孔洞(knob-in-hole)"工程改造(参见例如美国专利第5,731,168号)。也可通过以下方法制得多特异性抗体:工程改造用于制备抗体Fc-异二聚体的静电导引作用(WO 2009/089004A1);将两种或两种以上抗体或片段交联(参见例如美国专利第4,676,980号，及Brennan等人Science(科学)，229:81(1985));使用亮氨酸拉链来产生双特异性抗体(参见例如Kostelny等人，J.Immunol.(免疫学杂志)，148(5):1547-1553(1992));使用"双抗体"技术来制得双特异性抗体片段(参见例如Hollinger等人，Proc.Natl.Acad.Sci.USA,90:6444-6448(1993));及使用单链Fv(sFv)二聚体(参见例如Gruber等人，

J.Immunol.(免疫学杂志),152:5368(1994));及如例如 Tutt 等人,J.Immunol.(免疫学杂志)147:60(1991) 中所述制备三特异性抗体。

[0287] 本文中也包括具有三个或三个以上功能性抗原结合位点的经工程改造抗体,包括“章鱼抗体 (Octopus antibodies)”(参见例如 US2006/0025576A1)。

[0288] 本文中的抗体或片段也包括包含结合至 PCSK9 以及另一不同抗原的抗原结合位点的“双重作用 FAb 或“DAF”(例如参见 US 2008/0069820)。

[0289] 7. 抗体变体

[0290] 在某些实施方案中,涵盖本文中所提供抗体的氨基酸序列变体。举例来说,可能需要其来提高抗体的结合亲和力和 / 或其他生物特性。可通过在编码抗体的核苷酸序列中引入适当修饰或通过肽合成来制备抗体的氨基酸序列变体。这些修饰包括例如抗体氨基酸序列内的残基缺失和 / 或插入其中和 / 或对其进行置换。可进行缺失、插入和置换的任何组合以获得最终构建体,其限制条件是最终构建体具有所需特征,例如抗原结合。

[0291] A) 置换,插入,和缺失变体

[0292] 在某些实施方案中,提供具有一个或多个氨基酸置换的抗体变体。用于置换性诱变的相关位点包括 HVRs 和 FRs。保守置换显示在表 A 中在“保守置换”的标题下。更多实质性变化于表 1 中的“示例性置换”的标题下提供且如下文关于氨基酸侧链种类进一步描述。可将氨基酸置换引入相关抗体中并筛选具有所需活性,例如保持 / 升高的抗原结合、降低的免疫原性、或升高的 ADCC 或 CDC 的产物。

[0293] 表 A

[0294]

原始残基	示例性置换	优选的置换
Ala (A)	Val；Leu；Ile	Val
Arg (R)	Lys；Gln；Asn	Lys
Asn (N)	Gln；His；Asp, Lys；Arg	Gln
Asp (D)	Glu；Asn	Glu
Cys (C)	Ser；Ala	Ser
Gln (Q)	Asn；Glu	Asn
Glu (E)	Asp；Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn；Gln；Lys；Arg	Arg
Ile (I)	Leu；Val；Met；Ala；Phe；正亮氨酸	Leu
Leu (L)	正亮氨酸；Ile；Val；Met；Ala；Phe	Ile
Lys (K)	Arg；Gln；Asn	Arg
Met (M)	Leu；Phe；Ile	Leu
Phe (F)	Trp；Leu；Val；Ile；Ala；Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val；Ser	Ser
Trp (W)	Tyr；Phe	Tyr
Tyr (Y)	Trp；Phe；Thr；Ser	Phe

[0295]

原始残基	示例性置换	优选的置换
Val (V)	Ile；Leu；Met；Phe；Ala；正亮氨酸	Leu

[0296] 可根据常见侧链特性将氨基酸分组：

[0297] (1) 疏水性：正亮氨酸、Met、Ala、Val、Leu、Ile；

[0298] (2) 中性亲水性：Cys、Ser、Thr、Asn、Gln；

[0299] (3) 酸性：Asp、Glu；

[0300] (4) 碱性 :His、Lys、Arg ;

[0301] (5) 影响链取向的残基 :Gly、Pro ;

[0302] (6) 芳香性 :Trp、Tyr、Phe。

[0303] 非保守性置换将需要将一种这些种类中的成员换成另一种类。

[0304] 一种类型的置换变体涉及置换亲本抗体(例如人源化抗体或人抗体)的一个或多个高变区残基。一般而言,选择用于进一步研究的所得变体的某些生物学特性相对于亲本抗体改变(例如提高)(例如亲和力增加、免疫原性降低)和 / 或将实质上保持亲本抗体的某些生物学特性。示例性置换变体是亲和力成熟抗体,其可例如使用基于噬菌体展示的亲和力成熟技术,例如本文中所述的那些,方便地产生。简言之,一个或多个 HVR 残基被突变,且变异抗体呈现于噬菌体上,并针对特定生物活性(例如结合亲和力)对其进行筛选。

[0305] 可在 HVR 中进行改变(例如置换),例如以提高抗体亲和力。这些改变可于 HVR“热点”也即由在体细胞成熟过程中经历高频率突变的密码子编码的残基中进行(参见例如 Chowdhury,Methods Mol.Biol.(分子生物学方法)207:179-196(2008));和 / 或于 SDRs(a-CDRs),其中测试所得变异 VH 或 VL 的结合亲和力。通过构建并且自第二文库重新选择而获得的亲和力成熟已于例如 Hoogenboom 等人,Methods in Molecular Biology(分子生物学方法)178:1-37(O'Brien 等人编 Human Press,Totowa,NJ,(2001)) 中描述。在亲和力成熟的一些实施方案中,通过多种方法(例如易错 PCR(error-prone PCR)、链重组(shuffling) 或寡核苷酸定向诱变)中任一种将多样性引入经选择以供成熟的可变基因中。随后产生第二文库。随后筛选该文库以鉴别具有所需亲和力的任何抗体变体。另一引入多样性的方法涉及 HVR 定向方法,其中随机选择数个 HVR 残基(例如每次 4-6 个残基)。可例如使用丙氨酸扫描诱变或模型化特定地鉴别抗原结合中所涉及的 HVR 残基。特定地,通常靶向 CDR-H3 及 CDR-L3。

[0306] 在某些实施方案中,置换、插入或缺失可在一个或多个 HVR 内进行,只要这些改变不实质上降低抗体结合抗原的能力即可。举例来说,可在 HVR 中进行不实质上降低结合亲和力的保守性改变(例如如本文中所提供的保守性置换)。这些改变可在 HVR“热点”或 SDR 外部。在上文提供的变异 VH 及 VL 序列的某些实施方案中,各 HVR 未经改变,或含有不超过一个、两个或三个氨基酸置换。

[0307] 适用于鉴别可靶向以供诱变的抗体的残基或区域的方法称作“丙氨酸扫描诱变”如 Cunningham 及 Wells(1989)Science(科学),244:1081-1085 所述。在此方法中,靶残基(例如诸如 arg、asp、his、lys 和 glu 的带电残基)的一个残基或一组经鉴别且由中性或带负电氨基酸(例如丙氨酸或聚丙氨酸)置换以确定是否影响抗体与抗原的相互作用。可在对初始置换显示功能敏感性的氨基酸位置处引入其他置换。备选地或另外地,抗原 - 抗体复合物的晶体结构用于鉴别抗体与抗原之间的接触点。这些接触残基及邻近残基可以作为置换候选物被靶向或消除。可以筛选变体以确定其是否含有所需特性。

[0308] 氨基酸序列插入物包括长度在一个残基至含有一百个或一百个以上残基的多肽范围内的氨基端和 / 或羧基端融合体,以及单个或多个氨基酸残基的序列内插入物。末端插入物的实例包括具有 N 端甲硫胺酰基残基的抗体。抗体分子的其他插入变体包括抗体的 N 端或 C 端与酶(例如对于 ADEPT 而言)或增加抗体的血清半衰期的多肽的融合体。

[0309] b) 糖基化变体

[0310] 在某些实施方案中，本文中所提供的抗体经改变以增加或降低抗体经糖基化的程度。对抗体的糖基化位点的添加或缺失可通过改变氨基酸序列以便产生或移除一或多个糖基化位点而方便地实现。

[0311] 若抗体包含 Fc 区，则与其连接的糖类可以被改变。由哺乳动物细胞产生的天然抗体通常包含一般通过 N- 连接与 Fc 区 CH2 结构域的 Asn297 连接的分支链双触角寡糖。参见例如 Wright 等人，TIBTECH 15:26-32(1997)。寡糖可包括多种糖类，例如甘露糖、N- 乙酰葡糖胺 (GlcNAc)、半乳糖及唾液酸，以及与双触角寡糖结构的“主干”中的 GlcNAc 连接的岩藻糖。在一些实施方案中，可对本发明抗体中的寡糖进行修饰以产生具有某些改良特性的抗体变体。

[0312] 在一实施方案中，提供具有缺乏与 Fc 区连接（直接或间接）的岩藻糖的糖结构的抗体变体。举例来说，该抗体中岩藻糖的量可以是 1％至 80％、1％至 65％、5％至 65％或 20％至 40％。通过相对于根据 MALDI-TOF 质谱法所测量的所有与 Asn 297 连接的糖结构（例如复合型、杂合型及高甘露糖型结构）的总和，计算糖链内 Asn297 处岩藻糖的平均量来测定岩藻糖的量，例如如 WO 2008/077546 中所述。Asn297 是指位于 Fc 区中约位置 297 处 (Fc 区残基的 Eu 编号）的天冬酰胺残基；然而，由于抗体中的微小序列变化，Asn297 也可能位于位置 297 上游或下游约 ±3 个氨基酸处，也即介于位置 294 与 300 之间。这些岩藻糖基化变体可具有升高的 ADCC 功能。参见例如美国专利公开号 US 2003/0157108(Presta, L.)；US 2004/0093621(Kyowa Hakko Kogyo Co., Ltd)。 与“去岩藻糖基”或“岩藻糖缺乏”抗体变体有关的公开文本的实例包括 US 2003/0157108；WO 2000/61739；WO 2001/29246；US 2003/0115614；US 2002/0164328；US 2004/0093621；US 2004/0132140；US 2004/0110704；US 2004/0110282；US 2004/0109865；WO 2003/085119；WO 2003/084570；WO 2005/035586；WO 2005/035778；WO 2005/053742；WO 2002/031140；Okazaki 等人，J.Mol.Biol.（分子生物学杂志）336:1239-1249(2004)；Yamane-Ohnuki 等人，Biotech.Bioeng.（生物技术和生物工程）87:614(2004)。能够产生脱除岩藻糖基的抗体的细胞系的实例包括蛋白质岩藻糖基化缺乏的 Lec13CHO 细胞 (Ripka 等人 Arch. Biochem.Biophys.249:533-545(1986)；美国专利申请号 US2003/0157108A1, Presta, L；及 WO 2004/056312A1, Adams 等人，尤其实施例 11)；及基因敲除细胞系，例如 α-1,6- 岩藻糖基转移酶基因、FUT8、基因敲除 CHO 细胞（参见例如 Yamane-Ohnuki 等人，Biotech.Bioeng.（生物技术和生物工程）87:614(2004)；Kanda, Y. 等人，Biotechnol.Bioeng.（生物技术和生物工程），94(4):680-688(2006)；及 WO 2003/085107)。

[0313] 进一步提供具有平分型寡糖 (bisected oligosaccharide) 的抗体变体，例如其中与抗体的 Fc 区连接的双触角寡糖经 GlcNAc 平分。这些抗体变体可具有降低的岩藻糖基化和 / 或升高的 ADCC 功能。这些抗体变体的实例例如于 WO 2003/011878(Jean-Mairet 等人）；美国专利第 6,602,684 号 (Umana 等人）；及 US 2005/0123546(Umana 等人）中描述。也提供在与 Fc 区连接的寡糖中具有至少一个半乳糖残基的抗体变体。这些抗体变体可具有升高的 CDC 功能。这些抗体变体于例如 WO 1997/30087(Patel 等人）；WO 1998/58964(Raju, S.）；及 WO 1999/22764(Raju, S.）中描述。

[0314] c)Fc 区域变体

[0315] 在某些实施方案中，可在本文中所提供抗体的 Fc 区中引入一个或多个氨基酸修

饰，以此产生 Fc 区变体，以便增强例如抗体治疗涉及异常血管发生和 / 或血管通透性或渗漏的疾病或病症的有效性。Fc 区变体可包含在一或多个氨基酸位置处包含氨基酸修饰（例如置换）的人 Fc 区序列（例如人 IgGl、IgG2、IgG3 或 IgG4Fc 区）。

[0316] 在某些实施方案中，本发明涵盖具有一些但非所有效应子功能的抗体变体，这使其成为某些应用的理想候选物，在所述应用中抗体的活体内半衰期是重要的，但某些效应子功能（例如补体及 ADCC）是不必要或有害的。可进行体外和 / 或体内细胞毒性测定以确认 CDC 和 / 或 ADCC 活性的降低 / 衰竭。举例来说，可进行 Fc 受体 (FcR) 结合测定以确保抗体缺乏 FcγR 结合（因此可能缺乏 ADCC 活性），但保持 FcRn 结合能力。介导 ADCC 的初级细胞、NK 细胞仅表达 Fc(RIII，而单核细胞表达 Fc(RI、Fc(RII 及 Fc(RIII。FcR 在造血细胞上的表达于 Ravetch 及 Kinet，Annu.Rev.Immunol.（免疫学年度综述）9:457-492(1991) 的第 464 页上的表 3 中总结。评定相关分子的 ADCC 活性的体外测定的非限制性实例于美国专利 5,500,362（参见例如 Hellstrom，I. 等人，Proc.Nat'l.Acad.Sci.USA 83:7059-7063(1986)) 及 Hellstrom，I 等人，Proc.Nat'l.Acad.Sci.USA 82:1499-1502(1985)；5,821,337（参见 Bruggemann，M. 等人，J.Exp.Med.（实验医学杂志）166:1351-1361(1987)) 中描述。或者，可采用非放射性测定方法（参见例如用于流式细胞术的 ACTI™ 非放射性细胞毒性测定 (CellTechnology，Inc.Mountain View，CA) 及 CytoTox 96® 非放射性细胞毒性测定 (Promega，Madison，WI))。适用于这些测定的效应细胞包括外周血单核细胞 (PBMC) 及自然杀伤 (NK) 细胞。备选地或另外地，相关分子的 ADCC 活性可于体内评定，例如在例如 Clynes 等人，Proc.Nat'l.Acad.Sci.USA 95:652-656(1998) 中所揭示的动物模型中评定。也可进行 Clq 结合测定以证明抗体无法结合 Clq 且因此缺乏 CDC 活性。参见例如 WO 2006/029879 及 WO 2005/100402 中的 Clq 及 C3c 结合 ELISA。为评定补体活化，可进行 CDC 测定（参见例如 Gazzano-Santoro 等人，J.Immunol.Methods（免疫学方法杂志）202:163(1996)；Cragg，M.S. 等人，Blood（血液）101:1045-1052(2003)；及 Cragg，M.S. 及 M.J.Glennie，Blood 103:2738-2743(2004))。也可使用本领域中已知的方法进行 FcRn 结合及活体内清除 / 半衰期测定（参见例如 Petkova，S.B. 等人，Int'l.Immunol.（国际免疫学）18(12):1759-1769(2006))。

[0317] 效应子功能降低的抗体包括 Fc 区残基 238、265、269、270、297、327 及 329 中一个或多个置换的那些抗体（美国专利 6,737,056)。这些 Fc 突变体包括在氨基酸位置 265、269、270、297 和 327 的两个或两个以上位置处具有置换的 Fc 突变体，包括残基 265 和 297 置换为丙氨酸的所谓的“DANA”Fc 突变体（美国专利号 7,332,581)。

[0318] 描述某些与 FcRs 的结合提高或减少的抗体变体。（参见例如美国专利号 6,737,056；WO 2004/056312，及 Shields 等人，J.Biol.Chem.（生物化学杂志）9(2):6591-6604(2001))。

[0319] 在某些实施方案中，抗体变体包含具有一个或多个提高 ADCC 的氨基酸置换的 Fc 区，例如在 Fc 区的位置 298、333 和 / 或 334（残基的 EU 编号）处置换。

[0320] 在一些实施方案中，在 Fc 区中进行改变，其导致 Clq 结合和 / 或补体依赖性细胞毒性 (CDC) 改变（也即，通过提高或降低），例如如美国专利第 6,194,551 号、WO 99/51642 和 Idusogie 等人，J.Immunol.（免疫学杂志）164:4178-4184(2000) 中所述。

[0321] US2005/0014934A1(Hinton 等人）中描述具有增加的半衰期及改善的与新生儿 Fc

受体 (FcRn) 的结合的抗体，其中 FcRn 负责将母体 IgG 转移至胎儿 (Guyer 等人 J. Immunol.（免疫学杂志）117:587(1976) 及 Kim 等人，J. Immunol.（免疫学杂志）24:249(1994))。那些抗体包含具有一或多个置换的 Fc 区，所述置换提高 Fc 区与 FcRn 的结合。这些 Fc 变体包括在 Fc 区残基：238、256、265、272、286、303、305、307、311、312、317、340、356、360、362、376、378、380、382、413、424 或 434 中的一或多者处具有置换（例如置换 Fc 区残基 434) 的那些 Fc 变体（美国专利第 7,371,826 号）。

[0322] 关于 Fc 区变体的其他实例，也参见 Duncan 及 Winter，Nature（自然）322:738-40(1988)；美国专利第 5,648,260 号；美国专利第 5,624,821 号；及 WO 94/29351。

[0323] d) 经半胱氨酸工程改造的抗体变体

[0324] 在某些实施方案中，可能需要产生经半胱氨酸工程改造的抗体，例如“硫代 MAb”，其中抗体的一或多个残基经半胱氨酸残基置换。在特定实施方案中，经置换的残基出现在抗体的可接近位点处。通过用半胱氨酸置换那些残基，反应性硫醇基团通过定位于抗体的可接近位点处且可用于将抗体结合至其他部分，例如药物部分或接头 - 药物部分，以产生如本文中进一步描述的免疫缀合物。在某些实施方案中，任一或多个以下残基可经半胱氨酸置换：轻链的 V205(Kabat 编号）；重链的 A118(EU 编号）；及重链 Fc 区的 S400(EU 编号）。可如例如美国专利第 7,521,541 号中所述，产生经半胱氨酸工程改造的抗体。

[0325] e) 抗体衍生物

[0326] 在某些实施方案中，本文中所提供的抗体可进一步经修饰为含有本领域中已知且轻易获得的其他非蛋白质部分。适合抗体衍生作用的部分包括，但不限于，水溶性聚合物。水溶性聚合物的非限制性实例包括，但不限于，聚乙二醇 (PEG)、乙二醇 / 丙二醇共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚 -1,3- 二噁烷、聚 1,3,6- 三噁烷、乙烯 / 马来酸酐共聚物、聚氨基酸（均聚物或无规共聚物）、及葡聚糖或聚 (n- 乙烯基吡咯烷酮）聚乙二醇、丙二醇均聚物、聚环氧丙烷 / 氧化乙烯共聚物、聚氧乙基化多元醇（例如甘油）、聚乙烯醇、及其混合物。聚乙二醇丙醛因其在水中的稳定性可能具有制造优势。聚合物可具有任何分子量，且可有分支或无分支。与抗体连接的聚合物的数目可以变化，且若连接一种以上聚合物，则其可以是相同或不同分子。一般而言，用于衍生作用的聚合物数目和 / 或类型可以基于包括，但不限于，以下的考虑因素来确定：要升高的抗体的特定特性或功能、抗体衍生物是否将用于指定条件下的疗法等。

[0327] 在另一实施方案中，提供抗体与可通过暴露于辐射来选择性加热的非蛋白质部分的缀合物。在一个实施方案中，非蛋白质部分是碳纳米管 (Kam 等人，Proc. Natl. Acad. Sci. USA（美国科学院学报）102:11600-11605(2005))。该辐射可具有任何波长，且包括但不限于，不损伤普通细胞但能将非蛋白质部分加热至可杀死抗体 - 非蛋白质部分附近的细胞的温度的波长。

[0328] B. 重组方法和组合物

[0329] 本文所述的抗 PCSK9 抗体可以使用例如在美国专利号 4,816,567 中描述的重组方法和组合物产生。在一个实施方案中，提供了编码本文描述的抗 PCSK9 抗体的分离的核酸。此类核酸可编码包含抗体 VL 的氨基酸序列和 / 或包含其 VH 的氨基酸序列（例如抗体的轻链和 / 或重链）。在某些实施方案中，提供编码抗 -PCSK9 重链可变区的分离的核酸，

其中所述核酸包含与 SEQ ID NO:38 或 SEQ ID NO:39 的核酸序列具有至少 90%,91%,92%,93%,94%,95%,96%,97%,98%,99%,或100%序列同一性的序列。在某些实施方案中,提供编码抗 -PCSK9 轻链可变区的分离的核酸,其中所述核酸包含与 SEQ ID NO:40 或 SEQ ID NO:41 的核酸序列具有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%,或 100%序列同一性的序列。在某些实施方案中,提供编码抗 -PCSK9 重链可变区和抗 -PCSK9 轻链可变区的分离的核酸,其中编码重链可变区的核酸包含与 SEQ ID NO:38 或 SEQ ID NO:39 的核酸序列具有至少90%,91%,92%,93%,94%,95%,96%,97%,98%,99%,或100%序列同一性的序列并且编码轻链可变区的核酸包含与SEQ ID NO:40或SEQ ID NO:41 的核酸序列具有至少 90%,91%,92%,93%,94%,95%,96%,97%,98%,99%,或 100%序列同一性的序列。在某些实施方案中,提供编码抗 -PCSK9 重链可变区的分离的核酸,其中所述核酸包含 SEQ ID NO:38 或 39。在某些实施方案中,提供编码抗 -PCSK9 轻链可变区的分离的核酸,其中所述核酸包含 SEQ ID NO:40 或 41。在某些实施方案中,提供编码抗-PCSK9 重链可变区和轻链可变区的分离的核酸,其中编码重链的核酸包含SEQ ID NO:38 并且编码轻链的核酸包含 SEQ ID NO:40。在某些实施方案中,提供编码抗 -PCSK9 重链可变区和轻链可变区的分离的核酸,其中编码重链的核酸包含SEQ ID NO:39并且编码轻链的核酸包含 SEQ ID NO:41。

[0330] 抗体 508.20.33b 全长重链核酸序列 (SEQ ID NO:38)

[0331]

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GAA GTTCAGCTGG TGGAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC
TGTGCAGCTT CTGGCTTCAC CTTCTCTAGT ACTGCTATTC ACTGGGTGCG TCAGGCCCCG
GGTAAGGGCC TGGAATGGGT TGCTAGGATT TCTCCTGCTA ACGGTAATAC TAACTATGCC
GATAGCGTCA AGGGCCGTTT CACTATAAGC GCAGACACAT CCAAAAACAC AGCCTACCTA
CAAATGAACA GCTTAAGAGC TGAGGACACT GCCGTCTATT ATTGTGCTCG TTGGATCGGG
TCCCGGGAGC TGTACATTAT GGACTACTGG GGTCAAGGAA CCCTGGTCAC CGTCTCCTCG
GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
GGACTCTACT CCCTCAGCAG CGTGGTGACT GTGCCCTCTA GCAGCTTGGG CACCCAGACC
TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC
AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG
GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGAAGAG
ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG
CTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA
```

[0332] 抗体 508.20.33b 重链可变区核酸序列 (SEQ ID NO:39)

[0333]

```
GAA GTTCAGCTGG TGGAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC
TGTGCAGCTT CTGGCTTCAC CTTCTCTAGT ACTGCTATTC ACTGGGTGCG TCAGGCCCCG

GGTAAGGGCC TGGAATGGGT TGCTAGGATT TCTCCTGCTA ACGGTAATAC TAACTATGCC
GATAGCGTCA AGGGCCGTTT CACTATAAGC GCAGACACAT CCAAAAACAC AGCCTACCTA
CAAATGAACA GCTTAAGAGC TGAGGACACT GCCGTCTATT ATTGTGCTCG TTGGATCGGG
TCCCGGGAGC TGTACATTAT GGACTACTGG GGTCAAGGAA CCCTGGTCAC CGTCTCCTCG
```

[0334] 抗体 508.20.33b 全长轻链核酸序列 (SEQ ID NO :40)

[0335]

```
GA TATCCAGATG ACCCAGTCCC CGAGCTCCCT GTCCGCCTCT GTGGGCGATA GGGTCACCAT
CACCTGCCGT GCCAGTCAGG ATGTGTCCAC TGCTGTAGCC TGGTATCAAC AGAAACCAGG
AAAAGCTCCG AAGCTTCTGA TTTACTCGGC ATCCTTCCTC TACTCTGGAG TCCCTTCTCG
CTTCTCTGGT AGCGGTTCCG GGACGGATTT CACTCTGACC ATCAGCAGTC TGCAGCCGGA
AGACTTCGCA ACTTATTACT GTCAGCAAGC CTATCCGGCC CTACACACGT TCGGACAGGG
TACCAAGGTG GAGATCAAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC
TGATGAGCAG TTGAAATCTG GAACTGCTTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC
CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA ACTCCCAGGA
GAGTGTCACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA CCCTGACGCT
GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC ATCAGGGCCT
GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT
```

[0336] 抗体 508.20.33b 轻链可变区核酸序列 (SEQ ID NO:41)

[0337]

```
GA TATCCAGATG ACCCAGTCCC CGAGCTCCCT GTCCGCCTCT GTGGGCGATA GGGTCACCAT
CACCTGCCGT GCCAGTCAGG ATGTGTCCAC TGCTGTAGCC TGGTATCAAC AGAAACCAGG
AAAAGCTCCG AAGCTTCTGA TTTACTCGGC ATCCTTCCTC TACTCTGGAG TCCCTTCTCG
CTTCTCTGGT AGCGGTTCCG GGACGGATTT CACTCTGACC ATCAGCAGTC TGCAGCCGGA
AGACTTCGCA ACTTATTACT GTCAGCAAGC CTATCCGGCC CTACACACGT TCGGACAGGG
TACCAAGGTG GAGATCAAAC GA
```

[0338] 在又一个实施方案中,提供了包含此类核酸的一个或多个载体 (例如表达载体)。在又一个实施方案中,提供了包含此类核酸的宿主细胞。在一个此类实施方案中,宿主细胞包含 (例如,用下述各项转化):(1) 包含核酸的载体,所述核酸编码包含抗体的 VL 的氨基酸序列和包含抗体的 VH 的氨基酸序列,或 (2) 包含核酸的第一载体,所述核酸编码包含抗体的 VL 的氨基酸序列,和包含核酸的第二载体,所述核酸编码包含抗体的 VH 的氨基酸序列。在一个实施方案中,宿主细胞是真核的,例如中国仓鼠卵巢 (CHO) 细胞或淋巴样细胞 (例如,Y0,NS0,Sp20 细胞)。在一个实施方案中,提供了制备抗 PCSK9 抗体的方法,其中所述方法包括,在适合抗体表达的条件下,培养包含编码所述抗体的核酸的宿主细胞,如上文所提供的,和任选地从所述宿主细胞 (或宿主细胞培养基) 回收所述抗体。

[0339] 为了重组产生抗 PCSK9 抗体,分离编码抗体 (例如上文所描述的抗体) 的核酸,并插入一个或多个载体,用于在宿主细胞中进一步克隆和 / 或表达。此类核酸易于使用常规规程分离和测序 (例如通过使用能够与编码抗体重链和轻链的基因特异性结合的寡核苷酸探针进行)。

[0340] 用于克隆或表达编码抗体的载体的适当宿主细胞包括本文描述的原核或真核细胞。例如,抗体可在细菌中产生,特别当不需要糖基化和 Fc 效应子功能时。对于抗体片段和多肽在细菌中的表达,见,例如,美国专利号 5,648,237,5,789,199 和 5,840,523。(还见 Charlton,分子生物学方法 (Methods in Molecular Biology),卷 248(B.K.C.Lo,编辑,Humana Press,Totowa,NJ,2003),第 245-254 页,描述抗体片段在大肠杆菌中的表达)。在表达后,抗体可以从可溶级分中的细菌细胞糊状物分离,并且可以进一步纯化。

[0341] 除了原核生物以外,真核微生物诸如丝状真菌或酵母也是关于编码抗体的载体的合适克隆或表达宿主,包括真菌和酵母菌株,其糖基化途径已经进行“人源化”,导致产生具有部分或完全人糖基化模式的抗体。参见 Gerngross,Nat.Biotech.(自然生物技术)22:1409-1414(2004),和 Li 等 ,Nat.Biotech.(自然生物技术)24:210-215(2006)。

[0342] 适于表达糖基化抗体的宿主细胞也衍生自多细胞生物体 (无脊椎动物和脊椎动

物）。无脊椎动物细胞的实例包括植物和昆虫细胞。已经鉴定了许多杆状病毒株，其可与昆虫细胞联合使用，特别是用于转染草地夜蛾 (Spodoptera frugiperda) 细胞。

[0343] 还可利用植物细胞培养物作为宿主。见例如，美国专利号 5,959,177，6,040,498，6,420,548，7,125,978 和 6,417,429（其描述了在转基因植物中产生抗体的 PLANTIBODIES™ 技术）。

[0344] 也可以将脊椎动物细胞用作宿主。例如，可以使用被改造以适合于悬浮生长的哺乳动物细胞系。有用哺乳动物宿主细胞系的其它实例是用 SV40 转化的猴肾 CV1 系 (COS-7)；人胚肾系 (293 或 293 细胞，如例如 Graham 等，遗传病毒学杂志 (J.Gen Virol.)36:59(1977) 中所描述的）；幼仓鼠肾细胞 (BHK)；小鼠塞托利 (sertoli) 细胞 (TM4 细胞，如例如在 Mather，Biol.Reprod.23:243-251(1980) 中描述的）；猴肾细胞 (CV1)；非洲绿猴肾细胞 (VERO-76)；人宫颈癌细胞 (HELA)；犬肾细胞 (MDCK；布法罗大鼠 (buffalo rat) 肝细胞 (BRL 3A)；人肺细胞 (W138)；人肝细胞 (Hep G2)；小鼠乳瘤 (MMT 060562)；TRI 细胞，如例如 Mather 等，Annals N.Y.Acad.Sci.383:44-68(1982) 中所描述的；MRC5 细胞；和 FS4 细胞。其它有用的哺乳动物宿主细胞系包括中国仓鼠卵巢 (CHO) 细胞，包括 DHFR-CHO 细胞 (Urlaub 等，美国国家科学院学报 (Proc.Natl.Acad.Sci.USA)77:4216(1980))；和骨髓瘤细胞系如 Y0，NS0 和 Sp2/0。关于适合产生抗体的某些哺乳动物宿主细胞系的综述见例如 Yazaki 和 Wu，分子生物学方法 (Methods in Molecular Biology)，卷 248(B.K.C.Lo，ed.，Humana Press，Totowa，NJ)，第 255-268 页 (2003)。

[0345] C. 测定

[0346] 本文中提供的抗 PCSK9 抗体可以对其物理 / 化学特性和 / 或生物活性，通过本领域中已知的不同测定来识别、筛选或表征。

[0347] 1. 结合测定和其他测定

[0348] 在一方面中，测试本发明的抗 PCSK9 抗体的 PCSK9 结合活性，例如，通过已知方法如 ELISA、蛋白印迹法等。可以使用多种类型的竞争性结合测定以确定抗 PCSK9 抗体是否彼此竞争，例如：固相直接或间接放射免疫测定 (RIA)，固相直接或间接酶免疫测定 (EIA)，夹心竞争测定（参见，例如，Stahli 等，1983，Methods in Enzymology 9:242-253)；固相直接生物素 - 亲和素 EIA（参见，例如，Kirkland 等，1986，J.Immunol.137:3614-3619) 固相直接标记测定，固相直接标记夹心测定（参见，例如，Harlow 和 Lane，1988，Antibodies，A Laboratory Manual（抗体，实验室手册），Cold Spring Harbor Press)；使用 I-125 标记的固相直接标记 RIA（参见，例如，Morel 等，1988，Molec.Immunol.25:7-15)；固相直接生物素 - 亲和素 EIA（参见，例如，Cheung 等，1990，Virology 176:546-552)；和直接标记 RIA(Moldenhauer 等，1990，Scand.J.Immunol.32:77-82)。典型地，这样的测定涉及使用与载有任何这些的固体表面或细胞结合的纯化的抗原：未标记的测试抗原结合蛋白和标记的参考抗原结合蛋白。通过确定在存在测试抗原结合蛋白的情况下与固体表面或细胞结合的标记的量来测量竞争性抑制。通常，测试抗原结合蛋白过量存在。通过竞争测定鉴定的抗原结合蛋白（竞争性抗原结合蛋白）包括结合与参考抗原结合蛋白相同的表位的抗原结合蛋白和结合与参考抗原结合蛋白结合的表位足够接近以致发生空间位阻的邻近表位的抗原结合蛋白。关于确定竞争性结合的方法的其他细节提供在本文的实施例中。通常，当竞争性抗原结合蛋白以过量存在时，它将抑制（例如，降低）参考抗原结合蛋白与共同抗原的特

异性结合达至少 40-45％，45-50％，50-55％，55-60％，60-65％，65-70％，70-75％或 75％以上。在某些实施方案中，所述结合被抑制达至少 80-85％，85-90％，90-95％，95-97％或 97％以上。

[0349] 在本发明的一方面中，竞争测定可以被用于鉴定与抗 PCSK9 抗体 508.20.04a,508.20.04b,508.20.06,508.20.28a,508.20.28b,508.20.33a,508.20.33b 或 508.20.84 竞争结合 PCSK9 的抗体。在某些实施方案中，这样的竞争性抗体结合由抗 PCSK9 抗体 508.20.04a,508.20.04b,508.20.06,508.20.28a,508.20.28b,508.20.33a,508.20.33b 和 / 或 508.20.84 结合的表位相同的表位（例如，线性或构象表位）。用于定位与抗体结合的表位的详细的示例性方法提供在 Methods in Molecular Biology（分子生物学中的方法）vol.66(Humana Press,Totowa,NJ) 中的 Morris(1996)“Epitope Mapping Protocols（表位定位实验方法）”中。

[0350] 在示例性竞争测定中，在溶液中温育固定的 PCSK9，所述溶液包含与 PCSK9 结合的第一标记抗体（例如，抗 PCSK9 抗体 508.20.04a,508.20.04b,508.20.06,508.20.28a,508.20.28b,508.20.33a,508.20.33b 或 508.20.84）和被测试其与所述第一抗体竞争结合 PCSK9 的能力的第二未标记抗体。所述第二抗体可以存在于杂交瘤上清液中。作为对照，在这样的溶液中温育固定的 PCSK9，所述溶液包含第一标记抗体但是不包含第二未标记抗体。在允许所述第一抗体与 PCSK9 结合的条件下温育后，除去过量的未结合的抗体，并且测量与固定的 PCSK9 结合的标记的量。如果相对于对照样品在测试样品中与固定的 PCSK9 结合的标记的量显著降低，则说明所述第二抗体与所述第一抗体竞争结合 PCSK9。参见 Harlow 和 Lane(1988)Antibodies :A Laboratory Manual（抗体 :实验室手册）ch.14(Cold Spring Harbor Laboratory,Cold Spring Harbor,NY)。

[0351] 2. 活性测定

[0352] 在一方面中，提供用于鉴别具有生物学活性的抗 PCSK9 抗体的测定。抗 PCSK9 抗体的生物学活性可以包括，例如，阻断、拮抗、抑制、干扰、调节和 / 或降低 PCSK9 的一种或多种生物学活性。还提供在体内和 / 或体外具有这样的生物学活性的抗体。

[0353] 在某些实施方案中，抗 PCSK9 抗体结合人 PCSK9 并防止与 LDLR 的相互作用。在某些实施方案中，抗 PCSK9 抗体特异性结合人 PCSK9 和 / 或基本上抑制人 PCSK9 与 LDLR 的结合达至少约 20％ -40％，40-60％，60-80％，80-85％以上（例如，通过在体外竞争性结合测定中测量结合）。在某些实施方案中，本发明提供分离的抗 PCSK9 抗体，其特异性地结合 PCSK9，并且当在体外使用本文中公开的 HepG2 细胞中的 LDLR 下调测定测量时，拮抗 PCSK9 介导的对 LDLR 水平的作用。

[0354] D. 免疫缀合物

[0355] 本发明也提供包含本文中的抗 PCSK9 抗体与一或多种细胞毒性剂，例如化疗剂或药物、生长抑制剂、毒素（例如蛋白质毒素、细菌、真菌、植物或动物来源的酶促活性毒素，或其片段）或放射性同位素缀合的免疫缀合物。

[0356] 在一实施方案中，免疫缀合物是抗体 - 药物缀合物 (ADC)，其中抗体与一种或多种药物缀合，所述药物包括，但不限于，美坦生类化合物（参见美国专利第 5,208,020 号、第 5,416,064 号及欧洲专利 EP 0425235B1）；奥利司他汀 (auristatin)，如单甲基奥利司他汀 (monomethylauristatin) 药物部分 DE 及 DF(MMAE 及 MMAF)（参见美国专利第

5,635,483 号及第 5,780,588 号，及第 7,498,298 号）；多拉司他汀 (dolastatin)；加利车霉素 (calicheamicin) 或其衍生物（参见美国专利第 5,712,374 号、第 5,714,586 号、第 5,739,116 号、第 5,767,285 号、第 5,770,701 号、第 5,770,710 号、第 5,773,001 号及第 5,877,296 号；Hinman 等人，Cancer Res.（癌症研究）53:3336-3342(1993)；及 Lode 等人，Cancer Res.（癌症研究）58:2925-2928(1998)）；蒽环类抗生素 (anthracycline) 如道诺霉素 (daunomycin) 或多柔比星（参见 Kratz 等人 Current Med.Chem.13:477-523(2006)；Jeffrey 等人，Bioorganic&Med.Chem.Letters 16:358-362(2006)；Torgov 等人，Bioconj.Chem.16:717-721(2005)；Nagy 等人，Proc.Natl.Acad.Sci.USA（美国科学院学报）97:829-834(2000)；Dubowchik 等人，Bioorg.&Med.Chem.Letters 12:1529-1532(2002)；King 等人，J.Med.Chem.45:4336-4343(2002)；及美国专利第 6,630,579 号）；甲氨喋呤 (methotrexate)；长春地辛 (vindesine)；紫杉烷 (taxane) 如多西他赛 (docetaxel)、紫杉醇 (paclitaxel)、拉洛紫杉醇 (larotaxel)、特赛紫杉醇 (tesetaxel) 及奥他紫杉醇 (ortataxel)；单端孢霉烯族化合物 (trichothecene)；及 CC1065。

[0357] 在另一实施方案中，免疫缀合物包含如本文中所述的抗体与酶促活性毒素或其片段缀合，酶促活性毒素或其片段包括，但不限于，白喉 (diphtheria)A 链、白喉毒素的非结合活性片段、外毒素 A 链（来自铜绿假单胞菌 (Pseudomonas aeruginosa)）、蓖麻毒蛋白 (ricin)A 链、相思豆毒蛋白 (abrin)A 链、蒴莲根毒蛋白 (modeccin)A 链、α-帚曲霉素 (sarcin)、油桐 (Aleutites fordii) 蛋白、香石竹毒蛋白 (dianthin protein)、美洲商陆 (Phytolaca americana) 蛋白 (PAPI、PAPII 和 PAP-S)、苦瓜 (Momordica charantia) 抑制剂、麻疯树毒蛋白 (curcin)、巴豆毒蛋白 (crotin)、肥皂草 (sapaonaria officinalis) 抑制剂、白树毒蛋白 (gelonin)、丝林霉素 (mitogellin)、局限曲菌素 (restrictocin)、酚霉素 (phenomycin)、依诺霉素 (enomycin) 和单端孢霉烯族化合物 (trichothecenes)。

[0358] 在另一实施方案中，免疫缀合物包含如本文中所述的抗体与放射性原子缀合形成放射性缀合物。多种放射性同位素可用于制备放射性缀合物。实例包括 At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}、Pb^{212} 及 Lu 的放射性同位素。当使用放射性缀合物进行检测时，其可包含用于闪烁摄影研究的放射性原子，例如 tc99m 或 I123 或用于核磁共振 (NMR) 成像（也称为磁共振成像，mri）的自旋标记物，如再次碘 -123、碘 -131、铟 -111、氟 -19、碳 -13、氮 -15、氧 -17、钆、锰或铁。

[0359] 抗体与细胞毒性剂的缀合物可使用多种双功能蛋白质偶合剂制得，例如 N- 琥珀酰亚胺基 -3-(2- 吡啶基二硫代）丙酸酯 (SPDP)、琥珀酰亚胺基 -4-(N- 马来酰亚氨甲基）环己烷 -1- 羧酸酯 (SMCC)、亚氨基硫烷 (IT)、亚氨酸酯（诸如盐酸二甲基己二亚酰胺化物）、活性酯类（诸如辛二酸二琥珀酰亚胺基酯）、醛类（诸如戊二醛）、双叠氮化合物（诸如双（对 - 叠氮苯甲酰基）己二胺）、双重氮衍生物（诸如双（对 - 重氮苯甲酰基）己二胺）、二异氰酸酯（诸如甲苯 2,6- 二异氰酸酯）、和双活性氟化合物（诸如 1,5- 二氟 -2,4- 二硝基苯）的双功能衍生物。举例来说，蓖麻毒蛋白免疫毒素可如 Vitetta 等人，Science（科学）238:1098(1987) 中所述来制备。碳 -14 标记的 1- 异硫氰酸苯甲基 -3- 甲基二亚乙基三胺五乙酸 (MX-DTPA) 是用于使放射性核苷酸与抗体结合的示例性螯合剂。参见 WO 94/11026。接头可以是促进细胞毒性药物在细胞中释放的“可裂解接头”。举例来说，可使

用酸不稳定接头、肽酶敏感性接头、光不稳定接头、二甲基接头或含二硫化物的接头 (Chari 等人，Cancer Res.（癌症研究）52:127-131(1992)；美国专利第 5,208,020 号）。

[0360] 本文中的免疫缀合物或 ADCs 明确涵盖，但不限于，用交联剂试剂制备的这些缀合物，该等交联剂试剂包括，但不限于：BMPS、EMCS、GMBS、HBVS、LC-SMCC、MBS、MPBH、SBAP、SIA、SIAB、SMCC、SMPB、SMPH、硫代 -EMCS、硫代 -GMBS、硫代 -KMUS、硫代 -MBS、硫代 -SIAB、硫代 -SMCC 及硫代 -SMPB 及 SVSB（琥珀酰亚胺基 -(4- 乙烯基砜）苯甲酸酯），交联剂试剂可商购获得（例如购自 Pierce Biotechnology，Inc.，Rockford，IL.，U.S.A）。

[0361] E. 用于诊断和检测的方法和组合物

[0362] 在某些实施方案中，本文中提供的任何抗 PCSK9 抗体可以用于检测 PCSK9 在生物样品中的存在。术语“检测”用于本文中时，包括定量或定性检测。在某些实施方案中，生物样品是血、血清或生物来源的其他液体样品。在某些实施方案中，生物样品包含细胞或组织。

[0363] 在一个实施方案中，提供用于诊断或检测方法的抗 PCSK9 抗体。在另一个方面中，提供检测 PCSK9 在生物样品中的存在的方法。在某些实施方案中，方法包含检测 PCSK9 蛋白在生物样品中的存在。在某些实施方案中，PCSK9 是人 PCSK9。在某些实施方案中，所述方法包括将生物样品与如本文所述的抗 PCSK9 抗体在允许抗 PCSK9 抗体与 PCSK9 结合的条件下接触，并检测在抗 PCSK9 抗体和 PCSK9 之间是否形成复合物。该方法可以是体外或体内方法。在一个实施方案中，抗 PCSK9 抗体被用于选择适合利用抗 PCSK9 抗体的治疗的受试者，例如其中 PCSK9 或 LDL- 胆固醇是用于选择患者的生物标记物。

[0364] 可以使用本发明的抗体诊断的示例性疾病包括胆固醇相关疾病（其包括“血清胆固醇相关疾病”），其包括以下的任何一种或多种：高胆固醇血症、心脏病、代谢综合征、糖尿病、冠状动脉心脏病、卒中、心血管疾病、阿尔茨海默病和一般性的异常脂血症（其显示为例如升高的总血清胆固醇、升高的 LDL、升高的甘油三酯、升高的极低密度脂蛋白 (VLDL) 和 / 或低的 HDL)。在一方面中，本发明提供治疗或预防个体中的高胆固醇血症和 / 或至少一种以下症状的方法：异常脂血症、动脉粥样硬化、心血管疾病 (CVD) 或冠状动脉心脏病，所述方法包括向所述个体施用有效量的抗 PCSK9 抗体。在某些实施方案中，本发明提供有效量的抗 PCSK9 抗体用于治疗或预防受试者中的高胆固醇血症和 / 或至少一种以下症状：异常脂血症、动脉粥样硬化、CVD 或冠状动脉心脏病。本发明还提供有效量的拮抗胞外或循环 PCSK9 的抗 PCSK9 抗体在制备药物中的用途，所述药物用于治疗或预防个体的高胆固醇血症和 / 或至少一种以下症状：异常脂血症、动脉粥样硬化、CVD 或冠状动脉心脏病。

[0365] 在某些实施方案中，提供标记的抗 PCSK9 抗体。标记包括但不限于，被直接检测的标记或部分（如荧光标记，发色团标记，电子致密标记，化学发光标记，和放射性标记），以及被间接检测的部分，如酶或配体，例如，通过酶促反应或分子相互作用。示例性标记包括但不限于，放射性同位素 ^{32}P，^{14}C，^{125}I，^{3}H，和 ^{131}I，荧光团如稀土螯合物或荧光素及其衍生物，罗丹明及其衍生物，丹酰 (dansyl)，伞形酮 (umbelliferone)，荧光素酶 (luceriferase)，例如，萤火虫荧光素酶和细菌荧光素酶（美国专利号 4,737,456)，荧光素 (luciferin)，2,3- 二氢酞嗪二酮，辣根过氧化物酶 (HRP)，碱性磷酸酶，β - 半乳糖苷酶，葡糖淀粉酶，溶解酶，糖类氧化酶，例如，葡萄糖氧化酶，半乳糖氧化酶，和葡萄糖 -6- 磷酸脱氢酶，杂环氧化酶如尿酸酶和黄嘌呤氧化酶，加上利用过氧化氢氧化染料前体的酶如 HRP，乳过氧化物

酶，或微过氧化物酶 (microperoxidase)，生物素 / 亲和素，自旋标记，噬菌体标记，稳定的自由基，等等。

[0366] F. 药物制剂

[0367] 本发明还包括包含抗 PCSK9 抗体的组合物（包括药物制剂）和包含编码抗 PCSK9 抗体的序列的多核苷酸。在某些实施方案中，组合物包含一种或多种结合 PCSK9 的抗体或一种或多种包含编码一种或多种结合 PCSK9 的抗体的序列的多核苷酸。这些组合物还可以包含合适的载体，如本领域中已知的药用赋形剂，包括缓冲剂。

[0368] 如本文所述的抗 PCSK9 抗体的药物制剂通过将具有所需纯度的所述抗体与一种或多种任选的药用载体 (Remington's Pharmaceutical Sciences，第 16 版，Osol，A. 编 (1980)) 混合来制备，以冻干制剂或水溶液的形式。药用载体通常在所用剂量及浓度对接受者无毒，并且包括但不限于 ：缓冲剂，如磷酸盐、柠檬酸盐及其他有机酸 ；抗氧化剂，包括抗坏血酸及甲硫氨酸 ；防腐剂（如氯化十八烷基二甲基苄铵、氯化六羟季铵、氯苄烷铵、苄索氯铵 ；苯酚、丁醇或苄醇 ；对羟基苯甲酸烷酯，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 ；及间甲酚）；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 ；亲水性聚合物，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、二糖和其他糖类，包括葡萄糖、甘露糖或糊精 ；螯合剂，如 EDTA ；糖类，例如蔗糖、甘露醇、海藻糖或山梨醇 ；成盐平衡离子，如钠 ；金属复合体（例如 Zn- 蛋白质复合体）；和 / 或非离子表面活性剂，如聚乙二醇 (PEG)。本文中的示例性药用载体还包括药物间质分散剂，如可溶性中性 - 活性透明质酸酶糖蛋白 (sHASEGP)，例如人可溶性 PH-20 透明质酸酶糖蛋白，如 rHuPH20（HYLENEX®，Baxter International，Inc.）。某些示例性 sHASEGPs 及使用方法，包括 rHuPH20，描述于美国专利公开号 2005/0260186 及 2006/0104968 中。在一方面中，sHASEGP 与一种或多种其他葡糖胺聚糖酶例如软骨素酶组合。

[0369] 示例性的冻干抗体制剂描述于美国专利号 6,267,958。水性抗体制剂包括美国专利号 6,171,586 和 WO2006/044908 中所述的那些，后一种制剂包括组氨酸 - 乙酸盐缓冲剂。

[0370] 本文的制剂还可以包含超过一种活性成分，所述活性成分是被治疗的特定适应证所需的，优选具有不会不利地影响彼此的互补活性的那些活性成分。例如，理想的是还提供他汀类。所述活性成分以对于目的用途有效的量合适地组合存在。

[0371] 可以将活性成分截留于例如通过凝聚技术或通过界面聚合所制备的微囊，例如，分别是羟甲基纤维素或明胶微囊及聚 -（甲基丙烯酸甲酯）微囊中、胶状药物传递系统（例如脂质体、白蛋白微球体、微乳液、纳米颗粒及纳米胶囊）中或粗滴乳状液中。这些技术披露于 Remington's Pharmaceutical Sciences，第 16 版，Osol，A. 编 (1980) 中。

[0372] 可制备持续释放制剂。持续释放制剂的合适实例包括含有抗体的固体疏水聚合物的半渗透基质，所述基质呈成形物品，例如薄膜或微囊形式。

[0373] 欲用于体内施用的制剂通常是无菌的。无菌可以例如借助通过无菌过滤膜的过滤而轻易地实现。

[0374] 在一个方面，本发明提供一种组合物，所述组合物包含约 100 至约 225mg/mL 的抗 -PCSK9 抗体，约 180 至约 220mM 的精氨酸琥珀酸盐，约 0.01％至约 0.03％的聚山梨酯，并且 pH 在约 5.2 至约 5.8。在某些实施方案中，所述组合物适于皮下给药。在某些实

施方案中，组合物的粘度在25℃小于约25cP，在25℃小于约20cP，在25℃小于约15cP，在25℃小于约12cP，或在25℃小于约10cP。在某些实施方案中，所述组合物在2-8℃稳定达至少一个月，至少两个月，至少三个月，至少四个月，至少五个月，或至少六个月。在一些实施方案中，所述组合物在0.5-mL,1-mL,1.25-mL,1.5-mL,1.75-mL,2-mL,2.25-mL，或2.5-mL预填充注射器中。在某些实施方案中，所述组合物中的抗体为约110,120,125,130,135,140,145,150,155,160,165,170,175,180,185,190,195,200,205,210,215,220，和225mg/mL中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物中的精氨酸琥珀酸盐为约180,185,190,200,210和220mM中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物中的聚山梨酯（例如，聚山梨酯20，聚山梨酯80）为约0.01％，0.015％，0.02％，0.025％，和0.03％中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物具有5.0,5.2,5.4,5.5,5.6,5.8,5.9,6.0,6.1和6.2中任一个的pH，包括这些值中任意值之间的pH。在某些实施方案中，组合物中的抗-PCSK9抗体为约150mg/mL，组合物中的精氨酸琥珀酸盐为约200mM，和组合物中的聚山梨酯20为约0.02％，并且pH为约5.5。

[0375] 在一个方面，本发明提供一种组合物，所述组合物包含约150至约225mg/mL的抗-PCSK9抗体，约10至约30mM的组氨酸乙酸盐，约150至约170mM的精氨酸乙酸盐，约0.01％至约0.03％的聚山梨酯，并且pH在约5.8至约6.2。在某些实施方案中，所述组合物适于皮下给药。在某些实施方案中，所述组合物的粘度在25℃小于约25cP，在25℃小于约20cP，在25℃小于约15cP，在25℃小于约12cP，或在25℃小于约10cP。在某些实施方案中，所述组合物在2-8℃稳定达至少一个月，至少两个月，至少三个月，至少四个月，至少五个月，或至少六个月。在一些实施方案中，所述组合物在0.5-mL,1-mL,1.25-mL,1.5-mL,1.75-mL,2-mL,2.25-mL，或2.5-mL预填充注射器中。在某些实施方案中，所述组合物中的抗体为约150,155,160,165,170,175,180,185,190,195,200,205,210,215,220，和225mg/mL中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物中的组氨酸乙酸盐为约10,15,20,25，和30mM中的任一个，包括这些浓度之间的浓度。在某些实施方案中，所述组合物中精氨酸乙酸盐为约150,155,160,165，和170mM中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物中的聚山梨酯（例如，聚山梨酯20，聚山梨酯80）为约0.01％，0.015％，0.02％，0.025％，和0.03％中的任一个，包括这些浓度中任意浓度之间的浓度。在某些实施方案中，所述组合物具有5.8,5.9,6.0,6.1和6.2中任一个的pH，包括这些值中任意值之间的pH。在某些实施方案中，所述组合物中的抗-PCSK9抗体为约200mg/mL，所述组合物中的组氨酸乙酸盐为约20mM，所述组合物中的精氨酸乙酸盐为约160mM，和所述组合物中的聚山梨酯20为约0.02％，并且pH为约6.0。

[0376] 本文中还提供的是皮下给药装置，所述装置含有本文中描述的组合物中的抗-PCSK9抗体，用于向个体递送200mg至1200mg抗体范围内的平稳剂量。单次施用的完全剂量可以在一个以上装置中。在某些实施方案中，所述装置中的抗体浓度为约200mg/mL。在某些实施方案中，所述装置是预填充注射器（例如，0.5-mL注射器，1-mL注射器，1.25-mL注射器，1.5-mL注射器，1.75-mL注射器，2-mL注射器，2.25-mL注射器，或2.5-mL注射器）或高容量、一次性、皮下输注装置（例如，用于递送

1-10mL,2-8mL,3-6mL,4-5mL, 或 4,5,6,7,8,9, 或 10mL)。

[0377] G. 治疗方法和组合物

[0378] 任何本文提供的抗 PCSK9 抗体可以用于治疗方法。

[0379] 在一方面中,提供用作药物的抗 PCSK9 抗体。在另一方面中,提供用于治疗与胆固醇相关疾病相关联的病症的抗 PCSK9 抗体。在某些实施方案中,提供用于治疗与升高的 LDL- 胆固醇水平相关联的病症的抗 PCSK9 抗体。在某些实施方案中,提供用于治疗方法中的抗 PCSK9 抗体。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于治疗具有与升高的 LDL- 胆固醇水平相关联的病症的个体的方法,所述方法包括向所述个体施用有效量的抗 PCSK9 抗体。在某些实施方案中,本文所述的方法和用途还包括向所述个体施用有效量的至少一种另外的治疗剂,例如,他汀类。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于降低受试者的 LDL- 胆固醇水平。在另外的实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于降低受试者的血清 LDL- 胆固醇水平。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于增加受试者的 LDLR 的利用度。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于抑制受试者中的 PCSK9 与 LDLR 的结合。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于降低个体的 LDL- 胆固醇水平的方法,所述方法包括向所述个体施用有效的抗 PCSK9 抗体以降低 LDL- 胆固醇水平。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于降低个体的血清 LDL- 胆固醇水平的方法,所述方法包括向所述个体施用有效的抗 PCSK9 抗体以降低血清 LDL- 胆固醇水平。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于增加个体的 LDLR 的利用度的方法,所述方法包括向所述个体施用有效的抗 PCSK9 抗体以提高 LDLR 的利用度。在某些实施方案中,本发明提供抗 PCSK9 抗体,所述抗 PCSK9 抗体用于抑制个体中的 PCSK9 与 LDLR 的结合的方法,所述方法包括向所述个体施用有效的抗 PCSK9 抗体以抑制 PCSK9 与 LDLR 的结合。根据任何本文描述的实施方案的“个体”或“受试者”优选是人。

[0380] 在其他方面中,本发明提供抗 PCSK9 抗体在生产或制备药物中的用途。在一个实施方案中,所述药物用于治疗胆固醇相关疾病。在某些实施方案中,胆固醇相关疾病是高胆固醇血症。在另一个实施方案中,所述药物用于治疗高胆固醇血症的方法,所述方法包括向具有高胆固醇血症的个体施用有效量的所述药物。

[0381] 在某些实施方案中,被治疗的疾病是可以通过消除、抑制或降低 PCSK9 的活性而被改善、减缓、抑制或预防的任何疾病或病症。在某些实施方案中,通常通过使用他汀类可解决的(可治疗或可预防的)疾病或病症也可以被治疗。在某些实施方案中,可以受益于阻止胆固醇合成或升高的 LDLR 表达的疾病或病症也可以通过本发明的抗 PCSK9 抗体治疗。在某些实施方案中,可通过本发明的抗 PCSK9 抗体和治疗方法治疗的个体包括适用 LDL 单采血液成分术 (apheresis) 的个体,具有 PCSK9 活化突变的个体(获得功能突变,"GOF"),具有杂合家族性高胆固醇血症 (heFH) 的个体,具有原发性高胆固醇血症、不耐受他汀类或是他汀类不受控的个体,和处于发生高胆固醇血症的风险、可以被预防性治疗的个体。其他适应征包括与继发性病因如 II 型糖尿病相关联的异常脂血症,胆汁淤积性肝病 (cholestatic liver diseases)(原发性胆汁性肝硬化 (primary biliary cirrhosis)),肾病综合征,甲状腺机能障碍,肥胖,和预防和治疗动脉粥样硬化和心血管疾病。在某些实

施方案中，可由本文中描述的抗-PCSK9抗体和治疗方法治疗的个体包括具有90-250mg/dL的LDL-c水平的和具有如实施例12中详细描述的冠心病(coronary heart disease)(CHD)或CHD风险替代症(risk equivalent)的个体。

[0382] 在某些实施方案中，本文中描述的方法包括向患有冠心病的个体施用抗-PCSK9抗体。在某些实施方案中，患有冠心病的个体具有有记载的心肌梗死(myocardial infarction)史。在某些实施方案中，患有冠心病的个体是指经历了之前的冠状动脉重建术(coronary revascularization)过程(例如，经皮冠状动脉介入或冠状动脉旁路搭桥)的个体。在某些实施方案中，患有冠心病的个体是指具有至少一次冠状动脉粥样硬化病变(coronary atherosclerotic lesion)同时≥50％直径狭窄的个体(例如，如通过冠状动脉血管造影术(包括侵入性冠状动脉血管造影术或心脏计算机断层扫描冠状动脉血管造影术)确定。

[0383] 在某些实施方案中，本文中描述的方法包括向具有至少一种CHD风险替代症的个体施用抗-PCSK9抗体。在某些实施方案中，具有CHD风险替代症的个体是具有一种以上动脉粥样硬化疾病形式的个体，所述动脉粥样硬化疾病形式比如，例如，外周动脉疾病(例如，<0.85的踝/上臂血压指数，之前的经皮或外科手术外周动脉血管重建步骤，之前由于外周动脉疾病造成的下肢非外伤性截肢，或之前的血管成像方面的≥50％直径狭窄)，颈动脉疾病(例如，颈动脉动脉粥样硬化病变同时≥50％直径狭窄或之前的皮肤或外科手术颈动脉血管重建步骤)，之前的缺血性卒中，或腹主动脉瘤(abdominal aortic aneurysm)。在某些实施方案中，具有CHD风险替代症的个体是患有II型糖尿病的个体。在某些实施方案中，具有CHD风险替代症的个体是患有I型糖尿病外加器官损伤(例如，视网膜病(retinopathy)，神经病(neuropathy)，或肾病(nephropathy)(包括微量白蛋白尿(microalbuminuria)))的个体。在某些实施方案中，具有CHD风险替代症的个体是患有中度到严重慢性肾病的个体。

[0384] 在某些实施方案中，本文中描述的方法包括向具有一个以上下述风险因素的个体施用抗-PCSK9抗体:年龄(对于男性≥45岁或对于女性≥55岁)，抽烟(在1个月内)，高血压(收缩压≥140mmHg，舒张压≥90mmHg，或服用抗高血压药物)，低HDL胆固醇(<40mg/dL)，或过早CHD的家族史。

[0385] 在某些实施方案中，本文所述的方法和用途还包括向所述个体施用有效量的至少一种另外的治疗剂，例如，他汀类。在某些实施方案中，所述另外的治疗剂用于预防和/或治疗动脉粥样硬化和/或心血管疾病。在某个实施方案中，所述另外的治疗剂用于降低复发的心血管事件的风险的方法。在某些实施方案中，所述另外的治疗剂用于提高受试者的HDL-胆固醇水平。

[0386] 在另一方面中，本发明提供药物制剂，所述药物制剂包含任何本文提供的抗PCSK9抗体，例如，用于任何以上的治疗方法。在一个实施方案中，所述药物制剂包含任何本文提供的抗PCSK9抗体和药用载体。在另一个实施方案中，所述药物制剂包含任何本文提供的抗PCSK9抗体和至少一种另外的治疗剂，例如，他汀类。

[0387] 在治疗中，本发明的抗体可以单独使用或与其他试剂组合使用。例如，本发明的抗体可以与至少一种另外的治疗剂一起共同给药。在某些实施方案中，这样的另外的治疗剂提升LDLR的水平。在某些实施方案中，另外的治疗剂是降低LDL-胆固醇的药物如他汀类，

例如，阿托伐他汀，氟伐他汀，洛伐他汀，美伐他汀，匹伐他汀，普伐他汀，罗舒伐他汀，辛伐他汀或其任意组合，例如，VYTORIN®， ADVICOR®或SIMCOR®。在某些实施方案中，另外的治疗剂是提高HDL-胆固醇的药物。

[0388] 这样的以上所述的组合疗法包括组合给药（其中两种以上治疗剂被包含在相同或分开的制剂中），和分别给药，其中，本发明的抗PCSK9抗体的给药可以发生在另外的治疗剂和/或佐剂的给药前、同时和/或之后。

[0389] 本发明的抗体（以及任何另外的治疗剂）可以通过任何合适的方法给药，包括肠胃外给药，肺内给药和鼻内给药，并且，如果局部治疗需要，病变内给药。肠胃外输注包括肌肉内、静脉内、动脉内、腹膜内或皮下给药。在一定程度上根据用药是短期或长期性而定，可通过任何适合途径，例如通过注射，例如静脉内或皮下注射用药。本文中涵盖各种用药时程，包括，但不限于，单次给药或在多个时间点多次给药、推注给药及脉冲输注。

[0390] 本发明的抗PCSK9抗体将以与良好医疗实践相一致的方式配制、给药和施用。在该背景下考虑的因素包括待治疗的具体病症、待治疗的具体哺乳动物、个体患者的临床状态、病症的原因、递送试剂的位点、施药方法、施药时间安排、和医疗从业者已知的其他因素。所述抗体不需要，但任选地，与目前用于预防或治疗待讨论病症的一种或多种试剂一起配制。所述其他试剂的有效量取决于制剂中存在的抗体的量、病症或治疗的类型、和以上讨论的其他因素。这些一般以相同剂量，并使用如本文中所述的施药途径，或以本文中所述的剂量的约1-99％，或以通过经验/临床确定合适的任意剂量和任何途径来使用。

[0391] 为了预防或治疗疾病，本发明的抗体的合适剂量（当单独或与一种或多种其他另外的治疗剂组合使用时）将取决于待治疗疾病的类型、抗体的类型、疾病的严重性和进程、所述抗体是以预防目的施用还是以治疗目的施用、以前的治疗、患者的临床病史和对所述抗体的应答，和主治医师的判断力。所述抗体以一次治疗或经过一系列治疗合适地施用于患者。根据疾病的类型和严重性，约1μg/kg-15mg/kg（例如0.1mg/kg-10mg/kg）的抗体可以是用于向患者施用的最初候选剂量，无论，例如，通过一次或多次分别施药，或通过连续输注。一个典型的每日剂量可以在约1μg/kg-100mg/kg或更多的范围内，其取决于上文提及的因素。为了重复施用数日或更长，根据病症，通常将持续治疗直至出现疾病症状的理想抑制。所述抗体的一个示范性剂量应该在约0.05mg/kg-约10mg/kg范围内。因此，约0.5mg/kg、2.0mg/kg、4.0mg/kg或10mg/kg（或其任意组合）的一个或多个剂量可以施用于患者。这样的剂量可以间隔地，例如每周或每三周施用（例如以使得患者接受约2个-约20个或例如约6个剂量的所述抗体）。可以施用最初较高的负荷剂量，随后是一个或多个较低的剂量。

[0392] 在某些实施方案中，使用无变化的平稳固定（flat-fixed）剂量给药方案来将抗PCSK9抗体施用于个体。取决于疾病的类型和严重性，示例性的无变化的平稳固定剂量可以为10至1200mg的抗PCSK9抗体。抗体的一个示例性剂量将为约10mg至约1000mg。抗体的另一个示例性剂量将为约100mg至约600mg。抗体的另一个示例性剂量将为约200mg至约800mg。抗体的另一个示例性剂量将为约350mg至约400mg。抗体的另一个示例性剂量将为约750mg至约800mg。在某些实施方案中，将150mg,200mg,220mg,300mg,380mg,400mg,500mg,600mg,700mg,760mg,800mg,1000mg,1140mg,或1200mg的抗-PCSK9抗体施用于个体。在某些实施方案中，每2周，每4周，每6周，每8周，每10周，或每12周施

用抗 -PCSK9 抗体的平稳剂量。在某些实施方案中，以不比每 2 周，每 4 周，每 6 周，每 8 周，每 10 周，或每 12 周一次更频繁的频率施用平稳剂量的抗 -PCSK9 抗体。在某些实施方案中，每个月，每 1.5 个月，每 2 个月，每 2.5 个月，或每 3 个月施用平稳剂量的抗 -PCSK9 抗体。在某些实施方案中，以不比每个月，每 1.5 月，每 2 月，每 2.5 月，或每 3 个月一次更频繁的频率施用平稳剂量的抗 -PCSK9 抗体。在某些实施方案中，皮下给药所述抗 -PCSK9 抗体。然而，其它剂量方案可以是有用的。本治疗的过程容易通过常规技术和测定监测。

[0393] 在某些实施方案中，以小于或等于 5mL, 4.5mL, 4mL, 3.8mL, 3.5mL, 3mL, 2.5mL, 2mL, 1.9mL, 1.5mL, 或 1mL 的体积提供要施用的平稳的 (flat)、固定的、皮下剂量。在某些实施方案中，平稳的、固定的皮下剂量为在小于或等于 4mL 的总体积中 800mg。在某些实施方案中，平稳的、固定的，皮下剂量为在小于或等于 3.8mL 的总体积中 760mg。在某些实施方案中，平稳的、固定的，皮下剂量为在小于或等于 3mL 的总体积中 600mg。在某些实施方案中，平稳的、固定的，皮下剂量为在小于或等于 2mL 的总体积中 400mg。在某些实施方案中，平稳的、固定的，皮下剂量为在小于或等于 1.9mL 的总体积中 380mg。

[0394] 在某些实施方案中，由本文中描述的方法治疗的个体中 LDL- 胆固醇水平从基线降低至少约 45％，至少约 50％，至少约 55％，或至少约 60％。在一些实施方案中，由描述的方法治疗的个体中 LDL- 胆固醇水平从基线降低至少约 45％，至少约 50％，至少约 55％，或至少约 60％，并在最后一次剂量给药之后维持在降低的水平达至少两周，至少一个月，至少两个月，或三个月。在一些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平在起始剂量给药的约 1 周，约 10 天，或约 2 周内从基线降低至少约 45％，至少约 50％，至少约 55％，或至少约 60％。在一些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平在起始剂量给药的约 1 周，约 10 天，或约 2 周内从基线降低至少约 45％，至少约 50％，至少约 55％，或至少约 60％，并在最后一次剂量给药之后维持在降低的水平达至少两周，至少一个月，至少两个月，或三个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 45％并维持在降低的水平达至少约六周，至少约 7 周或至少约 1.5 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量给药起约 1 周降低至少约 45％并维持在降低的水平达至少约六周，至少约 7 周或至少约 1.5 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 50％并维持在降低的水平达至少约四周或至少约 1 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量给药起约 10 天内降低至少约 50％并维持在降低的水平达至少约四周或至少约 1 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 50％并维持在降低的水平达至少约八周或至少约 2 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量给药起约 10 天内降低至少约 50％并维持在降低的水平达至少约八周或至少约 2 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 55％并维持在降低的水平达至少约两周。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平在起始剂量给药的约 2 周内降低至少约 55％并维持在降低的水平达至少约两周。如本文中使用的，个体中的“基线”水平（比如 LDL- 胆固醇水平的基线水平）是指向个体施用本文中描述的抗 -PCSK9 抗体之前的水平。在某些实施方案中，基线可以

是在施用抗 -PCSK9 抗体前获得的两次以上测量值的平均数 (mean) 或平均值 (average)。

[0395] 在某些实施方案中，由本文中描述的方法治疗的个体中的 LDL- 胆固醇水平从基线降低至少约 60mg/dL，至少约 70mg/dL，至少约 75mg/dL，至少约 80mg/dL，或至少约 90mg/dL。在一些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从基线降低至少约 60mg/dL，至少约 70mg/dL，至少约 75mg/dL，至少约 80mg/dL，或至少约 90mg/dL，并在最后一次剂量给药之后维持在降低的水平达至少两周，至少一个月，至少两个月，或三个月。在一些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平在起始剂量给药约 1 周，约 10 天，或约 2 周内从基线降低至少约 60mg/dL，至少约 70mg/dL，至少约 75mg/dL，至少约 80mg/dL，或至少约 90mg/dL。在一些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平在起始剂量给药约 1 周，约 10 天，或约 2 周内从基线降低至少约 60mg/dL，至少约 70mg/dL，至少约 75mg/dL，至少约 80mg/dL，或至少约 90mg/dL，并在最后一次剂量给药之后维持在降低的水平达至少两周，至少一个月，至少两个月，或三个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 60mg/dL 或 70mg/dL 并维持在降低的水平达至少约六周，至少约 7 周或至少约 1.5 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量给药起约 1 周内降低至少约 60mg/dL 或 70mg/dL 并维持在降低的水平达至少约六周，至少约 7 周或至少约 1.5 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 80mg/dL 和维持在降低的水平达至少约四周或至少约 1 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量起约 10 天内降低至少约 80mg/dL 并维持在降低的水平达至少约四周或至少约 1 个月。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平降低至少约 90mg/dL 并维持在降低的水平达至少约两周。在某些实施方案中，由描述的方法治疗的个体中的 LDL- 胆固醇水平从起始剂量给药起约 2 周内降低至少约 90mg/dL 并维持在降低的水平达至少约两周。

[0396] 在某些实施方案中，LDL- 胆固醇水平的降低在剂量给药之间维持在一定范围内。在某些实施方案中，在施用一个剂量抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线的至少约 45％，至少约 50％，至少约 55％，或至少约 60％的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 40％，45％，50％，55％，或 60％。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线的至少约 45％的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 40％或 45％。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线的至少约 50％的最低点并在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下的约 40％，45％，或 50％。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至从基线的至少约 55％的最低点并在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 40％，45％，50％，或 55％。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线的至少约 60％的最低点并在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 40％，45％，50％，55％，或 60％。

[0397] 在某些实施方案中，在剂量给药之间，LDL- 胆固醇水平的降低维持在一定范围内。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线以下至少约 60mg/dL，至少约 70mg/dL，至少约 75mg/dL，至少约 80mg/dL，或至少约

90mg/dL 的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL,60mg/dL,65mg/dL,70mg/dL,75mg/dL,80mg/dL 或 90mg/dL。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至基线以下至少约 60mg/dL 的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL 或 60mg/dL。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，降低 LDL- 胆固醇水平至至少约 70mg/dL 基线以下的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL,60mg/dL,65mg/dL, 或 70mg/dL。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至至少约 75mg/dL 基线以下的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL,60mg/dL,65mg/dL,70mg/dL, 或 75mg/dL。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至至少约 80mg/dL 基线以下的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL,60mg/dL,65mg/dL,70mg/dL,75mg/dL, 或 80mg/dL。在某些实施方案中，在施用一个剂量的抗 -PCSK9 抗体时，LDL- 胆固醇水平降低至至少约 90mg/dL 基线以下的最低点并且在下一次剂量给药抗 -PCSK9 抗体之前不增加超过基线以下约 55mg/dL,60mg/dL,65mg/dL,70mg/dL,75mg/dL,80mg/dL 或 90mg/dL。

[0398] 在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 800mg 的剂量施用于受试者，其中受试者中 LDL- 胆固醇水平在 10 天内降低基线以下至少 50％并且在下次剂量给药之前不增加至多于基线以下 40％或 45％。在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 760mg 的剂量施用于受试者，其中受试者中的 LDL- 胆固醇水平在 14 天内降低基线以下至少 45％并且在下次剂量给药前不增加至多于基线以下 35％或 40％。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的剂量施用于受试者，其中受试者中的 LDL- 胆固醇水平在 10 天内降低基线以下至少 50％并且在下次剂量给药之前不增加至多于基线以下 45％或 50％。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的剂量施用于受试者，其中受试者中的 LDL- 胆固醇水平在 10 天内降低基线以下至少 50％并且在下次剂量给药之前不增加至多于基线以下 45％或 50％。

[0399] 在某些实施方案中，监测接受抗 -PCSK9 抗体的受试者的 LDL-c 水平并且如果他们的水平降低至低于 25 或 15mg/dL, 则通过将施用的抗体的总量降低至施用的起始剂量的大约 50％或 25％并保持注射频率不变，通过保持施用的抗体的总量相同，但以 2 倍或 4 倍降低频率（例如，从每 4 周一次至每 8 周或 16 周一次），或其组合（例如，通过降低剂量并改变施用频率）将他们的剂量调低至起始剂量的大约 50％或 25％。在某些实施方案中，将抗 -PCSK9 抗体以每 8 周 800mg 的起始剂量施用于受试者。监测受试者并且如果受试者的 LDL-c 水平降低至低于 25 或 15mg/dL, 则将受试者转变为每 8 周 400mg, 每 16 周 400mg, 每 8 周 380mg, 每 16 周 380mg, 每 8 周 200mg, 每 4 周 200mg, 每 8 周 190mg, 每 4 周 190mg, 每 16 周或 4 个月 760mg, 或每 24 周或 6 个月 760mg，每 16 周或 4 个月 800mg, 或每 24 周或 6 个月 800mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 800mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL, 则将受试者转变为每 8 周 200mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 800mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL, 则将受试者转变为每 8 周 200mg 的剂量。在某些实施方案中，将抗 -PCSK9 抗体

以每 8 周 760mg 的起始剂量施用于受试者。监测受试者并且如果受试者的 LDL-c 水平降低至低于 25 或 15mg/dL，那么将受试者转变为每 8 周 380mg，每 16 周 380mg，每 4 周 200mg，每 8 周 200mg，每 8 周 190mg，每 4 周 190mg，每 16 周或 4 个月 760mg，或每 24 周或 6 个月 760mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 760mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 8 周 190mg 或 200mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 8 周 760mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 8 周 190mg 或 200mg 的剂量。在某些实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者。监测受试者并且如果受试者的 LDL-c 水平降低至低于 25 或 15mg/dL，则将受试者转变为每 4 周 200mg，每 8 周 200mg，每 4 周 100mg，每 8 周 400mg，每 16 周或 3 个月 400mg，每 2 周 50mg，或每 2 周 25mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 4 周 100mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 4 周 100mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 8 周 200mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 8 周 200mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 2 周 50mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 2 周 50mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 400mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 2 周 25mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体施以每 4 周 400mg 的起始剂量用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 2 周 25mg 的剂量。在某些实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者。监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL 或 15mg/dL，则将受试者转变为每 4 周 200mg，每 8 周 200mg，每 4 周 190mg，每 4 周 100mg，每 4 周 90mg，每 8 周 380mg，每 16 周或 3 月 380mg，每 2 周 50mg，或每 2 周 25mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 4 周 100mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 4 周 100mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 8 周 200mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 8 周 200mg

的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 8 周 190mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 8 周 190mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 2 周 50mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 4 周 50mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 25mg/dL，将受试者转变为每 2 周 25mg 的剂量。在一个实施方案中，将抗 -PCSK9 抗体以每 4 周 380mg 的起始剂量施用于受试者，监测受试者并且如果受试者的 LDL-c 水平降低至低于 15mg/dL，将受试者转变为每 2 周 25mg 的剂量。

[0400] 要理解，任何以上制剂或治疗方法可以使用本发明的免疫缀合物进行以代替抗 PCSK9 抗体或作为抗 PCSK9 抗体的补充。

[0401] H. 制品和试剂盒

[0402] 在本发明的另一方面中，提供一种制品或试剂盒，所述制品或试剂盒包含可用于治疗、预防和 / 或诊断上述病症的材料。在某些实施方案中，所述制品或试剂盒包括含有一种以上本文中描述的抗 -PCSK9 抗体或组合物的容器。在某些实施方案中，所述制品或试剂盒包括容器和在容器上或与容器一起的标签或包装说明书 (package insert)。适合的容器包括，例如，瓶子、小瓶、注射器、IV 溶液包等。所述容器可以由各种材料诸如玻璃或塑料制成。容器装有组合物，所述组合物是单独地或与可有效用于治疗、预防和 / 或诊断所述病症的另一种组合物结合，对于所述疾患的治疗有效的组合物，并且可以具有无菌的存取口（例如，所述容器可以是具有可被皮下注射针刺穿的塞子的静脉内溶液袋或小瓶）。组合物中至少一种活性试剂是本发明的抗 PCSK9 抗体。标签或包装说明书标明该组合物是用于治疗特定的病症。此外，所述制品或试剂盒可以包含 (a) 其中包含组合物的第一容器，其中所述组合物包含本发明的抗 PCSK9 抗体；和 (b) 其中包含组合物的第二容器，其中所述组合物包含另一种细胞毒性剂或其他的治疗剂。在某些实施方案中，第二容器包含第二治疗剂，其中所述第二治疗剂是“他汀类”类的降胆固醇药。在某些实施方案中，他汀类是和 / 或包含阿托伐他汀（例如，LIPITOR®或 Torvast），氟伐他汀（例如，LESCOL®），洛伐他汀（例如，MEVACOR®，ALTOCOR™或 ALTOPREV®），美伐他汀（匹伐他汀（例如，LIVALO®或 PITAVA®），普伐他汀（例如，PRAVACHOL®，SELEKTINE®，LIPOSTAT®），罗舒伐他汀（例如，CRESTOR®），辛伐他汀（例如，ZOCOR®，LIPEX®）或其任意组合，例如，VYTORIN®，ADVICOR®或 SIMCOR®。

[0403] 本发明的该实施方案中的制品或试剂盒还可以包括包装说明书，所述包装说明书指明所述组合物可以用于治疗特定病症。备选地，或另外地，所述制品还可以包括第二（或第三）容器，所述第二（或第三）容器包含药用缓冲剂，如抑菌注射用水 (BWFI)，磷酸盐缓

冲盐水,Ringer 氏溶液和葡萄糖溶液。从商业和用户立场,它还可以包括所需的其他材料,包括其他缓冲剂、稀释剂、滤膜、针头和注射器。

[0404] 要理解,任何以上制品或试剂盒可以包括本发明的免疫缀合物以代替抗 PCSK9 抗体或作为抗 PCSK9 抗体的补充。

[0405] III. 实施例

[0406] 以下是本发明的方法和组合物的实施例。要理解,根据以上提供的一般性描述,可以实施多种其他实施方案。

[0407] 实施例 1 :抗 PCSK9 抗体的产生

[0408] 残基编号是根据 Kabat(Kabat 等, Sequences of proteins of immunological interest,第 5 版,Public Health Service,National Institutes of Health,Bethesda, MD(1991)) 进行。

[0409] 文库分选和筛选以鉴定抗 PCSK9 抗体

[0410] 将内部生产的生物素化的人 PCSK9 用作用于文库分选的抗原。针对处于溶液相的生物素化的 PCSK9 进行五轮噬菌体文库分选。对于第一轮的分选,将 20μg/mL 生物素化的 PCSK9 添加到用噬菌体封闭缓冲液 PBST(磷酸盐缓冲盐水 (PBS) 和 1% (w/v) 牛血清清蛋白 (BSA) 和 0.05% (v/v) TWEEN® 20) 预封闭的抗体噬菌体文库 VH(参见,例 如, Lee 等, J.Immunol.Meth.284:119-132,2004) 和 VH/VL(参 见 Liang 等, JMB.366 : 815-829,2007),并且在室温过夜温育。第二天,向每个文库中加入 120μl 预吸收 PBST/BSA 的 DYNABEADS® MyOne™ Streptavidin T1(Invitrogen,Carlsbad,CA),并且在室温温育 1 小时。然后用 PBT(含有 0.05% TWEEN®20 的 PBS) 将小珠洗涤三次,并用 1mL 50mM HCl 和 500mM NaCl 洗脱结合的噬菌体 30 分钟,并用 400μL 的 1M Tris 碱 (pH7.5) 中和。回收的噬菌体在大肠杆菌 (E.coli)XL-1Blue 细胞中扩增。在随后的选择轮次期间,抗体噬菌体与生物素化的 PCSK9 的温育减少至 2-3 小时,并且在 neutravidin 包被的 (货号 89890, 10μg/ml,Fisher Scientific,Waltham,MA) 或链霉亲和素包被的 (货号 21125,10μg/ml, Fisher Scientific,Waltham,MA)Nunc 96 孔 Maxisorp™ 免疫平板上捕获与噬菌体结合的抗原达 30 分钟。逐渐提高平板洗涤的严格性。

[0411] 在 5 轮淘选后,观察到显著的富集。各从 VH 和 VH/VL 文库分选中挑取 96 个克隆以确定它们是否特异性地结合人 PCSK9。对这些克隆的可变区进行 PCR 测序以鉴定独特序列克隆。选择以超过背景至少 5x 结合人 PCSK9 的独特噬菌体抗体,并且使其转型 (reformatted) 为全长 IgGs 以用于在体外细胞测定中进行评估。

[0412] 通过以下方法使目的克隆转型为 IgG :分别将个体克隆的 VL 和 VH 区克隆到 LPG3 和 LPG4 载体中,并且在哺乳动物 CHO 细胞中瞬时表达,并且利用蛋白质 A 柱进行纯化。

[0413] 构建文库用于使来源于 VH 文库的克隆的亲和力提高

[0414] 在所有 CDR-L3 位置含有终止密码子 (TAA) 并且在 M13 噬菌体的表面上展示单价 Fab 的噬菌粒 pW0703(来源于噬菌粒 pV0350-2b(Lee 等, J.Mol.Biol.340, 1073-1093(2004)) 充当用于从 VH 文库嫁接目的克隆的重链可变结构域 (VH) 以用于亲和力成熟的文库模板。硬性随机化和软性随机化的策略均用于亲和力成熟。对于硬性 (hard) 随机化,使用设计为模拟天然人抗体的氨基酸,对一个具有三个轻链 CDR 的选定

位置的轻链文库进行随机化，并且所设计的DNA简并性如在Lee等(J.Mol.Biol.340，1073-1093(2004))中所述。对于软性(soft)随机化，靶向CDR-L3的位置91-96，CDR-H1的30-33、35，CDR-H2的50、52、53-54、56和58，CDR-H3的95-100、100A和100C处的残基；并且选择CDR环的三个不同组合，H1/L3、H2/L3和H3/L3，用于随机化。为获得软性随机化条件(其在选定位置引入约50％的突变率)，利用有利于野生型核苷酸的碱基的70-10-10-10混合物合成诱变的DNA(Gallop等，Journal of Medicinal Chemistry 37:1233-1251(1994))。

[0415] 产生亲和力提高的噬菌体分选策略

[0416] 对于亲和力提高选择，以增加的严格性对噬菌体文库进行五轮溶液分选。对于第一轮的溶液分选，将在1％BSA和0.05％TWEEN® 20中的30.D./ml的噬菌体输入与在100μl含有1％SUPERBLOCK®(Pierce Biotechnology)和0.05％TWEEN® 20的缓冲液中的100nM生物素化的PCSK9(浓度是基于亲本克隆噬菌体IC50值)在室温温育2小时。用1％SUPERBLOCK®将混合物进一步稀释10X，并且将100μl/孔施用到neutravidin包被的孔(10μg/ml)中，在室温30分钟并伴以轻微振荡。用PBS-0.05％TWEEN® 20将所述孔洗涤十次。为了确定背景结合，在neutravidin包被的平板上捕获含有噬菌体的对照。用150μl/孔50mM HCl、500mM KCl洗脱结合的噬菌体30分钟，并且随后通过50μl/孔的1M TrispH8中和，滴定，并且繁殖以用于次轮。以增加的选择严格性再进行四轮溶液分选。前两轮通过将生物素化的靶标蛋白质浓度从100nM降至1nM进行结合速率(on-rate)选择，后两轮是通过在室温加入过量的未生物素化的靶标蛋白质(300-1000倍以上)以竞争掉较弱的结合者来进行解离速率(off-rate)选择。

[0417] 高通量亲和力筛选ELISA(单点竞争)

[0418] 从第五轮筛选中挑取菌落。将克隆在37℃在150μl/孔的含有50μg/ml羧苄青霉素和1E10/ml KO7的2YT培养基中在96孔板(Falcon)中过夜培养。从同一个平板，挑取XL-1感染的亲本噬菌体的菌落作为对照。将96孔Nunc Maxisorp™平板用100μl/孔的neutravidin(10μg/ml)在PBS中在4℃过夜。将所述平板用150μl在PBS中的1％BSA和0.05％TWEEN® 20封闭1小时。

[0419] 用35μl含有或不含15nM PCSK9的ELISA(酶偶联免疫吸附测定)缓冲液(含0.5％BSA，0.05％TWEEN® 20的PBS)稀释35μl的噬菌体上清液，并且在室温在F平板(NUNC)中温育1小时。然后将35μl的3μg/ml生物素化的PCSK9加入每孔中，并且在室温温育15分钟。将95μl的混合物并排转移至neutravidin包被的平板。将所述平板温和振动15分钟以允许捕获与生物素化的PCSK9结合的噬菌体至平板。将平板用PBS-0.05％TWEEN® 20洗涤十次。通过加入辣根过氧化物酶(HRP)缀合的抗-M13抗体于ELISA缓冲液中(1:2500)并在室温温育30分钟来量化结合。将所述平板用PBS-0.05％TWEEN® 20洗涤十次。接下来，将100μl/孔的1:1比率的3,3',5,5'-四甲基联苯胺(TMB)过氧化物酶底物和过氧化物酶溶液B(H_2O_2)(Kirkegaard-Perry Laboratories(Gaithersburg，MD))添加到所述孔中，并且在室温温育5分钟。通过向每孔加入100μl 0.1M磷酸(H_3PO_4)并允许在室温温育5分钟来终止反应。使用标准ELISA平板读数器在450nm测定每孔中黄色的OD(光密度)。通过以下公式计算OD下降(％)：

[0420] OD_{450nm}下降(%)=[(含有竞争者的孔的OD_{450nm})/(不含竞争者的孔的OD_{450nm})]*100。

[0421] 与亲本噬菌体的孔(100%)的OD_{450nm}下降(%)相比,挑取具有的OD_{450nm}下降(%)低于50%的克隆用于序列分析。选择独特克隆用于噬菌体制备以通过与亲本克隆(克隆508.20b)的比较确定针对PCSK9的结合亲和力(噬菌体IC_{50})。然后将亲和力提高最大的克隆(508.20.04b,508.20.06,508.20.28b,508.20.33b和508.20.84)转型为人IgG1用于抗体产生以及进一步的BIAcore结合动力学分析和其他体外或体内测定。参见图1和2。

[0422] 实施例2:通过BIAcore表征抗PCSK9抗体

[0423] 抗PCSK9抗体的结合亲和力通过表面等离子共振(SPR)使用BIAcore™-3000仪器来测量。抗PCSK9人抗体由包被在CM5生物传感器芯片上的小鼠抗人Fc抗体(货号BR-1008-39,GE Healthcare,Piscataway,NJ)来捕获从而获得约200个应答单位(RU)。对于动力学测量,在25℃以30μl/min的流速注射在PBT缓冲液(含0.05% TWEEN® 20的PBS)中的人、鼠、恒河猴和食蟹猴PCSK9(Genentech,South San Francisco,CA)的两倍连续稀释液(500nM至0.245nM)。使用简单一对一Langmuir结合模型(BIAcore Evaluation Software 3.2版本)计算结合速率(k_{on})和解离速率(k_{off})。平衡解离常数(K_D)被计算作比率k_{off}/k_{on}。见图3。

[0424] 实施例3:LDLR-PCSK9结合测定

[0425] 进行竞争结合ELISA以研究抗PCSK9抗体在阻断人PCSK9与LDLR结合方面的活性。简言之,将1μg/mL的可溶人LDLR胞外结构域(R&DSystems,Minneapolis,MN)在4℃过夜包被在384孔MaxiSorp™板(NALGENE® NUNC™ International,Rochester,NY)上。然后向所述板中加入0.25μg/mL的与不同浓度的抗PCSK9抗体和对照抗体预混的生物素化的人PCSK9,并且在室温温育2小时。通过加入链霉亲和素-HRP(GEHealthcare)和底物3,3',5,5'-四甲基联苯胺(TMBE-1000,Moss,Inc.,Pasadena,MD)来检测PCSK9与包被的LDLR的结合。结合结果(OD)对抗体浓度作图,并且使用KaleidaGraph软件产生IC_{50}值。见图4。

[0426] 实施例4:抗体防止HepG2细胞上LDLR的下调

[0427] 以$1x10^5$将HepG2细胞接种到48孔板中。次日,将培养基换成10%无脂蛋白血清(LPDS,Frederick,Maryland)。次日,将15μg/ml PCSK9+/-抗PCSK9抗体加入到细胞中,在37℃达4小时。用PBS清洗细胞并使用2.5mM EDTA进行分离。将细胞与1:20抗LDLR(Progen Biotechnik,Heidelberg,Germany)温育15分钟,用PBS洗涤并与1:200来自Invitrogen(Carlsbad,CA)的山羊抗小鼠ALEXAFLUOR®488温育15分钟。将细胞洗涤并重悬在PBS加10μg/ml碘化丙啶中。然后利用双波长激光流式细胞仪(FACScan™,Becton Dickinson,Franklin Lakes,NJ)分析样品。数据显示所有五种抗PCSK9抗体(508.20.04b,508.20.06,508.20.28b,508.20.33b和508.20.84)阻止LDLR的下调。见图5。

[0428] 实施例5:小鼠肝中的LDLR下调

[0429] 通过静脉内给药用3、30或60μg的PCSK9处理正常C57/BL6小鼠(Charles River,Wilmington,MA)。使用来自Calbiochem(Gibbstown,NJ)的PROTEOEXTRACT®

天然膜蛋白提取试剂盒，根据制造商的说明，在 PCSK9 静脉内给药后 15 分钟、1 小时或 4 小时收获来自每只小鼠的肝脏并提取蛋白。作为对照，将 5 只小鼠仅用载体处理，并且汇集 8μg 的各肝溶胞产物用于分析。通过 SDS-PAGE 在 8％ tris-gly 凝胶 (Invitrogen，Carlsbad，CA) 上分析溶胞产物。使用 IBLOT ® (Invitrogen) 将蛋白转移至硝酸纤维膜。将所述膜用 5％脱脂奶封闭 1 小时，然后与 1:500 抗 LDLR(Abcam，Cambridge，MA) 在 5％脱脂奶中在 4℃过夜温育。次日，将所述膜用 TBS-T 洗涤三次，与 1:5000 抗兔 HRP(GE Healthcare，Piscataway，NJ) 温育 1 小时，并用 TBS-T 洗涤三次。使用 ECL-Plus(GE Healthcare) 并暴露于 XAR 胶片 (KODAK®，Rochester，NY) 来使蛋白可视。在过夜曝光后，将所述膜用 TBS-T 洗涤，与 1:500 抗运铁蛋白受体抗体 (Invitrogen) 温育 1 小时，用 TBS-T 洗涤，与 1:5000 抗小鼠 HRP(GE Healthcare) 温育 1 小时，用 TBS-T 洗涤并用 ECL-Plus 进行可视化。使用抗 LDLR 抗体的蛋白印迹显示用 30μg 的 PCSK9 处理 1 小时显著地下调小鼠肝中的 LDLR 水平。见图 6。

[0430] 实施例 6 :抗体防止肝 LDLR 下调

[0431] 用赋形剂或 5mg/kg 抗 PCSK9 抗体注射正常的 C57/BL6 小鼠 (Charles River)，24 小时后用 30μg PCSK9 处理 1 小时。使用 PROTEOEXTRACT® 天然膜蛋白提取试剂盒 (Calbiochem) 根据制造商的说明收获来自每只小鼠的肝脏。通过 SDS-PAGE 在 8％ bis-tris 凝胶上分析溶胞产物。使用 IBLOT® (Invitrogen) 将蛋白转移至硝酸纤维膜。将所述膜用 5％脱脂奶封闭 1 小时，然后与 1:500 抗 LDLR(Abcam) 在 5％脱脂奶中在 4℃过夜温育。次日，将所述膜用 TBS-T 洗涤三次，与 1:5000 抗兔 HRP(GE Healthcare) 温育 1 小时，并用 TBS-T 洗涤三次。使用 ECL-Plus(GE Healthcare) 并暴露于 XAR 胶片 (KODAK®) 来使蛋白可视。使用抗 LDLR 抗体的蛋白印迹显示所有五种抗 PCSK9 抗体 (508.20.84，508.20.33b，508.20.04b，508.20.28b，508.20.06) 都防止小鼠肝中的 LDLR 下调。见图 7。

[0432] 实施例 7 :抗 PCSK9 抗体的药物代谢动力学

[0433] 使用抗人 IgG Fc ELISA 来测定在小鼠 PK 研究样品中的抗 PCSK9 抗体浓度。简言之，将驴抗人 IgG Fc(Jackson ImmunoResearch，West Grove，PA) 用于包被测定平板，并将山羊抗人 IgG Fc HRP 缀合物 (Jackson ImmunoResearch，West Grove，PA) 用作检测抗体。该测定能够测量在小鼠血清基质中多达 10％的抗 PCSK9 抗体，测定范围为 0.31-20ng/mL。见图 8 和 9。

[0434] 通过抗 PCSK9 抗体 ELISA 使用重组人 PCSK9(Genentech，Inc.South San Francisco，CA) 作为捕获抗体而山羊抗人 IgG(H+L)HRP 作为检测抗体来测定食蟹猴 PK 研究样品中的血清抗 PCSK9 抗体浓度。该测定能够测量在食蟹猴血清基质中多达 2％的抗 PCSK9 抗体，并且测定范围为 0.313-50ng/mL。参见图 10 和 11。

[0435] 实施例 8 :抗体降低小鼠中的血清胆固醇水平

[0436] 八周龄雄性 C57BL/6J 小鼠商购自 Jackson Laboratory。将小鼠在保藏室中圈养 (on housing) 达一周之后开始实验。在麻醉下对所有小鼠进行预放血并使用 INFINITY™ 胆固醇试剂 (INFINITY™ Cholesterol Reagent)(Fisher Diagnostics，Middletown，VA) 测定小鼠的总胆固醇水平。将小鼠随机分成 6 个不同的具有相同水平的平均胆固醇水平的组。所有小鼠接受单剂量 10mg/kg 体重的对照抗体或抗 PCSK9 抗体。在第 3 天、第 7 天、

第 10 天和第 15 天对小鼠进行放血，并使用 INFINITY™ 胆固醇试剂 (Fisher Diagnostics, Middletown, VA) 测定血清总胆固醇水平。

[0437] 当施用 10mg/kg 的单剂量时，所有五种抗 PCSK9 抗体 (508.20.04b, 508.20.06, 508.20.28b, 508.20.33b, 508.20.84) 都显示总胆固醇水平的下降。在第 3 天和多至第 10 天，当与接受对照抗体的小鼠比较时，抗 PCSK9 抗体的给药导致总胆固醇水平的显著下降。见图 12。

[0438] 实施例 9 :他汀类疗效的增强

[0439] 本实验证明 :与单独的抗 PCSK9 抗体治疗或单独的他汀类治疗相比，抗 PCSK9 抗体和他汀类的组合导致总胆固醇水平更大的下降。见例如，图 13。八周龄雄性 C57BL/6J 小鼠购自 Jackson Laboratory。将小鼠分成 2 个不同的组。无他汀类小鼠接受对照饮食，而他汀类组在抗体施用前接受饮食中的 0.2％洛伐他汀 (Bioserve, Frenchtown, NJ) 2 周。对所有小鼠进行预放血，并且基于相等的平均胆固醇水平对小鼠进行随机化。在第 3 天对小鼠进行放血，并且使用 INFINITY™ 胆固醇试剂 (Fisher Diagnostics, Middletown, VA) 测定总胆固醇水平。

[0440] 抗 PCSK9 抗体显示显著的降胆固醇效果。与无他汀类组相比，单独的他汀类治疗导致总胆固醇水平的适度下降。与单独的抗 PCSK9 相比，他汀类加抗 PCSK9 抗体的组合导致总胆固醇水平的额外下降。见图 13。

[0441] 实施例 10 :与抗 PCSK9 抗体的 Fab 片段结合的 PCSK9 的 X 射线晶体结构

[0442] 蛋白纯化和结晶

[0443] 将来自 10L 大肠杆菌 (E.coli) 表达的 210g 冷冻的细胞糊在 1L 裂解缓冲液 (PBS/25mM EDTA/1mM PMSF) 中解冻。通过 Tissuemizer(30 秒) 使细胞破裂，并将所得的浆液两次通过微流体化机。通过离心沉淀不溶的物质。将澄清的细胞裂解液 (一次 250mL) 以 5mL/min 加样到蛋白 G 柱 (货号 17-0618-05, GE Healthcare) 上。然后用 100mL 的裂解缓冲液对所述柱进行洗涤，之后用 150mL 的洗脱缓冲液 (0.58％乙酸) 洗脱结合的抗 PCSK9 抗体的 Fab 片段。在洗脱期间收集 25mL 级分。在 SDS PAGE 分析后，汇集含有抗 PCSK9 抗体的 Fab 片段的级分。

[0444] 用 50ml 缓冲液 A(20mM MES pH5.5) 平衡 5mL 预装的 SPHP 柱 (GE Healthcare, 货号 17-1152-01)。以 3mL/min 将来自前一步的汇集的级分加样到柱上。用缓冲液 A 将该柱洗涤至基线。使用 20 个柱体积的缓冲液 B(20mM MES pH 5.5, 1M NaCl) 使用从 0％至 100％缓冲液 B 的梯度洗脱结合的 Fab 片段。在洗脱期间收集 2mL 级分。汇集含有所述蛋白的级分 (使用 SDS-PAGE 确定), 并且浓缩至 5mL, 之后加样到已经用分选缓冲液 (sizing buffer) (20mM Hepes 7.2, 150mM NaCl) 预平衡的 320mL S75 凝胶过滤柱上。以 1.5mL/min 连续运行分选缓冲液 220 分钟，同时收集 2mL 级分。使用 SDS-PAGE 分析峰级分 (A280)。

[0445] 使用标准分子生物学技术，将含有组氨酸 $(His)_8$C 端标签 (SEQ ID NO:32) 的人 PCSK9(Genbank EF692496) 互补脱氧核糖核酸 (cDNA) 插入到带有巨细胞病毒 (CMV) 启动子的哺乳动物表达载体 (pRK5) 中。通过瞬时转染中国仓鼠卵巢 (CHO) 细胞来表达蛋白，并且利用在镍 - 氮基三乙酸 - 琼脂糖柱 (Qiagen) 上的亲和层析以及之后在Sephacryl® S-200 柱 (GE Healthcare) 上的凝胶过滤从条件培养基中进行纯化。纯化的蛋白的正确的质量通过十二烷基硫酸钠聚丙烯酰胺凝胶电泳 (SDS-PAGE) 证实，并且氨基酸序列的准确性通过 N

端测序确认。

[0446] 将纯化的抗 PCSK9 抗体的 Fab 片段和 6.9mg 的 PCSK9 蛋白在 2 倍摩尔过量的 Fab 片段中混合，并在 4℃温育 1 小时，之后浓缩至 5mL。然后将浓缩的混合物上样到用分选缓冲液预平衡的 Superdex 200 尺寸排阻柱（货号 17-1071-01，GE Healthcare）上。以 1.5mL/min 连续运行分选缓冲液 220 分钟，同时收集 2mL 级分。汇集含有 PCSK9 和抗 PCSK9 抗体的 Fab 片段两者 (SDS-PAGE) 的峰级分 (A280)，并浓缩至 20mg/mL。将浓缩的复合物用于进行结晶尝试。由蛋白和池液 (reservoir)（池液含有 1.3M 磷酸钾 / 钠，pH 7）之间 1:1 混合物，使用坐滴，形成最初的晶体。通过改变悬滴中蛋白：池液比来优化晶体。用补充以 25％甘油的母液处理选择的晶体，并保存在液氮中。

[0447] PCSK9：抗 PCSK9 抗体的 Fab 片段复合物的结构测定

[0448] 在同步加速器束线 SSRL 7-1 收集扩展至约 3.5 Å 分辨率的衍射数据，并进行积分，并在空间群 I222 中换算 (scaled)。通过分子置换的方法，使用之前报道的 PCSK9 的结构 (Hampton 等，PNAS 104:14609-9(2007)，pdb 登记号 2QTW) 和之前报道的抗体 Fv 片段的结构 (Eigenbrot 等，J Mol Biol229:969-95(1993)，pdb 登记号 1FVC) 获得近似相位。在部分置换已经改善相位后，使用之前报道的同源结构 (Eigenbrot 等，同上，pdb 登记号 1FVD) 的部分，将抗 PCSK9 抗体的 Fab 片段的恒定区作为刚体放置。最后精修的结构的晶体学 R- 值为 25&30％。数据收集和修正统计数据显示在以下表 1 中。

[0449] 表 1.

[0450] 数据收集

[0451]

空间群	I222
晶胞(Å,°)	***a***= 92.283，***b***= 142.523，***c***= 253.983
V_M (Å^3/道尔顿)	2.8
分辨率(Å)	40 – 3.5 (3.63 – 3.50)
Rsym[a,b]	0.184 (0.807)
观测的数目	157526
独特反射	21579
完整度(%)[b]	100 (100)
I/σI[b]	11 (2.6)
Wilson B (Å^2)	58

[0452] 精修

[0453]

分辨率(Å)	40 – 3.5
反射的数目 (F>0σ(F))	20644
最终 R^c，R_{FREE}	0.247，0.295
复合物/不对称单元	1
蛋白残基	994
溶剂分子	0
原子	7463
平均 B 因子($Å^2$)	86
Rmsd 键(Å)	0.007
Rmsd 角(°)	1.1
Rmsd 成键的 B ($Å^2$)	2.4/1.9
TLS 群的数目	4
Ramachandran (%)	81.5/16.8/0.6/1.1

[0454] [a]Rsym = Σ||I|-|<I>||/Σ|<I>|，其中 I 是单次观察的强度，而 <I> 是对称等效观察的平均强度。

[0455] [b] 括号中是针对最高分辨率壳层。

[0456] [c]R = Σ|FO-Fc|/Σ|Fo，其中 Fo 和 Fc 分别是观察的和计算的结构因子振幅。R_{FREE} 被计算为不包括在精修中的反射的 R。

[0457] 从 X 射线结构确定 PCSK9 上的表位

[0458] 使用分子分析程序 PyMOL，使用4 Å标准。在抗 PCSK9 抗体的 Fab 片段的任何部分的4 Å内的 PCSK9 残基被确定为表位。基于该分析，表位包含以下残基中的一个或多个：人 PCSK9 的 R194、E195、D238、A239、A341、Q342、E366、D367、I369、S376、T377、C378、F379、S381 和 H391。

[0459] 实施例 11：人临床试验，单个和多个递增剂量

[0460] 首先进行随机的，双盲的，安慰剂对照的，单剂量和多剂量研究以评估通过向具有升高的血清低密度脂蛋白胆固醇 (LDL-c) 浓度的健康志愿者皮下 (SC) 注射施用的单剂量和多（每周四次）剂量的研究药物（重构为人 IgG_1 的 YW508.20.33b，其具有 SEQ ID NO:35 的重链和 SEQ ID NO:36 的轻链）的安全性和耐受性。将 80 名具有升高的血清 LDL-c 浓度 (130-220mg/dL) 的健康成年志愿者（男性和女性）随机分为 10 个群组，每个群组包含 8 名受试者。将各个群组中的受试者随机接受研究药物或安慰剂之一（每个群组 6 名活性剂受试者和 2 名安慰剂受试者）。

[0461] 如图 14 和表 2 中所示对所述群组剂量给药。使用注射器典型地在腹部或大腿皮下给药所有剂量。将药品配制为溶于 200mM 精氨酸琥珀酸盐，0.02％聚山梨酯 20 的 150mg/mL 抗体，pH 5.5。对于多剂量群组，研究药物每周施用一次，连续四周。他汀类群组 (H 和

I)，最初以一天一次 20mg 口服施用阿托伐他汀 (atorvastatin) 至少 7 天，随后进行安全性和耐受性评价。如果 20mg 剂量被良好耐受，将剂量增加至每天 40mg 并持续最少 21 天，之后起始第 1 天的研究药物。群组 H 和 I 中的受试者继续用阿托伐他汀 (atorvastatin)（每日 40mg PO) 直到并包括第 35 天。在研究过程中的任何点，对于直接 LDL-c 水平降低至低于 25mg/dL 的任何受试者，终止治疗。

[0462] 表 2. 研究剂量群组概览。

[0463]

群组	剂量 (mg)	施用的总剂量	随访持续时间 [a]	阿托伐他汀 (Atorvastatin)
A	10	1	8 周	否
B	40	1	8 周	否
C	150	1	12 周	否
D	300	1	12 周	否
E	600	1	16 周	否
F	40	4	16 周	否
G	150	4	16 周	否
H	40	4	16 周	是
I	150	4	16 周	是
J	800	1	16 周	否

[0464] a ＝研究药物的第一次剂量给药和最后研究随访之间的时间。

[0465] 在研究药物起始后对受试者进行 8 至 16 周的有关频率安全性，PK 和 PD 评价的跟踪。评估以下数据：安全性结果（不良事件，血液学异常，临床化学，和尿分析，和抗－治疗性抗体发生率），药物代谢动力学 (PK) 属性（包括 C_{max}，总血清表观清除 (CL/F)，表观容积分布 (V/F)，总暴露 (AUC)，t_{max}，$t_{1/2}$，和剂量比例性（基于 AUC)），药效学结果（单剂量群组中第 15 天和多剂量群组中第 36 天 LDL-c 从基线的百分比和绝对降低），和总胆固醇，LDL-c，HDL-c，非 HDL-c，甘油三酯，和脂质颗粒亚级分随时间从基线的百分比和绝对数值变化。

[0466] 研究的早期结果尚未鉴定出不良事件的药物相关的，临床上重要的模式。无严重的或剧烈的不良事件，对于不良事件不停药，并且无剂量限制的毒性。测试的剂量未明确最大耐受剂量。报道了两个中等不良事件：一个是头痛 (10-mg 单剂量群组中用研究药物治疗的受试者）和一个是桡骨骨折 (600-mg 单剂量群组中用研究药物治疗的受试者）。因为 LDL-c 水平低于方案规定的 25mg/dL 的阈值，所以五个用研究药物治疗的受试者（都属于多剂量群组和都伴随以阿托伐他汀 (atorvastatin) 治疗），中止研究药物治疗。在这些受试者中无相关不良事件。

[0467] 如图 15（左图）中所示，对于研究药物从 10-600mg 存在暴露的剂量相关的增加。

他汀类治疗和未治疗组之间未观察到PK的差异（图15，右图）。存在研究药物的可饱和清除，Km为5.94ug/mL。

[0468] 如在图16-19和表3和4中所示的，研究药物单独和与他汀类组合，在健康志愿者中产生临床上有意义的LDL-c降低。药效(PD)数据显示剂量依赖性的LDL-c降低，其在除了10mg单剂量群组的所有群组中均是统计显著的。在最高剂量组（单剂量群组中，300－800mg）中，LDL-c从160－170mg/dL的平均基线LDL-c降低80－90mg/dL。在阿托伐他汀(atorvastatin)（群组H和I）和非他汀类群组（群组F和G）之间观察到LDL-c水平的类似降低（见图18和19和表3和4）。群组I和G（在第10天）和F和H（在第36天）之间的差异统计学上不显著。如在图16和17中所示的，在≥300mg的剂量，最大LDL-c效果似乎饱和，而效果的持续时间延长。该数据支持每个月或更低频率剂量给药。

[0469] 表3. 在单剂量和多剂量群组中LDL-c水平从基线的绝对数值变化。

[0470]

	LDL的平均(SD)变化 (mg/dL)		
组	活性剂	安慰剂	P-值[a]
单剂量(第15天)			
A (10 mg)	-18 (21)	-5.6 (15)	0.22
B (40 mg)	-45 (32)		0.03
C (150 mg)	-61 (17)		<0.001
D (300 mg)	-88 (28)		<0.001
E (600 mg)	-82 (22)		<0.001
J (800 mg)	-91 (14)		<0.001
多剂量 (第36天)			
F (40 mg x 4)	-50 (28)	-9.7 (13)	0.016
G (150 mg x 4)	-71 (26)		0.001

[0471]

H (A^b + 40 mg x 4)	-38 (10)	-5 (14)	0.009
I (A^b + 150 mg x 4)	N/A^c	-15 (21)	N/A^c

[0472] a＝群组I和G（第10天）和F和H（第36天）之间的差异统计学上不显著。b＝A是阿托伐他汀。c＝由于LDL水平降低至低于<25mg/dL方案阈值，群组I(150mg x 4+阿托伐他汀(Atorvastatin))中的多个受试者在第10天后中止。

[0473] 表4. 单剂量和多剂量群组中LDL-c水平从基线的百分比改变。

[0474]

	LDL 的平均 % (SD)改变		
组	活性剂	安慰剂	P-值
单剂量(第 15 天)			
A (10 mg)	-9.4 (11)	-3.7 (10)	0.3
B (40 mg)	-23 (12)		0.008
C (150 mg)	-37 (11)		<0.001
D (300 mg)	-53 (10)		<0.001
E (600 mg)	-51 (18)		<0.001
J (800 mg)	-58 (4)		<0.001
多剂量 (第 36 天)			
F (40 mg x 4)	-34 (19)	-6.2 (8)	0.016
G (150 mg x 4)	-49 (10)		0.001
H (A^a + 40 mg x 4)	-48 (17)	-5.7 (16)	0.005
I (A^a + 150 mg x 4) (第 10 天)	-65 (13)	-12 (24)	0.014

[0475] a = A 是阿托伐他汀。

[0476] 实施例 12 :患有冠心病 (CHD) 或具有高 CHD 风险的患者中的人临床试验

[0477] 将进行研究药物 (重构于人 IgG_1 中的 YW508.20.33b,其具有 SEQ ID NO:35 的重链和 SEQ ID NO:36 的轻链) 的随机、双盲、安慰剂对照的研究以评估在具有 90-250mg/dL 的 LDL-c 水平和患有冠心病 (CHD) 或 CHD 风险替代症之一的患者中,在标准护理 (SOC) 他汀类之上,研究药物的安全性和有效性。将具有 90-250mg/dL 的 LDL-c 浓度和患有 CHD 或 CHD 风险替代症之一的大约 224 名患者 (成年男性和女性) 随机分到要施用研究药物的五个研究组或安慰剂组中的一个 , 如下文表 5 中列出的。所有剂量将使用注射器皮下给药。将药品配制为溶于 200mM 精氨酸琥珀酸盐 , 0.02％聚山梨酯 20 的 150mg/mL 抗体 , pH 5.5。

[0478] 表 5. 研究剂量群组的概览。

[0479]

	研究药物剂量方案		计划的患者数量	
组	剂量 (mg)	频率 (周)	活性药物	安慰剂
A	400	4	56	--
B	200	8	14	--
C	400	8	28	--
D	800	8	56	--
E	800	12	14	--
F	安慰剂	--	--	56
(A-F) 总	--	--	168	56

[0480] 所述研究将包括用于筛查 (0-4 周), 准备 (run-in)(0-6 周, 如果需要), 治疗 (24 周 ;天 1 - 169), 和随访 (12 周) 的连续期。随访期 (第 253 天) 最后的研究完成访问将在研究药物的最终剂量给药 (第 141 天) 之后 16 周发生。所有患者, 不论治疗任务, 将接受 SOC 治疗, 包括他汀类 (除非他汀类不耐受)。在整个治疗和随访期,所有患者将以他们在准备期和在招募时 (at enrollment) 接受的相同剂量继续 SOC 他汀类治疗。不容许其它处方和非处方 (OTC) 调脂治疗。在筛查时期接受稳定剂量 SOC 他汀类治疗 (或无他汀类并对于两种以上他汀类具有有记载的不耐受) 并未接受其它调脂治疗达至少 4 周 (或在贝特类药物 (fibrates) 的情况下 6 周) 的患者将不需要准备期。

[0481] 将监测患者以基于在第 169 天 LDL-c 浓度从基线的绝对数值变化确定疗效。此外, 将监测患者以确定次要疗效结果,包括对于每组在该组的最低点 LDL-c 浓度从基线的绝对数值变化 ;对于每组的 LDL-c 的变化 (绝对和百分比变化) 随时间的平均值 (直到第 169 天, 通过连续的 LDL-c 测量之间的周数加权);在第 169 天和在每组的最低点的 LDL-c 浓度从基线的百分比变化 ;在所有其它指定的时间点 LDL-c 浓度从基线的百分比和绝对数值变化 ;和在第 169 天和在各组最低点总胆固醇, 非 HDL-c, 和载脂蛋白 B 从基线的百分比和绝对数值变化。最后, 还将监测患者的安全性,包括不良事件的发生率、性质和严重度 ; 在研究药物施用期间和之后生命体征, 身体研究结果, 和临床实验结果方面变化的发生率和性质 ;和针对研究药物的抗 - 治疗抗体的发生率。

[0482] 将以不知情 (blinded)、探索性的方式定期评估低 LDL-c 值的安全性。将拒绝给予具有两个连续的 <15mg/dL 的 LDL-c 值的患者研究药物。这将不被认为是不良事件。此种患者将以不知情的方式用安慰剂替代治疗, 直到 LDL-c 增加至≥ 50mg/dL, 这之后患者将转变为最低剂量 (200mg 每 8 周)。将根据研究药物施用时间表给予所有剂量的活性药物或安慰剂, 即, 仅在 1,29 天 (±2 天),57 天 (±2 天),85 天 (±2 天),113 天 (±4 天), 和 141 天 (±4 天)。

[0483] 主要的疗效结果是在第 169 天 LDL-c 从基线的变化。基线 LDL-c 被定义为研究药物的第一次剂量给药之前收集的最后两次测量值的平均值。研究药物剂量之间和各个研究药物剂量和安慰剂之间的治疗比较将基于协方差 (ANCOVA) 的分析, 其将通过调整以下两个协变量的线性回归模型进行 :基线 LDL-c 浓度 (<120mg/dL, ≥ 120mg/dL) 和糖尿病状态

（是，否）。ANCOVA 模型的置信区间，以及最小平方评估，将用于辅助解释研究结果。

[0484] 基于欧洲心脏病学会 (European Society of Cardiology)(ESC)/ 欧洲动脉粥样硬化学会 (European Atherosclerosis Society)(EAS) 和国家胆固醇教育计划成人治疗小组 (National Cholesterol Education Program Adult Treatment Panel)III(NCEP ATP III) 降脂指导原则中的风险类别，资格标准限定具有高心血管和 CHD 风险的患者群体。研究致力于招收具有根据这些指导原则的 70mg/dL 的治疗目标 LDL-c 水平的资格，但因为 SOC 不充足或因为他汀类不耐受而造成的尽管接受 SOC 他汀类治疗尚未接近该目标的患者。这些患者需要额外的安全和有效的降 LDL-c 治疗。

[0485] CHD 是指有记载的心肌梗死史，之前的冠状动脉重建术过程（经皮冠状动脉介入或冠状动脉旁路搭桥）史，或之前的冠状动脉血管造影术（侵入性冠状动脉血管造影术或心脏计算机断层扫描冠状动脉血管造影术）史，其显示至少一种具有≥ 50％直径狭窄的冠状动脉粥样硬化病变。

[0486] CHD 风险替代病症是以下各项中的至少一种：

[0487] 1. 临床动脉粥样硬化疾病的一种以上形式：

[0488] a. 外周动脉疾病（在前有记载的踝 / 上臂血压指数 <0.85，之前的经皮或外科手术外周动脉血管重建步骤，之前的由于外周动脉疾病导致的下肢非外伤性截肢，或之前血管成像上≥ 50％直径狭窄），

[0489] b. 颈动脉疾病（在前有记载的颈动脉动脉粥样硬化病变，成像时具有≥ 50％直径狭窄或之前的皮肤或外科手术颈动脉血管重建步骤），

[0490] c. 之前的缺血性卒中，通过 CT 或 MRI 脑成像记载，其在研究者看来不由心源性栓塞（例如，心房颤动，瓣膜病，或左心室附壁血栓）引起，或

[0491] d. 以之前的外科手术或血管内修复的腹主动脉瘤。

[0492] 2.2 型糖尿病，

[0493] 3. 伴有目标器官损伤（如由研究者确定的视网膜病，神经病，或肾病（包括微白蛋白尿））的 1 型糖尿病，

[0494] 4. 中度到严重肾病（通过 15-60mL/min/1.73m^2 的估算的肾小球过滤速率，持续经至少三个测量间隔至少 3 个月（包括筛查实验室）使用肾病中饮食调整 (Modification of Diet in Renal Disease) 方程显示），或

[0495] 5. 下文列出的 CHD 风险因素中的两种以上并且 CHD 事件的绝对 10- 年风险≥ 20％（如通过 Framingham 风险评分的国家胆固醇教育计划成人治疗小组 III 指导原则修改确定）或者第一致命动脉粥样硬化事件的 10- 年风险≥ 10％（如通过全身性冠心病的风险评估系统确定）：

[0496] a. 对于男性年龄≥ 45 岁或对于女性年龄≥ 55，

[0497] b. 最近吸烟 (1 个月内），

[0498] c. 高血压（筛查收缩压≥ 140mmHg，舒张压≥ 90mmHg，或服用抗高血压药物以治疗高血压）

[0499] d. 低 HDL 胆固醇 (<40mg/dL)，或

[0500] e. 过早 CHD 的家族史（在一级亲属 <55 岁年龄的男性或一级亲属 <65 年龄的女性中的心肌梗死或冠状动脉重建）。

[0501] 护理标准他汀类治疗是指满足一个以下条件的治疗:(1) 高剂量辛伐他汀(simvastatin)(40mg 每日),阿托伐他汀 (atorvastatin)(40–80mg 每日),或罗舒伐他汀 (rosuvastatin)(20–40mg 每日),(2) 低剂量辛伐他汀 (simvastatin),阿托伐他汀 (atorvastatin),或罗舒伐他汀 (rosuvastatin) 和有记载地对高剂量的该他汀类或任何剂量的另一种他汀类不耐受,(3) 其他他汀类(任何剂量)和有记载的对辛伐他汀(simvastatin),阿托伐他汀 (atorvastatin),或罗舒伐他汀 (rosuvastatin)(任何剂量)的不耐受,或 (4) 无他汀类和有记载的对至少两种他汀类(任何他汀类,任何剂量)不耐受。

[0502] 将根据患者医疗记录或历史,或根据筛查实验室测试结果,基于以下各项中的任一项的存在确定糖尿病状态:(1)HbA_{1c}>6.5%,(2) 空腹血糖≥ 126mg/dL(7.0mmol/L),(3) 在口服葡萄糖耐量试验过程中,之前 2- 小时血糖≥ 200mg/dL(11.1mmol/L)(应该如由世界卫生组织描述的,使用含有溶于水的 75g 无水葡萄糖的当量的葡萄糖载量,进行该试验),或 (4) 目前接受对于糖尿病的诊断结果的口服或可注射治疗。

[0503] 实施例 13:稳定,高浓度抗体制剂的开发

[0504] 使用抗 -PCSK9 抗体制剂(重构于人 IgG_1 中的 YW508.20.33b,其具有 SEQ ID NO:35 的重链和 SEQ ID NO:36 的轻链)进行最初临床研究(参见实施例 11 和 12),所述抗体被配制为溶于 200mM 精氨酸琥珀酸盐,0.02% (w/v) 聚山梨酯 20,pH 5.5 的 150mg/mL 抗体。然而,需要具有更高蛋白浓度(≥ 200mg/mL) 和增加的稳定性的制剂以利于施用可以每个月或更低频率递送的更高的皮下剂量。

[0505] 抗 -PCSK9 制剂的粘度

[0506] 在不同蛋白浓度评估 200mM 精氨酸琥珀酸盐,0.02% (w/v)PS20,pH5.5 抗 -PCSK9 制剂的粘度。在各个蛋白浓度,使用具有 10001/s 的剪切速率的流变仪 (Anton Paar Physica MCR 501) 在 5,15,25 和 40℃测量粘度。

[0507] 粘度是皮下剂量给药药物溶液的重要参数。对于使用注射器皮下递送的所需粘度限度是在环境温度 <10cP。溶于 200mM 精氨酸琥珀酸盐,0.02% (w/v)PS20,pH 5.5 的 100 至 300mg/mL 的抗 -PCSK9 的粘度显示在表 6 中。对于抗 -PCSK9, 粘度是蛋白浓度和温度依赖的。随蛋白浓度增加,粘度也增加。然而,在各个浓度,可以通过增加温度降低粘度。通过增加蛋白浓度超过 200mg/mL, 抗 -PCSK9 的粘度成倍增加(图 20)。因此,200mg/mL 的抗 -PCSK9 被选为目标浓度。

[0508] 表 6.100 至 300mg/mL 抗体浓度的抗 -PCSK9 的粘度。

[0509]

温 度 (°C)	粘度 (cP)[1]						
	100 mg/mL	150 mg/mL	200 mg/mL	225 mg/mL	250 mg/mL	275 mg/mL	300 mg/mL
5	4.6 ± 0.27	8.0 ± 0.16	18.6 ± 0.17	50.2 ± 0.99	76.9 ± 0.83	306 ± 5.8	603 ± 9.2
15	3.1 ± 0.10	5.2 ± 0.08	11.7 ± 0.15	31.9 ± 0.33	46.7 ± 0.91	179 ± 2.8	357 ± 4.8
25	2.5 ± 0.06	3.8 ± 0.09	8.3 ± 0.13	22.6 ± 0.14	31.0 ± 0.84	115 ± 0.6	225 ± 4.1
40	1.8 ± 0.01	2.7 ± 0.10	5.7 ± 0.26	15.5 ± 0.2	18.8 ± 0.63	74.8 ± 4.3	135 ± 3.2

[0510] [1]200mM 精氨酸琥珀酸盐，0.02％ PS20，pH 5.5。

[0511] 搅拌研究

[0512] 进行搅拌研究以评估阻止或最小化溶于 200mM 精氨酸琥珀酸盐，pH5.5 的 150mg/mL 抗 -PCSK9 聚集所需的表面活性剂的最小量。将聚山梨酯 20(PS20) 添加至所述制剂以获得 0.01,0.02,0.04,0.06,0.08 和 0.1％ (w/v) 的浓度。将所有样品无菌过滤，并且将 0.5mL 的各个样品装入 2-cc 玻璃小瓶。使用设置为 50 个循环 / 分钟 Glas-Col 台式摇床以 11cm 的样品位移将样品在室温 (RT) 搅拌 24 小时。将在相应配置下的合适的样品对照（无震荡）置于摇床的相同的附近。通过尺寸排阻层析分析 (SEC) 分析所有样品并通过在 340-360nm 吸光度 (abs) 的 UV 测量分析浊度。

[0513] 结果显示于图 21。在制剂中无 PS20 的情况下，当与未震荡的对照小瓶相比时，搅拌 24 小时的样品（在室温）具有明显的可见变化。搅拌的样品具有乳白色外管，浊度增加并且 SEC 主峰降低 6％。将≥ 0.01％ PS20 加入至制剂，通过 SEC 和浊度测量未在小瓶中的对照（无搅拌）和搅拌的样品之间观察到差异。这些结果提示使用 0.01％ PS20 足以阻止搅拌诱导的 200mg/mL 抗 -PCSK9 的聚集形成。然而，选择 0.02％ PS20 的浓度作为目标浓度以在产品储存过程中负责可能的表面活性剂降解。

[0514] 抗 -PCSK9 制剂的氧化可能

[0515] 通过胰蛋白酶 - 肽图确定抗 -PCSK9 的氧化并且通过 LC-MS 表征氧化位点。抗 -PCSK9 的氧化由升高的温度，光和氧化剂比如过氧化氢和 2,2'- 偶氮双 (2- 脒基丙烷）二盐酸盐 (AAPH) 引起。用于制备氧化的样品的降解条件总结于表 7。还通过测量其抑制 PCSK9 结合低密度脂蛋白受体结构域 Fc(LDLR$_D$-Fc) 融合蛋白的能力评估这些氧化的抗 -PCSK9 样品由于氧化造成的可能的效力损失，如实施例 3 所描述。

[0516] 对于肽制图，用 1M 二流苏糖醇还原样品，以 2.9M 碘乙酰胺烷基化，并且在消化前替换缓冲液。利用 1 :25 酶对蛋白比例使用胰蛋白酶在 37℃消化 1.5 小时。以 10％三氟乙酸 (TFA) 终止消化至最终 pH 2-3。通过反相液相层析分析产生的肽消化混合物，通过具有 LTQ Orbitrap XL 的质谱检测 (LC-MS)。与维持在 55℃的 Phenomenex Jupiter C18 柱 (5μm,2x250mm, 300Å) 一起，肽图使用以 0.25mL/min 经 160 分钟的 0-40％线性梯度。移动相 A 和 B 分别由溶于水的 0.1％ TFA 和溶于乙腈的 0.09％ TFA 组成。在 MS 分析前还检测肽在 214 和 280nm 处的 abs。通过 Mascot 软件处理 LC-MS 数据以鉴定肽和抗 -PCSK9 的各自氧化位点。样品中氧化的量被表达为“每个位点的总氧化”或积累的氧化，因为 Trp 和 Met 产生多个氧化产物和 / 或氧化状态。

[0517] 蛋氨酸 (Met/M) 和色氨酸 (Trp/W) 是两个常见的在蛋白药品中容易被氧化的氨基酸残基。位于重链的互补决定区 (CDR)III 中的 W_{99} 和 M_{108} 和邻近 CDR 的三个 Trp 残基 (W_{36}, W_{111} 和 W_{486}) 是抗 -PCSK9 可能的氧化位点。由于其接近 CDRs，这些氨基酸残基的氧化可以导致药物效力的损失。降解的样品的肽制图分析揭示抗 -PCSK9 的氧化主要发生在 Fc 部分的 M_{256}, M_{362}, M_{432} 和 M_{455} 残基处 (图 22)。当抗 -PCSK9 通过暴露于光 (室或 UV) 和氧化剂比如 H_2O_2 和 AAPH 而降解时，对于 CDR 中的或邻近 CDR 的 Met 或 Trp 残基，每个位点的相对氧化量小于 3%，对效力无显著影响 (图 23)。因此，抗 -PSCK9 被认为不对氧化不敏感并且在蛋白制剂中不需要使用抗氧化剂。

[0518] 表 7. 用于氧化分析的抗 -PCSK9 降解条件。

[0519]

降解模式	暴露条件	预期的降解
热	2 周 @ 40°C	氧化
光	室内灯 24 小时	光氧化
	120 万勒时	
氧化剂	1000 ppM H_2O_2 (24 小时 @ 5°C)	蛋氨酸氧化
	5 mM AAPH (24 小时 @ 40°C)	蛋氨酸 + 色氨酸氧化

[0520] pH 属性和赋形剂研究

[0521] 在 200mg/mL 的蛋白浓度评估制剂 pH 和赋形剂对抗 -PCSK9 的影响。对含有精氨酸琥珀酸盐，组氨酸 HCl 或组氨酸乙酸盐作为缓冲液种类和精氨酸 HCl 或精氨酸乙酸盐作为增溶剂的制剂中 5.0 至 6.5 的 pH 范围，评估在 40℃的加速 (accelerated) 稳定性 (参见表 9) 和在 5℃和 25℃的粘度 (参见表 8)。使用以下测定用于评价 :SEC, 离子交换层析 (IEC), 毛细管电泳 - 十二烷基硫酸钠 (CE-SDS) 和效力。评估总共七种制剂。

[0522] 在 Agilent 1100HPLC 上进行 IEC 并且以 0.9mL/min 的流速在 50 分钟内使用 Dionex ProPac™ WCX-10 柱 (4x 250mm)，使用流动相 A(20mM HEPES,pH 7.9) 和梯度从 1% -34%的流动相 B(20mM HEPES,100mM NaCl,pH 7.9)。将柱维持在 35℃。样品载量为 40μg, 并且在 280nm abs 监测分离。

[0523] pH 的影响

[0524] 从 pH 5.0,5.5 和 6.0 评估 pH 对溶于 200mM 精氨酸琥珀酸盐，0.02% PS20 的 200mg/mL 抗 -PCSK9 的稳定性的影响。如通过 SEC,IEC 和 CE-SDS 分析的，将制剂 pH 从 5.0 增加至 6.0 增加了在 40℃ 1 个月后抗 -PCSK9 的稳定性。如通过 IEC 确定的，与 pH 5.0 和 5.5 相比，pH 6.0 的制剂具有更少的酸性和碱性峰形成。pH 6.0 的制剂还具有高分子量种类 (HMWS)(如通过 SEC 确定) 和低分子量种类的减少 (通过 SEC 和 CE-SDS 二者确定)。对于 pH 6.5 的制剂，将抗 -PCSK9 配制为 200mg/mL 溶于 20mM 组氨酸 HCl,160mM 精氨酸 HCl, 和 0.02% PS20。对于所有制剂，抗 -PCSK9 在 40℃在 pH 5.0 至 6.5 的降解率显示于表 9 并且对于 IEC 和 SEC 的 pH 速率曲线显示于图 24 中。基于 pH 速率曲线和降解率，选择目标

pH 6.0。

[0525] 表 8. 在不同制剂中 200mg/mL 抗 -PCSK9 的粘度。

[0526]

制剂	缓冲液	稳定剂/赋形剂	pH	粘度 (cP)	
				5°C	25°C
1	200 mM 精氨酸琥珀酸盐	0.02% PS20	5.0	18.2	7.7
2			5.5	**18.6**	**8.3**
3			6.0	16.4	7.9
4	20 mM 组氨酸 HCl	160 mM 精氨酸 HCl, 0.02% PS20	6.0	18.0	7.6
5			6.5	17.5	7.3
6	20 mM 组氨酸乙酸盐	160 mM 精氨酸乙酸盐, 0.02% PS20	5.5	16.4	7.7
7			6.0	15.9	7.6

[0527] 缓冲液种类的影响

[0528] 在含有以下三种缓冲体系的制剂中评估缓冲液种类对 pH 6.0 下 200mg/mL 抗 -PCSK9 的加速稳定性的影响 :(1)160mM 精氨酸琥珀酸盐 ,(2)20mM 组氨酸 HCl 和 160mM 精氨酸 HCl, 和 (3)20mM 组氨酸乙酸盐和 160mM 精氨酸乙酸盐。所有三种制剂含有 0.02% PS20。在 40℃ 1 个月之后 , 抗 -PCSK9 在三种缓冲体系之间具有相当的 CE-SDS 曲线 (图 26, 上图)。通过 SEC 在组氨酸 HCl/ 精氨酸 HCI 和组氨酸乙酸盐 / 精氨酸乙酸盐缓冲系统之间未观察到差异 , 而使用精氨酸琥珀酸盐缓冲液具有 HMWS 峰的稍微增加 (图 26, 中图)。通过 IEC 分析 , 当与组氨酸乙酸盐 / 精氨酸乙酸盐缓冲体系和精氨酸琥珀酸盐比较时,在制剂中使用组氨酸 HCI/ 精氨酸 HCl 缓冲体系具有更少的酸性峰形成 (图 26, 下图)。然而 , 通过 SEC,IEC 和 CE-SDS 确定的抗 -PCSK9 在 40℃的整体降解率与 pH 6.0 下的全部三个缓冲体系相当 (表 9)。

[0529] 表 9. 在不同制剂中 200mg/mL 抗 -PCK9 在 40℃的降解率。

[0530]

% 损失/月，在 40℃	200 mM 精氨酸琥珀酸盐			20 mM HisHCl, 160 mM ArgHCl		20 mM HisAce, 160 mM ArgAce	
	pH 5.0	pH 5.5	pH 6.0	pH 6.0	pH 6.5	pH 5.5	pH 6.0
SEC 主峰	3.8	2.7	2.2	2.1	2.9	2.1	2.1
IEC 主峰	33	22	19	15.9	18	20.9	19
CE-SDS 主峰	5.1	4.5	3.6	3.8	3.5	4.0	4.3

[0531] 还评估了两种制剂(组氨酸HCl,pH 6.0和组氨酸乙酸盐,pH 6.0)中的抗-PCSK9在1mL注射器中的稳定性。

[0532] 在5℃，两种制剂稳定达高达6个月(表10和11)。在加速和压力条件下，对于液体制剂中的抗-PCSK9,酸性变体的形成和聚集的形成是主要的降解途径。在30℃/65％相对湿度(RH)和40℃/75％ RH,如通过IEC确定的,在pH 6.0,蛋白在组氨酸乙酸盐比在组氨酸HCl中降解地更快(表11)。在相同储存条件下,对于任一制剂通过SEC和CE-SDS均未观察到聚集率的差异(表12)。当在5℃储存高达6个月时,对于两种首要制剂都未观察到氧化的增加。尽管在30℃/65％ RH 6个月后,在两种制剂中,存在Fc部分中Met256氧化的稍微增加(～2％),但是未观察到其它Met和Trp残基的氧化的增加。对于在5℃和30℃/65％ RH达6个月,在任一种制剂中都未观察到效力损失。使用2.25mL注射器获得类似结果。

[0533] 表10. 溶于20mM组氨酸HCl,160mM精氨酸HCl,0.02％ PS20,pH6.0的200mg/mL抗-PCSK9在1-mL注射器中的稳定性数据。

[0534]

			IEC			SEC				
温度℃/%RH	时间点天/月	强度mg/mL	% 酸性的	%主峰	% 碱性的	% HMWS	% 主峰	% LMWS	CE-SDS%主峰	效力%相对效力
NA	T = 0/0	209	11.6	73.1	15.1	0.7	99.2	0	96.1	114
5	28/1	210	11.9	73.5	14.5	0.6	99.3	0	96.2	101
5	61/2	206	11.7	72.9	15.3	0.6	99.3	0	96.0	100
5	91/3	208	11.6	73.3	15.0	0.6	99.3	0	96.0	101
5	183/6	208	12.4	71.9	15.6	0.7	99.2	0	95.3	103
30/65	28/1	210	15.0	69.5	15.3	0.7	99.1	0.1	95.6	NT
30/65	61/2	206	19.0	64.0	16.9	0.9	98.8	0.2	94.7	91
30/65	91/3	204	21.4	61.9	16.5	1.0	98.5	0.4	94.0	84
30/65	183/6	209	33.9	48.7	17.3	1.4	97.7	0.8	91.2	92
40/75	7/0.25	206	15.0	68.6	16.3	0.8	99.0	0.1	95.5	NT
40/75	14/0.5	206	18.9	64.3	16.7	0.9	98.8	0.2	95.0	NT
40/75	28/1	209	25.9	57.4	16.6	1.1	98.4	0.4	93.6	105

[0535] NT ＝未测试。

[0536] 表 11. 溶于 20mM 组氨酸乙酸盐，160mM 精氨酸乙酸盐，0.02％ PS20，pH 6.0 的 200mg/mL 抗 -PCSK9 在 1-mL 注射器中的稳定性数据。

[0537]

			IEC			SEC				
温度 (℃)	时间点 天/ 月	强度 mg/mL	% 酸性的	% 主峰	% 碱性的	% HMWS	% 主峰	% LMWS	% 主峰	% 相对效力
NA	T = 0/0	211	11.8	72.7	15.4	0.6	99.3	0	96.2	100
5	28/1	203	12.1	72.8	14.9	0.6	99.4	0	96.2	106
5	61/2	208	11.7	72.8	15.4	0.6	99.3	0	96.0	97
5	91/3	208	11.6	72.9	15.3	0.6	99.3	0	96.0	92
5	183/6	207	12.5	71.6	15.8	0.7	99.2	0	95.6	98
30/65	28/1	209	16.8	67.5	15.6	0.7	99.1	0.1	95.6	NT
30/65	61/2	210	21.6	61.8	16.4	0.9	98.8	0.2	94.7	98
30/65	91/3	205	26.1	57.4	16.3	1.0	98.6	0.3	94.3	87
30/65	183/6	205	41.4	42.5	16.0	1.5	97.6	0.8	91.1	91
40/75	7/0.25	206	17.0	66.6	16.2	0.8	99.0	0.1	95.4	NT
40/75	14/0.5	204	22.5	60.0	16.2	0.9	98.8	0.2	94.5	NT
40/75	28/1	196	31.8	51.9	16.1	1.1	98.4	0.4	93.5	106

[0538] NT ＝未测试。

[0539] 表 12. 在加速的稳定性条件下 1-mL 注射器中的抗 -PCSK9 的降解率。

[0540]

% 每个月的变化		组氨酸 HCl[1]	组氨酸乙酸盐[2]
IEC	30℃/65%RH	4.0	5.0
	40℃/75%RH	16.7	22.0
SEC	30℃/65%RH	0.2	0.2
	40℃/75%RH	0.8	0.9
CE-SDS	30℃/65%RH	0.8	0.8
	40℃/75%RH	2.6	2.8

[0541] [1] 组氨酸 HCl ＝ 200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸 HCl,160mM 精氨酸 HCl,0.02％ PS20,pH 6.0

[0542] [2] 组氨酸乙酸盐＝ 200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸乙酸盐 ,160mM 精氨酸乙酸盐 ,0.02％ PS20,pH 6.0

[0543] 冷冻稳定性

[0544] 将抗 -PCSK9 以 200mg/mL 配制在以下两种制剂中 :(1)20mM 组氨酸 HCl,160mM 精

氨酸 HCl,0.02％ PS20,pH 6.0;和 (2)20mM 组氨酸乙酸盐,160mM 精氨酸乙酸盐,0.02％ PS20,pH 6.0。对于每种制剂,将 20mL 的药物溶液装入 25-cc 316L 不锈钢小罐中。将所有小罐随后置于 -20℃高达 6 个月,以用于稳定性分析。

[0545] 对于在 -20℃达 6 个月的两种制剂,通过 IEC,CE-SDS 和效力都未观察到差异。然而,当与在相同储存条件下组氨酸乙酸盐制剂中聚集仅增加 0.5％相比,6 个月的冷冻储藏后,组氨酸 HCl 中聚集增加 1.4％(见图 25)。由于在冷冻储藏条件下组氨酸 HCl 制剂的更快速的聚集,选择组氨酸乙酸盐制剂作为优选的缓冲液。

[0546] 蔗糖对冷冻稳定性的影响

[0547] 评估在冷冻储藏过程中蔗糖对稳定抗 -PCSK9 的影响。使用实验室规模的装有 LCGC10 盒的 Millipore 正切流动过滤 (Tangential Flow Filtration)(TFF) 系统,在以下两种含蔗糖制剂中测试抗 -PCSK9:(1)200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸 HCl,130mM 精氨酸 HCl,60mM 蔗糖,0.02％ PS20(w/v),pH 6.0;和 (2)200mg/mL 抗 -PCSK9 溶于 20mM 组氨酸乙酸盐,100mM 精氨酸乙酸盐,60mM 蔗糖,0.02％ PS20(w/v),pH6.0。将两种制剂中的抗 -PCSK9 样品置于 -20℃高达 3 个月并通过 SEC 分析聚集。

[0548] 添加蔗糖 (60mM) 对降低组氨酸乙酸盐制剂中的 aPCSK9 聚集无影响,但其的确帮助减缓在 -20℃经 3 个月组氨酸 HCl 制剂中的聚集达 0.7％。然而,对于两种制剂,添加蔗糖还将在 25℃的粘度从 7-8cP 增加到 11-13cP,其是皮下制剂所不需要的。因此,未选择蔗糖作为制剂的稳定剂。

[0549] 基于上文描述的结果,选择由溶于 20mM 组氨酸乙酸盐,160mM 精氨酸乙酸盐,0.02％ PS20(w/v),pH 6.0 的 200mg/mL 抗 -PCSK9 组成的液体制剂。该制剂具有在 2-8℃和在 -20℃储藏的最佳稳定性和当与在 pH 5.5 的最初制剂相比时改善的稳定性。

[0550] 实施例 14:患有冠心病 (CHD) 或具有 CHD 高风险的患者中的人临床试验

[0551] 本实施例描述了 II 期临床研究并且图 27-37 显示至少 50％的患者在 12 周的中间结果。该研究招募了 248 名患者,包括用研究药物治疗的 183 患者和用安慰剂治疗的 64 名患者。一名患者在第一次治疗前退出并且 13 名患者在研究的第 85 天之前终止治疗。234 患者完成了至少 12 周的研究。

[0552] 进行～ 3:1 研究药物(重构于人 IgG_1 中的 YW508.20.33b,其具有 SEQ ID NO:35 的重链和 SEQ ID NO:36 的轻链)的随机、双盲、安慰剂对照的研究以评估在护理标准 (SOC) 他汀类之上具有 90-250mg/dL 的空腹血清 LDL-c(直接)水平和患有冠心病 (CHD) 或 CHD 风险替代症之一的患者中研究药物的安全性和疗效。另外的资格标准包括重量≥ 45kg(100lb);体重指数 18-37kg/m^2;和年龄在 18 和 80 之间。通过 LDL-c>120mg/dL 和糖尿病状态将随机化分类。

[0553] 该 II 期临床研究的资格标准基于欧洲心脏病学会 (European Society of Cardiology)(ESC)/ 欧洲动脉粥样硬化学会 (European Atherosclerosis Society)(EAS) 和国家胆固醇教育计划成人治疗小组 (National Cholesterol Education Program Adult Treatment Panel)III(NCEP ATP III) 降脂指导原则中的风险目录限定具有心血管和 CHD 风险的患者群体。该研究招募符合根据这些指导原则的 70mg/dL 的治疗目标 LDL-c 水平的,但尽管接受稳定 SOC 他汀类治疗,而由于 SOC 不足量或由于他汀类不耐受,尚未接近该目标的患者。

[0554] 简言之，CHD 是指有记载的心肌梗死史，之前的冠状动脉重建术过程（经皮冠状动脉介入或冠状动脉旁路搭桥）史，或之前的冠状动脉血管造影术（侵入性冠状动脉血管造影术或心脏计算机断层扫描冠状动脉血管造影术）史，其显示至少一种具有≥ 50％直径狭窄的冠状动脉粥样硬化病变。

[0555] 患有 CHD 风险替代病症的患者具有以下各项中的至少一种：

[0556] 1. 临床动脉粥样硬化疾病的一种以上形式：

[0557] a. 外周动脉疾病（在前有记载的踝 / 上臂血压指数 <0.85，之前的经皮或外科手术外周动脉血管重建步骤，之前的由于外周动脉疾病导致的下肢非外伤性截肢，或之前血管成像上≥ 50％直径狭窄），

[0558] b. 颈动脉疾病（在前有记载的颈动脉动脉粥样硬化病变，成像时具有≥ 50％直径狭窄或之前的皮肤或外科手术颈动脉血管重建步骤），

[0559] c. 之前的缺血性卒中，通过 CT 或 MRI 脑成像记载，其在研究者看来不由心源性栓塞（例如，心房颤动，瓣膜病，或左心室附壁血栓）引起，或

[0560] d. 以之前的外科手术或血管内修复的腹主动脉瘤。

[0561] 2.2 型糖尿病，

[0562] 3. 伴有目标器官损伤（如由研究者确定的视网膜病，神经病，或肾病（包括微白蛋白尿））的 1 型糖尿病，

[0563] 4. 中度到严重肾病（通过 15-60mL/min/1.73m^2 的估算的肾小球过滤速率，持续经至少三个测量间隔至少 3 个月（包括筛查实验室）使用肾病中饮食调整 (Modification of Diet in Renal Disease) 方程显示），或

[0564] 5. 下文列出的 CHD 风险因素中的两种以上并且 CHD 事件的绝对 10- 年风险≥ 20％（如通过 Framingham 风险评分的国家胆固醇教育计划成人治疗小组 III 指导原则修改确定）或者第一致命动脉粥样硬化事件的 10- 年风险≥ 10％（如通过全身性冠心病的风险评估系统确定）：

[0565] a. 对于男性年龄≥ 45 岁或对于女性年龄≥ 55，

[0566] b. 最近吸烟 (1 个月内），

[0567] c. 高血压（筛查收缩压≥ 140mmHg，舒张压≥ 90mmHg，或服用抗高血压药物以治疗高血压）

[0568] d. 低 HDL 胆固醇 (<40mg/dL)，或

[0569] e. 过早 CHD 的家族史（在一级亲属 <55 岁年龄的男性或一级亲属 <65 年龄的女性中的心肌梗死或冠状动脉重建）。

[0570] 根据患者医疗记录或历史，或根据筛查实验室测试结果，基于以下各项中的任一项的存在确定糖尿病状态：(1)HbA_{1c}>6.5％，(2) 空腹血糖≥ 126mg/dL(7.0mmol/L)，(3) 在口服葡萄糖耐量试验过程中，之前 2- 小时血糖≥ 200mg/dL(11.1mmol/L)（应该如由世界卫生组织描述的，使用含有溶于水的 75g 无水葡萄糖的当量的葡萄糖载量，进行该试验），或 (4) 目前接受对于糖尿病的诊断结果的口服或可注射治疗。

[0571] 排除标准包括：研究过程中计划的冠状动脉，颈动脉或外周动脉血管重建步骤或外科手术；3 个月内或筛查中的如方案中列出的不受控制的临床显著医学疾病；任何获得性或先天性免疫抑制；除了角膜移植之外的任何器官移植；据研究者的判断，预期寿命 <2

年；空腹血清甘油三酯水平＞＝400mg/dL；筛查的一年的酗酒或药瘾史；筛查的3个月内使用非法药物；怀孕或不愿意使用高效避孕方法；过敏性或过敏反应史。

[0572] 将具有90-250mg/dL的血清LDL-c浓度和患有CHD或CHD风险替代症之一的248患者（成年男性或女性）随机分到五个研究组之一并施用研究药物或安慰剂组（组F）。每4周向第一研究组（组A）中的患者施用400mg的抗-PCSK9抗体；每8周向第二研究组（组B）中的患者施用200mg的抗-PCSK9抗体；每8周向第三研究组（组C）中的患者施用400mg的抗-PCSK9抗体；每8周向第四研究组（组D）中的患者施用800mg的抗-PCSK9抗体；并且每12周向研究组（组E）中的患者施用800mg的抗-PCSK9抗体。在图27中提供研究剂量群组，研究药物剂量方案，和每组患者数的总览。所有剂量都使用注射器皮下给药。将药品配制为溶于200mM精氨酸琥珀酸盐，0.02％聚山梨酯20,pH 5.5的150mg/mL抗体。

[0573] 研究中患者的人口统计在下文列于表13中，表明组间无差异。患者的基线特征在下文列于表14中，表明组间无差异。

[0574] 表13：患者人口统计（平均值(SD)，除非注明）

[0575]

	400 mg /4W	200 mg /8W	400 mg /8W	800 mg /8W	800 mg /12W	安慰剂	mITT
	(n=57)	(n=23)	(n=30)	(n=50)	(n=23)	(n=64)	(n=247)
年龄 (岁)	66 (8.5)	63 (10.0)	63 (8.1)	64 (8.9)	64 (7.2)	63 (7.8)	64 (8.4)
重量 (kg)	89 (15.4)	89 (15.3)	85 (11.7)	83 (17.7)	83 (17.1)	87 (15.1)	86 (15.6)
BMI (kg/m)	31 (4.3)	30 (4.5)	30 (4.2)	29 (5.2)	29 (3.7)	30 (5.0)	30 (4.7)

[0576]

女性 (%)	24 (42%)	8 (35%)	16 (53%)	24 (48%)	10 (44%)	24 (38%)	106 (43%)
拉美裔美国籍的 (%)	1 (2%)	1 (4%)	1 (3%)	1 (2%)	1 (4%)	5 (8%)	10 (4%)
种族：白 (%)	55 (97%)	19 (83%)	27 (90%)	44 (88%)	23 (100%)	59 (92%)	227 (92%)
种族：黑 (%)	1 (2%)	2 (9%)	2 (7%)	5 (10%)	0	3 (5%)	13 (5%)
种族:亚洲 (%)	0	0	1 (3%)	1 (2%)	0	1 (2%)	3 (1%)
种族:其它 (%)	1 (2%)	1 (4%)	0	0	0	1 (2%)	3 (1%)
种族:土著 (%)	0	1 (4%)	0	0	0	0	1 (0.4%)

[0577] 表 14 :患者基线特征（平均值 (SD), 除非注明）

[0578]

	400 mg /4W	200 mg /8W	400 mg /8W	800 mg /8W	800 mg /12W	安慰剂	mITT
	(n=57)	(n=23)	(n=30)	(n=50)	(n=23)	(n=64)	(n=247)
前期糖尿病 (% FBG ≥ 100 mg/dl)	68%	65%	60%	54%	65%	59%	62%
使用他汀类 (%)	88%	78%	73%	76%	74%	89%	82%

[0579]

LDL-c ≥ 120 (%)	46%	48%	60%	54%	52%	45%	50%
LDL-c (mg/dL)	123 (31.3)	123 (25.3)	133 (35.2)	127 (31.5)	134 (43.8)	122 (31.4)	126 (32.7)
中值 LDL-c (mg/dL)	117	117	123	118	123	111	117
甘油三酯 (mg/dL)	156 (66.3)	146 (60.0)	152 (54.3)	173 (90.8)	144 (37.0)	141 (63.1)	153 (67.8)
中值 Trig. (mg/dL)	142	132	142	149	145	132	142
CHD 家族史 (%有)	26 (46%)	5 (22%)	13 (43%)	20 (40%)	9 (39%)	18 (28%)	91 (37%)
吸烟：从不 (%)	23 (40%)	6 (26%)	13 (43%)	17 (34%)	7 (30%)	25 (39%)	91 (37%)

[0580] 如在图 27 中显示的，所述研究包括筛查 (0-4 周)，准备 (0-6 周，如果需要)，治疗 (24 周；天 1 - 169)，和随访 (12 周) 的连续期。在随访期 (第 253 天) 最后的研究完成访问在研究药物的最终剂量之后 16 周 (第 141 天) 发生。所有患者，不考虑治疗分配，接受护理标准 (SOC) 治疗，包括他汀类，除非他汀类不耐受。SOC 他汀类治疗是指满足一个以下条件的治疗：(1) 高剂量辛伐他汀 (simvastatin)(40mg 每日)，阿托伐他汀 (atorvastatin)(40 - 80mg 每日)，或罗舒伐他汀 (rosuvastatin)(20 - 40mg 每日)，(2) 低剂量辛伐他汀 (simvastatin)，阿托伐他汀 (atorvastatin)，或罗舒伐他汀 (rosuvastatin) 和有记载地对高剂量的该他汀类或任何剂量的另一种他汀类不耐受，(3) 其他他汀类 (任何剂量) 和有记载的对辛伐他汀 (simvastatin)，阿托伐他汀 (atorvastatin)，或罗舒伐他汀 (rosuvastatin)(任何剂量) 的不耐受，或 (4) 无他汀类和有记载的对至少两种他汀类 (任何他汀类，任何剂量) 不耐受。在整个治疗和随访期，所有患者将以他们在准备期和在招募时接受的相同剂量继续 SOC 他汀类治疗。不容许其它处方和非处方 (OTC) 调脂治疗 (例如，红曲米，ω-3 脂肪酸补充等)。在筛查时期接受稳定剂量 SOC 他汀类治疗 (或无他汀类并对于两种以上他汀类具有有记载的不耐受) 并未接受其它调脂治疗达至少 4 周 (或在贝特类药物 (fibrates) 的情况下 6 周) 的患者将不需要准备期。

[0581] 根据研究药物施用时间表给予活性药物或安慰剂的所有剂量，即，仅在第 1, 29 天 (±2 天)，第 57 天 (±2 天)，第 85 天 (±2 天)，第 113 天 (±4 天)，和第 141 天 (±4 天)。见图 28。监测患者以基于在第 169 天 LDL-c 浓度从基线的绝对数值变化确定疗效。

此外，监测患者以确定次要疗效结果，包括对于每组在该组的最低点 LDL-c 浓度从基线的绝对数值变化；对于每组的 LDL-c 的变化（绝对和百分比变化）随时间的平均值（直到第 169 天，通过连续的 LDL-c 测量之间的周数加权）；在第 169 天和在每组的最低点的 LDL-c 浓度从基线的百分比变化；在所有其它指定的时间点 LDL-c 浓度从基线的百分比和绝对数值变化；和在第 169 天和在各组最低点总胆固醇，非 HDL-c，和载脂蛋白 B 从基线的百分比和绝对数值变化。

[0582] 主要的疗效结果包括在第 169 天 LDL-c 从基线的变化。基线 LDL-c 被定义为研究药物的第一次剂量给药之前收集的最后两次测量值的平均值。研究药物剂量之间和各个研究药物剂量和安慰剂之间的治疗比较基于协方差 (ANCOVA) 的分析，其将通过调整以下两个协变量的线性回归模型进行：基线 LDL-c 浓度 (<120mg/dL, ≥ 120mg/dL) 和糖尿病状态（是，否）。ANCOVA 模型的置信区间，以及最小平方评估，用于辅助解释研究结果。次要疗效结果测量值包括在最低点和所有时间点的 LDL-c 绝对数值变化；每周 LDLc 改变的加权平均值；在第 169 天、最低点和所有探访时 LDL-c 从基线的百分比变化；在第 169 天和在最低点总胆固醇，非 HDL-c，和载脂蛋白 B 的绝对和百分比变化。

[0583] 下文表 15 显示治疗 12 周后的患者倾向。

[0584] 表 15：治疗 12 周之后的倾向。

[0585]

	400 mg /4W	**200 mg /8W**	**400 mg /8W**	**800 mg /8W**	**800 mg /12W**	安慰剂	**ITT***
	(n=57)	**(n=23)**	**(n=30)**	**(n=51)**	**(n=23)**	**(n=64)**	**(n=248)**
完成研究	0	0	0	0	0	0	0
终止研究	2 (4%)	0	1 (3%)	3 (6%)	0	1 (2%)	7 (3%)
终止药物	3 (5%)	0	1 (3%)	4 (8%)	0	2 (3%)	10 (4.0%)
不良事件	1 (2%)	0	0	0	0	0	1 (0.4%)
方案违背	2 (4%)	0	0	2 (4%)	0	0	4 (1.6%)
受试者选择	0	0	1 (3%)	1 (2%)	0	1 (2%)	3 (1.2%)
发起人选择	0	0	0	1 (2%)	0	0	1 (0.4%)
其它	0	0	0	0	0	1 (2%)	1 (0.4%)

[0586] 该研究的暂时性数据总结于下表 16 和图 28-36。

[0587] 表 16：患者的总胆固醇，非 HDL-c，和载脂蛋白 B，从基线测量到最低点

[0588]

	400 mg /4W (n=57)	*200 mg /8W (n=23)*	*400 mg /8W (n=30)*	*800 mg /8W (n=50)*	*800 mg /12W (n=23)*	*安慰剂 (n=63)*
TC, 平均绝对数值变化 (mg/dL)	-99.9	-73.9	-92.3	-102.0	-92.3	-24.4
从安慰剂的降低	**74.9**	**48.7**	**64.7**	**75.5**	**66.2**	
95% 置信区间	65.2, 84.6	36.0, 61.5	52.9, 76.4	65.5, 85.5	53.4, 79.0	
TC, 平均相对变化 (%)	-49.4	-37.7	-43.6	-48.6	-44.8	-12.4
从安慰剂的降低	**36.7**	**25.2**	**30.8**	**35.8**	**32.1**	
95% 置信区间	32.6, 40.8	19.8, 30.7	25.7, 35.8	31.6, 40.1	26.6, 37.6	
非 HDLc, 平均 abs.	-101.6	-76.2	-96.3	-103.3	-95.0	-24.1

[0589]

ch. (mg/dL)						
从安慰剂的降低	**76.7**	**51.3**	**68.7**	**76.9**	**69.0**	
95% 置信区间	66.9, 86.5	38.4, 64.1	56.9, 80.5	66.9, 87.0	56.1, 81.9	
非 HDLc, 平均 rel. 变化 (%)	-67.3	-52.1	-60.5	-65.9	-59.8	-16.6
从安慰剂的降低	**50.3**	**35.5**	**43.7**	**49.1**	**43.0**	
95% 置信区间	45.2, 55.4	28.8, 42.2	37.5, 49.8	43.9, 54.3	36.3, 49.7	
Apo-B, 平均 abs. 变化 (mg/dL)	-64.3	-48.5	-59.1	-65.6	-62.8	-15.8
从安慰剂的降低	**48.1**	**32.3**	**41.4**	**48.5**	**46.0**	
95% 置信区间	42.0, 54.3	24.2, 40.4	33.9, 48.8	42.2, 54.9	37.8, 54.1	
Apo-B, 平均相对变化 (%)	-63.1	-49.2	-55.8	-62.7	-58.3	-15.7
从安慰剂的降低	**47.0**	**33.4**	**39.9**	**46.8**	**42.3**	
95% 置信区间	42.2, 51.7	27.1, 39.6	34.2, 45.7	41.9, 51.7	36.1, 48.6	

[0590] 图 28 提供平均药物代谢动力学 (+/- 标准偏差) (左图) 和平均总 PCSK9, 例如结合药物和游离 PCSK9 二者 (+/- 标准误差) (右图)。

[0591] 图 29 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的直接 LDL 胆固醇从基线的绝对数值变化。图 30 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的直接 LDL 胆固醇从基线的相对变化。每 4 周接受 400mg 抗 -PCSK9 抗体的患者和每 8 周接受 800mg 抗 -PCSK9 抗体的患者呈现直接 LDL-c 的最大降低。一周的治疗内观察到该效果。每 12 周接受 800mg 的抗 -PCSK9 抗体的患者呈现直接 LDL-c 的最低减少。

[0592] 图 31 显示参与此研究的患者中观察到的总胆固醇从基线的绝对数值变化。图 32 显示在接受抗 -PCSK9 抗体或安慰剂的患者中观察到的总胆固醇从基线的相对变化。每 4 周接受 400mg 抗 -PCSK9 抗体的患者和每 8 周接受 800mg 抗 -PCSK9 抗体的患者呈现总胆固醇的最大降低。在一周的治疗内观察到该效果。每 12 周接受 800mg 抗 -PCSK9 抗体的患者呈现总胆固醇的最低减少。

[0593] 图 33 显示参与此研究的患者中非 HDL 胆固醇从基线的绝对数值变化。图 34 显示参与此研究的患者中非 HDL 胆固醇从基线的相对变化。每 4 周接受 400mg 抗 -PCSK9 抗体的患者和每 8 周接受 800mg 抗 -PCSK9 抗体的患者呈现非 HDL 胆固醇最高降低。在一周的治疗内观察到该效果。每 12 周接受 800mg 抗 -PCSK9 抗体的患者呈现非 HDL 胆固醇的最低减少。

[0594] 图 35 显示参与此研究的患者中载脂蛋白 B 从基线的绝对数值变化。图 36 显示参与此研究的患者中载脂蛋白 B 从基线的相对变化。每 4 周接受 400mg 抗 -PCSK9 抗体的患者和每 8 周接受 800mg 抗 -PCSK9 抗体的患者呈现载脂蛋白 B 的最高降低。在一周的治疗内观察到该效果。每 12 周接受 800mg 抗 -PCSK9 抗体的患者呈现载脂蛋白 B 的最低减少。

[0595] 关于研究药物的疗效的结论总结于此。在每 4 周接受 400mg 抗 -PCSK9 抗体的患者和每 8 周接受 800mg 抗 -PCSK9 抗体的患者中观察到在第 85 天，在最低点，和 AUC 的 LDL-c 的最高的剂量依赖性降低。在每 12 周接受 800mg 抗 -PCSK9 抗体的患者中观察到基于第 85 天分析的 LDL-c 的最小的剂量依赖型降低。在每 8 周接受 200mg 抗 -PCSK9 抗体的患者中观察到基于最低点和 AUC 分析的 LDL-c 的最小的剂量依赖型降低。在一周的治疗内降低明显。在第 85 天和在最低点观察到总胆固醇，非 HDL-c，和载脂蛋白 -B 的剂量依赖型降低，并且所述降低在一周的治疗内也是明显的。

[0596] 最后，还监测对患者的安全性，包括不良事件的发生率，性质，和严重度；在研究药物施用过程中和之后的生命体征，身体研究结果，和临床实验结果的改变的发生率和性质；和针对研究药物的抗 - 治疗抗体的发生率。

[0597] 以不知情、探索性方式定期评估低 LDL-c 值的安全性。图 37A 显示对于接受抗 -PCSK9 抗体或安慰剂之后的至少一次探访，直接 LDL-c 值小于或等于 15mg/dL 的患者的比例，并且图 37B 显示对于接受抗 -PCSK9 抗体或安慰剂之后的至少一次探访，直接 LDL-c 值小于或等于 25mg/dL 的患者的比例。LDL-c ≤ 15mg/dL 或 LDL-c ≤ 25mg/dL 的最高百分比的患者是每四周接受 400mg 药物或每 8 周接受 800mg 药物。LDL-c ≤ 25mg/dL 的最低百分数的患者是每 8 周接受 200mg 药物。不给予具有两个连续的 <15mg/dL 的 LDL-c 值的患者以研究药物。这不认为是不良事件。将此种患者替代以用不知情的方式用安慰剂治疗，直到 LDL-c 增加至≥ 50mg/dL，此后将这些患者变换为最低剂量（每 8 周 200mg）。

[0598] 关于研究药物的安全性的结论总结于此。简言之，在年龄在 37-80 之间的具有升高的基线 LDL-c(90-250mg/dL)，诊断为患有 CHD 或 CHD 风险替代症，和接受稳定剂量的他汀类或他汀类不耐受的患者中，抗 -PCSK9 抗体是良好耐受的。相对接受安慰剂 (9％)，注射位点反应在接受研究药物 (25％) 的患者中更常见。仅 2 个注射位点反应是中度的 (1 个安慰剂，1 个研究药物)，并且其余的在严重度方面是温和的。在研究药物治疗和安慰剂治疗的患者之间未观察到治疗 - 突发事件的其它临床上显著失衡。未观察到实验室异常方面的临床上相关失衡。未确定安全性信号。未报到死亡，并未观察到新的安全性问题。未检测到安全性实验室结果的模式。

[0599] 尽管为了清晰的理解，已经通过举例说明和实施例的方式详细描述了在前提到的发明，说明书和实施例不应该被认为限制本发明的范围。本文引用的所有专利和科学文献的公开均明确以其整体通过引用并入。

[0001]

序列表

<110> GENENTECH, INC.
F. HOFFMANN-LA ROCHE AG
WU, Yan
CHIU, Cecilia Pui Chi
KIRCHHOFER, Daniel K.
PETERSON, Andrew
KOLUMAM, Ganesh A.
BELTRAN, Monica Kong
MORAN, Paul
LI, Wei
LAM, Xanthe
LUIS, Lin
HUI, Ada
TINGLEY, Whittemore
DAVIS, John Douglas
BUDHA, Nageshwar R.

<120> 抗-PCSK9 抗体，制剂，剂量给药，和使用方法

<130> 146392014540

<140> 未指定
<141> 同时提交

<150> US 61/660,605
<151> 2012-06-15

<150> US 61/786,280
<151> 2013-03-14

<160> 45

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<213> 人工序列

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[0002]

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<212> PRT
<213> 人工序列

<220>
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Gly

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<212> PRT
<213> 人工序列

<220>
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<210> 6
<211> 11
<212> PRT
<213> 人工序列

<220>
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<400> 6
Arg Ala Ser Gln Asp Val Ser Ser Ala Val Ala
 1   5   10

<210> 7
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 7
Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
 1   5   10

<210> 8
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 8
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<210> 9
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

[0003]

<400> 9
Gln Gln Ser Tyr Thr Thr Pro Pro Thr
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<210> 10
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

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<210> 11
<211> 9
<212> PRT
<213> 人工序列

<220>
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<400> 11
Gln Gln Ser Tyr Pro Ser Pro Ala Thr
1 5

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<211> 9
<212> PRT
<213> 人工序列

<220>
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<400> 12
Gln Gln Ser Tyr Arg Ile Gln Pro Thr
1 5

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<220>
<223> 合成的构建体

<400> 13
Gln Gln Ser Tyr Pro Ala Leu His Thr
1 5

<210> 14
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<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 14
Gln Gln Ser Tyr Pro Ala Pro Ser Thr
1 5

<210> 15
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<213> 人工序列

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[0004]

<223> 合成的构建体

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            20                  25                  30
Ala Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Gly Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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<210> 16
<211> 121
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 16

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 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Arg His
            20                  25                  30
Thr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 17
<211> 121
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 17

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 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Thr
            20                  25                  30
Ala Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 18

[0005]

<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Ser Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Ser Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 19
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 19
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Pro Ala Pro Ala
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 20
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Pro Ser Pro Ala
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 21
<211> 107

[0006]

<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 21
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Arg Ile Gln Pro
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 22
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 22
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Pro Ala Leu His
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 23
<211> 107
<212> PRT
<213> 人工序列

<220>
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<400> 23
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Pro Ala Pro Ser
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 24
<211> 692
<212> PRT

[0007]

<213> 智人

<400> 24

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Met Gly Thr Val Ser Ser Arg Arg Ser Trp Trp Pro Leu Pro Leu Leu
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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
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
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
```

[0008]

```
            580                 585                 590
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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<212> DNA
<213> 智人

<400> 25

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[0009]

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catttatctt ttgggtctgt cctctctgtt gccttttac agccaacttt tctagacctg 3540
ttttgctttt gtaacttgaa gatatttatt ctgggttttg tagcattttt attaatatgg 3600
tgacttttta aaataaaaac aaacaaacgt tgtcct 3636

<210> 26
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 26
Ser Ala Ser Ser Leu Tyr Ser
1 5

<210> 27
<211> 121
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 27
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Gly Tyr
20 25 30
Ala Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 28
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<221> 变体
<222> 5
<223> Xaa = Ser或Thr

<221> 变体
<222> 6
<223> Xaa = Gly, Arg或Ser

<221> 变体
<222> 7
<223> Xaa = His, Thr或Tyr

<221> 变体
<222> 8
<223> Xaa = Ala或Thr

<400> 28
Gly Phe Thr Phe Xaa Xaa Xaa Xaa Ile His
1 5 10

[0010]

<210> 29
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<221> 变体
<222> 8
<223> Xaa = Ser或Thr

<400> 29
Arg Ala Ser Gln Asp Val Ser Xaa Ala Val Ala
1 5 10

<210> 30
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<221> 变体
<222> 4
<223> Xaa = Phe或Ser

<400> 30
Ser Ala Ser Xaa Leu Tyr Ser
1 5

<210> 31
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<221> 变体
<222> 5
<223> Xaa = Pro, Arg或Thr

<221> 变体
<222> 6
<223> Xaa = Ala, Ile, Ser或Thr

<221> 变体
<222> 7
<223> Xaa = Leu, Pro或Gln

<221> 变体
<222> 8
<223> Xaa = Ala, His, Pro或Ser

<400> 31
Gln Gln Ser Tyr Xaa Xaa Xaa Xaa Thr
1 5

<210> 32
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 32
His His His His His His His His
1 5

[0011]

<210> 33
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 33
Gln Gln Ala Tyr Pro Ala Leu His Thr
1 5

<210> 34
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 34
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Tyr Pro Ala Leu His
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 35
<211> 451
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 35
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Thr
20 25 30
Ala Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

[0012]

```
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225                 230                 235                 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    290                 295                 300
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
        355                 360                 365
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385                 390                 395                 400
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                405                 410                 415
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420                 425                 430
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445
Pro Gly Lys
    450
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<210> 36
<211> 214
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 36

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Tyr Pro Ala Leu His
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val 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
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
            180                 185                 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
Phe Asn Arg Gly Glu Cys
    210
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<210> 37
<211> 9
<212> PRT
<213> 人工序列

[0013]

<220>
<223> 合成的构建体

<221> 变体
<222> 5
<223> Xaa = Pro, Arg或Thr

<221> 变体
<222> 6
<223> Xaa = Ala, Ile, Ser或Thr

<221> 变体
<222> 7
<223> Xaa = Leu, Pro或Gln

<221> 变体
<222> 8
<223> Xaa = Ala, His, Pro或Ser

<400> 37
Gln Gln Ala Tyr Xaa Xaa Xaa Xaa Thr
1 5

<210> 38
<211> 1353
<212> DNA
<213> 人工序列

<220>
<223> 合成的构建体

<400> 38
gaagttcagc tggtggagtc tggcggtggc ctggtgcagc cagggggctc actccgtttg 60
tcctgtgcag cttctggctt caccttctct agtactgcta ttcactgggt gcgtcaggcc 120
ccgggtaagg gcctggaatg ggttgctagg atttctcctg ctaacggtaa tactaactat 180
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tcggcctcca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540
tcaggactct actccctcag cagcgtggtg actgtgccct ctagcagctt gggcacccag 600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660
cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga actcctgggg 720
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aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 960
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gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 1260
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acgcagaaga gcctctccct gtctccgggt aaa 1353

<210> 39
<211> 363
<212> DNA
<213> 人工序列

<220>
<223> 合成的构建体

<400> 39
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tcctgtgcag cttctggctt caccttctct agtactgcta ttcactgggt gcgtcaggcc 120
ccgggtaagg gcctggaatg ggttgctagg atttctcctg ctaacggtaa tactaactat 180
gccgatagcg tcaagggccg tttcactata agcgcagaca catccaaaaa cacagcctac 240
ctacaaatga acagcttaag agctgaggac actgccgtct attattgtgc tcgttggatc 300
gggtcccggg agctgtacat tatggactac tggggtcaag gaaccctggt caccgtctcc 360
tcg 363

<210> 40
<211> 642

[0014]

<212> DNA
<213> 人工序列

<220>
<223> 合成的构建体

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atcacctgcc gtgccagtca ggatgtgtcc actgctgtag cctggtatca acagaaacca 120
ggaaaagctc cgaagcttct gatttactcg gcatccttcc tctactctgg agtcccttct 180
cgcttctctg gtagcggttc cgggacggat ttcactctga ccatcagcag tctgcagccg 240
gaagacttcg caacttatta ctgtcagcaa gcctatccgg ccctacacac gttcggacag 300
ggtaccaagg tggagatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 360
tctgatgagc agttgaaatc tggaactgct tctgttgtgt gcctgctgaa taacttctat 420
cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 642

<210> 41
<211> 324
<212> DNA
<213> 人工序列

<220>
<223> 合成的构建体

<400> 41
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atcacctgcc gtgccagtca ggatgtgtcc actgctgtag cctggtatca acagaaacca 120
ggaaaagctc cgaagcttct gatttactcg gcatccttcc tctactctgg agtcccttct 180
cgcttctctg gtagcggttc cgggacggat ttcactctga ccatcagcag tctgcagccg 240
gaagacttcg caacttatta ctgtcagcaa gcctatccgg ccctacacac gttcggacag 300
ggtaccaagg tggagatcaa acga 324

<210> 42
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 42
Gly Phe Thr Phe Thr Arg His Thr Ile Asn
1 5 10

<210> 43
<211> 121
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 43
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Arg His
20 25 30
Thr Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Ser Pro Ala Asn Gly Asn Thr Asn Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Trp Ile Gly Ser Arg Glu Leu Tyr Ile Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 44

[0015]

<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<400> 44
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 45
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 合成的构建体

<221> 变体
<222> 5
<223> Xaa = Ser或Thr

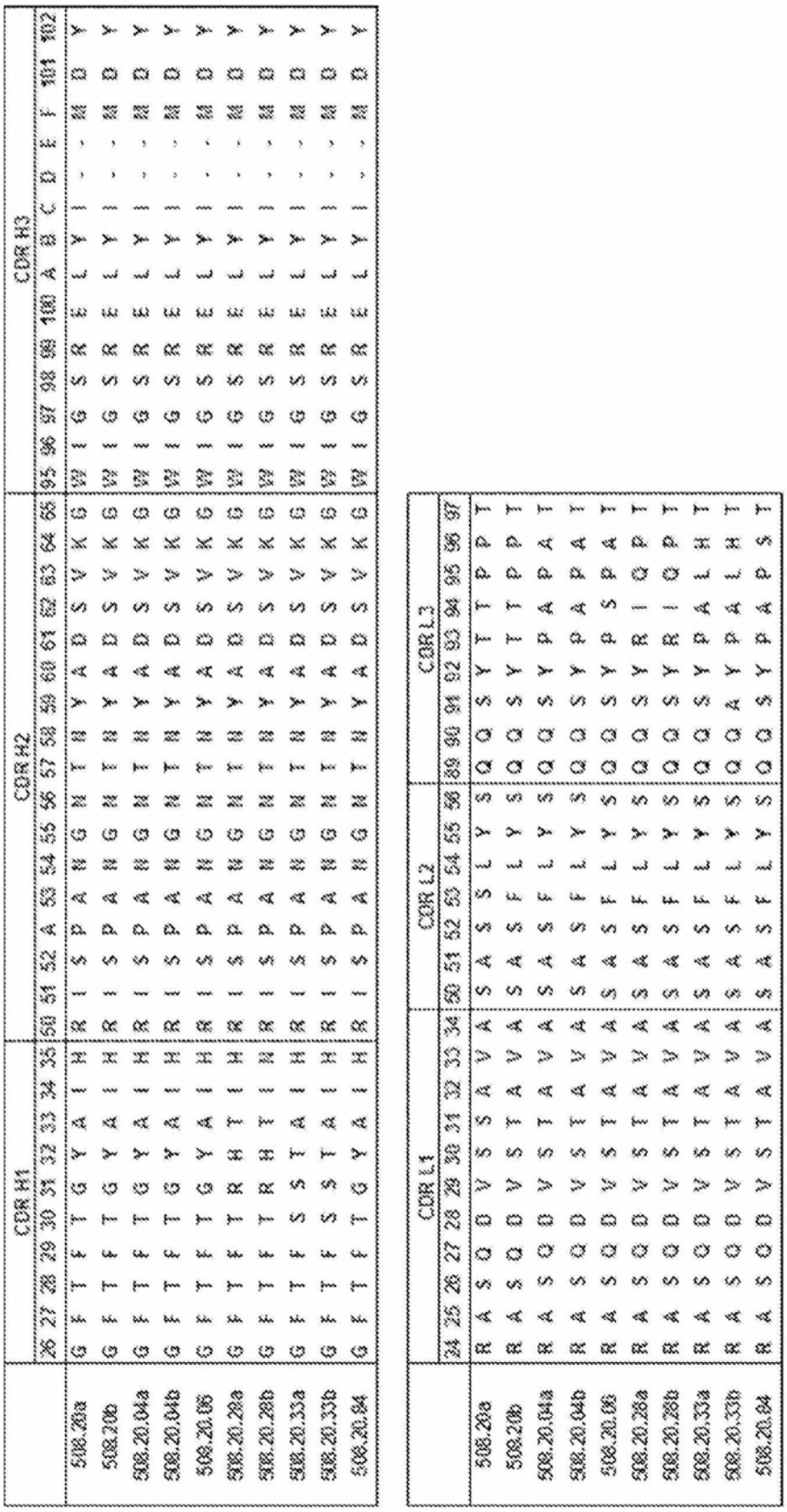
<221> 变体
<222> 6
<223> Xaa = Gly, Arg或Ser

<221> 变体
<222> 7
<223> Xaa = His, Thr或Tyr

<221> 变体
<222> 8
<223> Xaa = Ala或Thr

<221> 变体
<222> 10
<223> Xaa = His或Asn

<400> 45
Gly Phe Thr Phe Xaa Xaa Xaa Xaa Ile Xaa
1 5 10



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508.20b	G	F	T	F	T	G	Y	A	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.04a	G	F	T	F	T	G	Y	A	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.04b	G	F	T	F	T	G	Y	A	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.06	G	F	T	F	T	G	Y	A	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.28a	G	F	T	F	T	R	H	T	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
508.20.28b	G	F	T	F	T	R	H	T	I	N	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y
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508.20.84	G	F	T	F	T	G	Y	A	I	H	R	I	S	P	A	N	G	N	T	N	Y	A	D	S	V	K	G	W	I	G	S	R	E	L	Y	I	-	-	M	D	Y

	CDR L1												CDR L2							CDR L3									
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508.20a	R	A	S	Q	D	V	S	S	A	V	A	S	A	S	S	L	Y	S	Q	Q	S	Y	T	T	P	P	T
508.20b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	T	T	P	P	T
508.20.04a	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	A	T
508.20.04b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	A	T
508.20.06	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	S	P	A	T
508.20.28a	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	R	I	Q	P	T
508.20.28b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	R	I	Q	P	T
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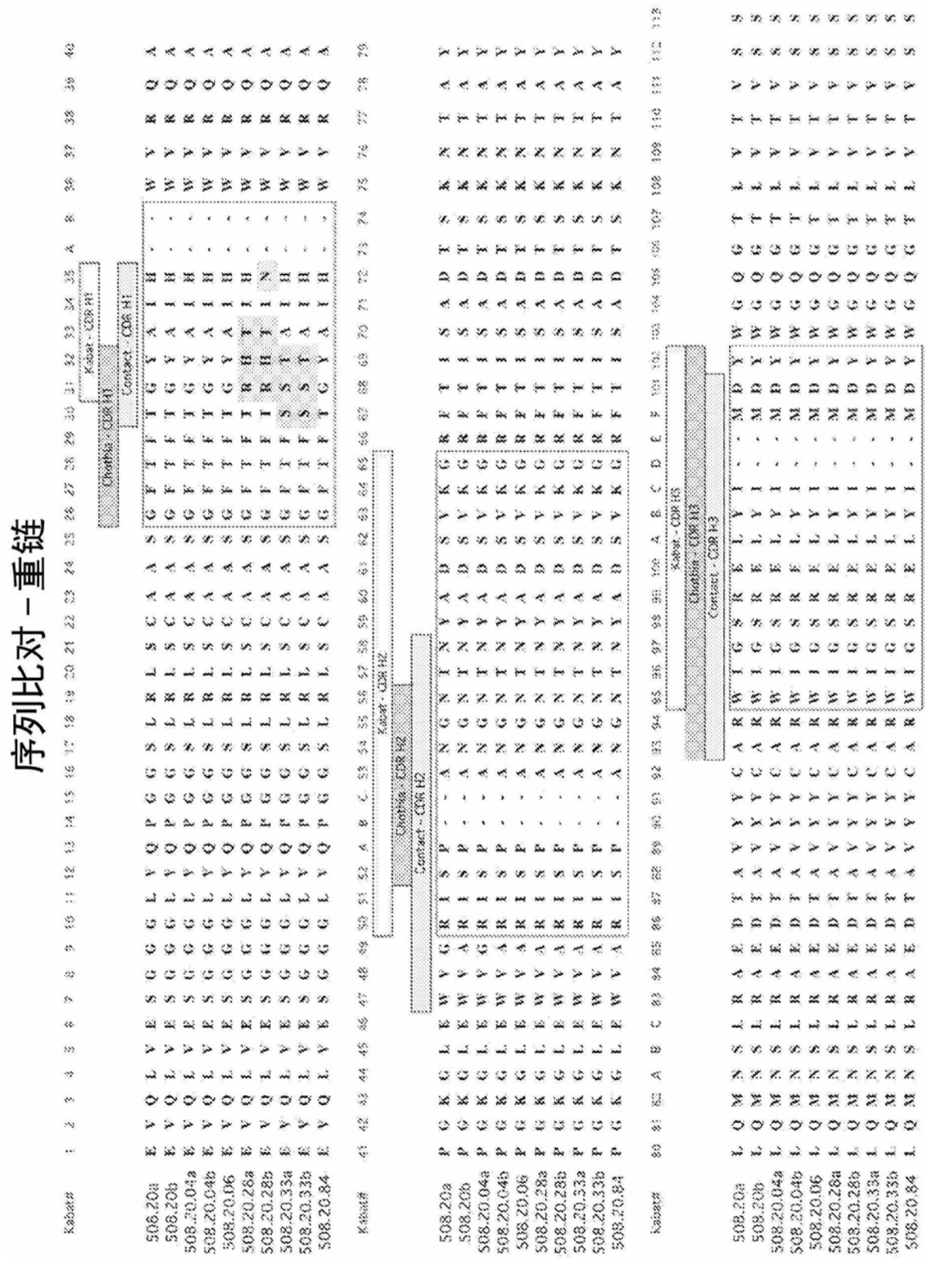
508.20.33b	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	A	Y	P	A	L	H	T
508.20.84	R	A	S	Q	D	V	S	T	A	V	A	S	A	S	F	L	Y	S	Q	Q	S	Y	P	A	P	S	T

图 1


序列比对 - 重链
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Chothia - CDR H1
Contact - CDR H1
Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2
Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3

图 2A

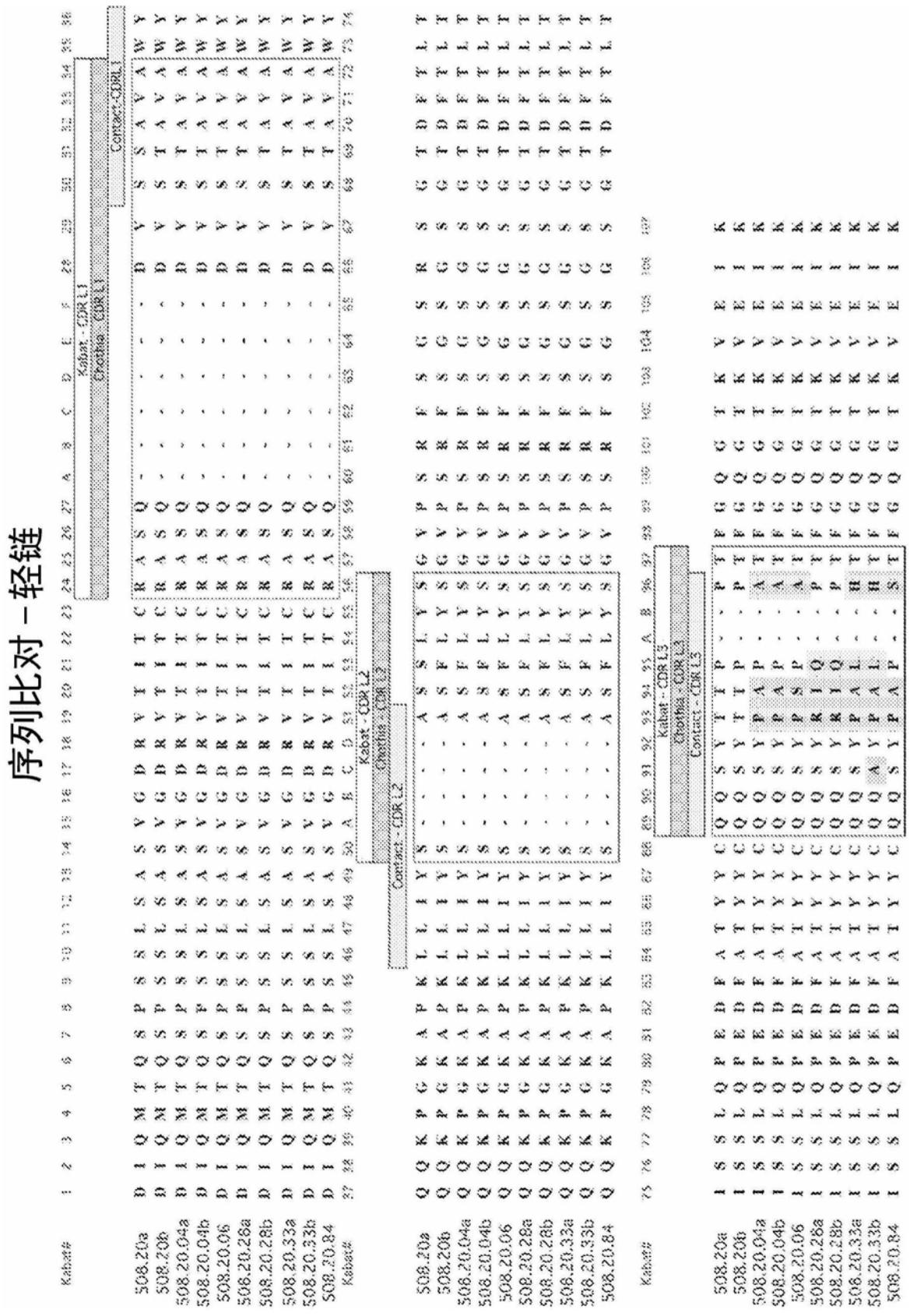

序列比对 – 轻链
Kabat - CDR L1
Chothia - CDR L1
Contact - CDR L1
Kabat - CDR L2
Chothia - CDR L2
Contact - CDR L2
Kabat - CDR L3
Chothia - CDR L3
Contact - CDR L3

图 2B

	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.04b	7.47E+04	5.65E-05	0.757
YW508.20.06	1.04E+05	6.51E-05	0.628
YW508.20.28b	5.98E+04	2.09E-05	0.349
YW508.20.33b	5.26E+04	2.15E-05	0.408
YW508.20.84	7.46E+04	3.59E-05	0.481

图 3A

	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.04b	2.70E+05	3.17E-05	0.117
YW508.20.06	2.50E+05	5.02E-05	0.201
YW508.20.28b	1.52E+05	4.90E-05	0.323
YW508.20.33b	1.57E+05	1.94E-06	0.0123
YW508.20.84	1.92E+05	2.59E-05	0.135

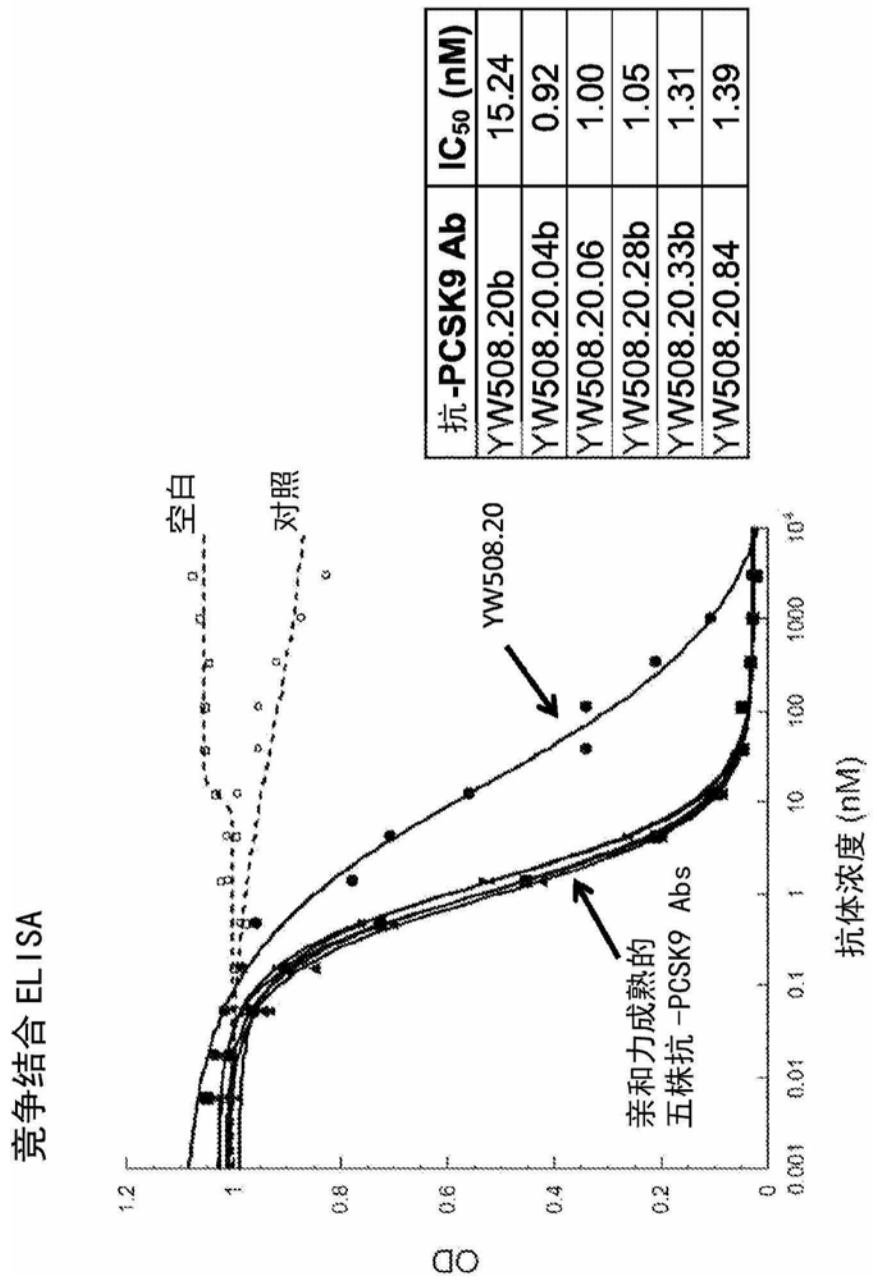
图 3B

YW508.20.33b	Ka (1/Ms)	Kd (1/s)	KD (pM)
食蟹猴	2.62E+04	1.63E-06	62.4
大鼠	6.81E+04	1.24E-06	18.2

图 3C

	Ka (1/Ms)	Kd (1/s)	KD (nM)
YW508.20.33b	8.35E+03	3.42E-05	4.09

图 3D



抗-PCSK9 Ab	IC_{50} (nM)
YW508.20b	15.24
YW508.20.04b	0.92
YW508.20.06	1.00
YW508.20.28b	1.05
YW508.20.33b	1.31
YW508.20.84	1.39

图 4

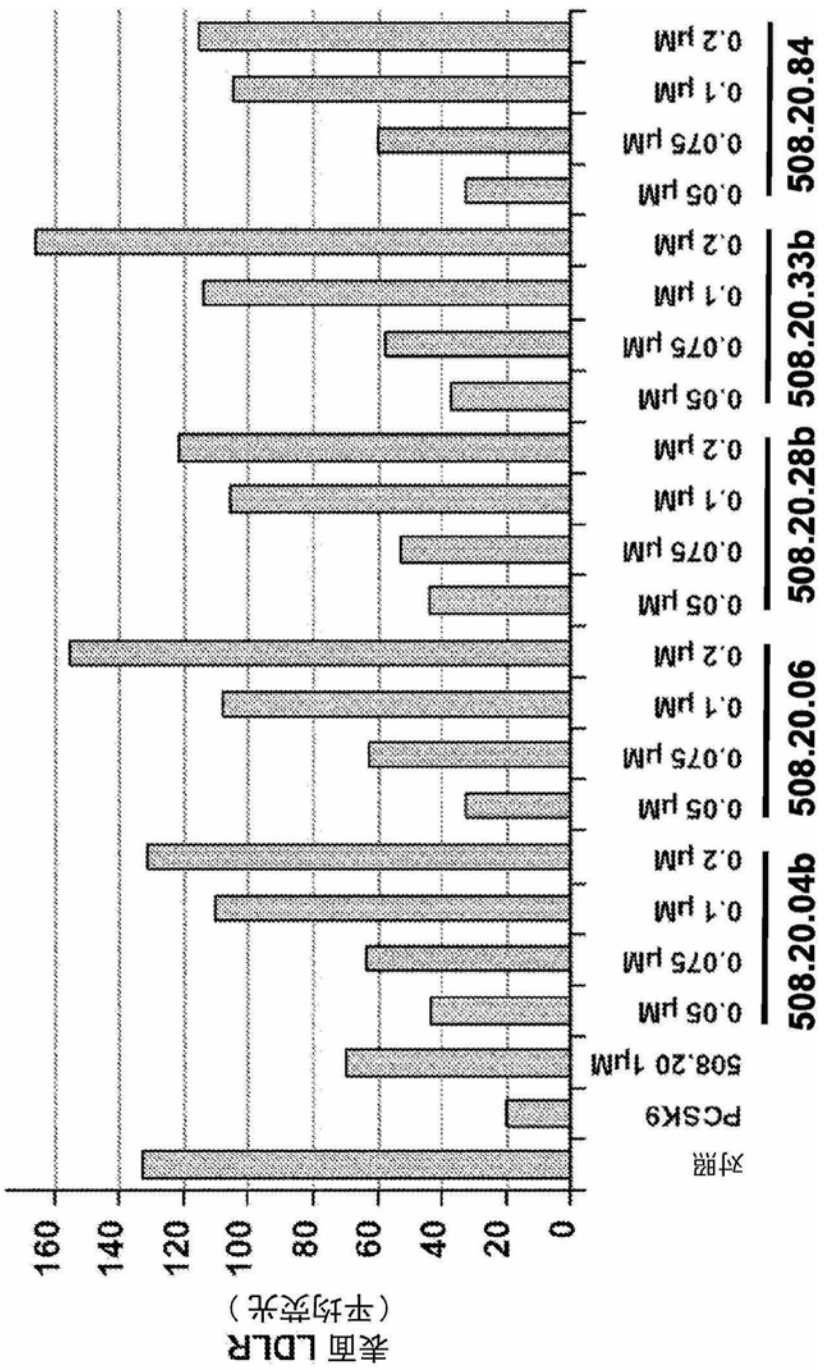

表面 LDLR
（平均荧光）
160
140
120
100
80
60
40
20
0
对照
PCSK9
508.20 1μM
508.20.04b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.06
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.28b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.33b
0.05 μM
0.075 μM
0.1 μM
0.2 μM
508.20.84
0.05 μM
0.075 μM
0.1 μM
0.2 μM

图 5

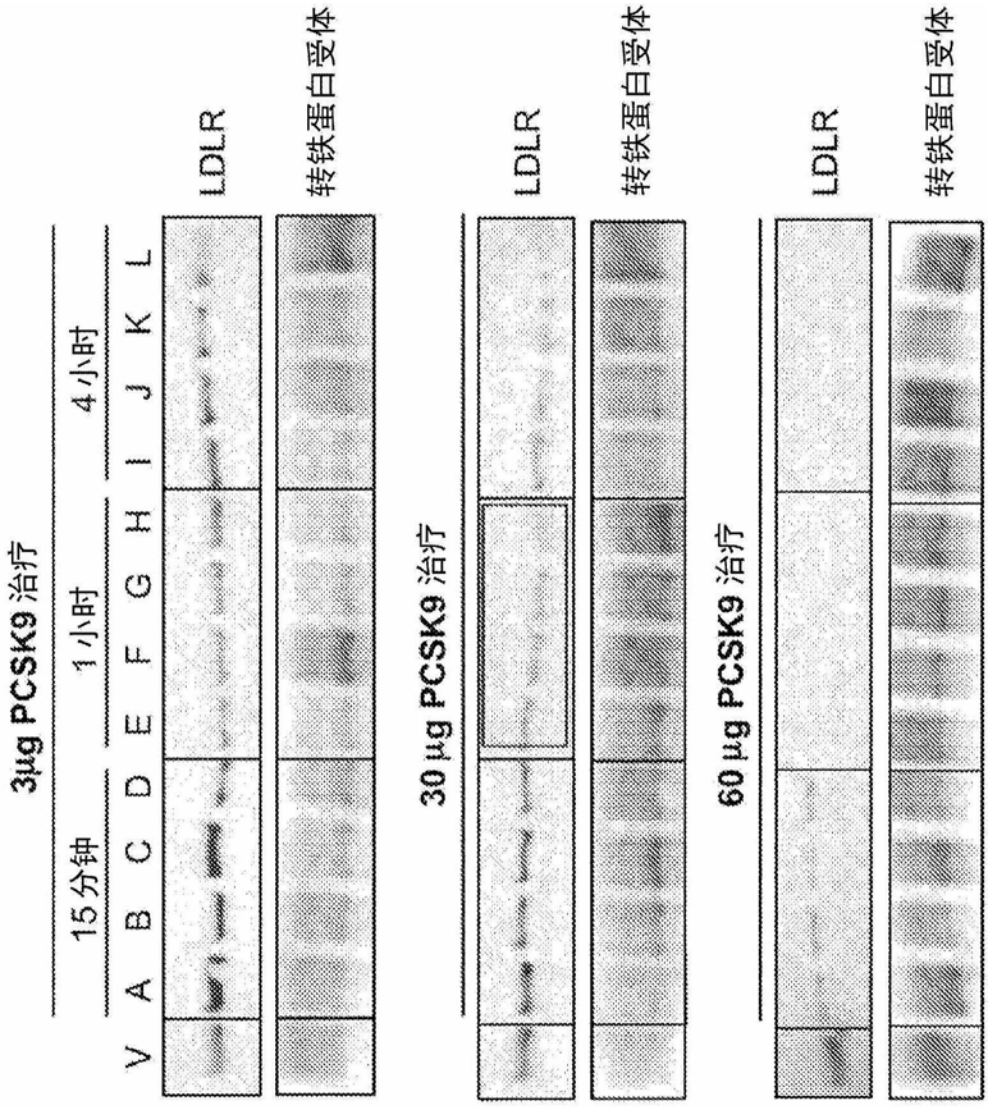

3μg PCSK9 治疗
30 μg PCSK9 治疗
60 μg PCSK9 治疗
15 分钟
1 小时
4 小时
V A B C D E F G H I J K L
LDLR
转铁蛋白受体

图 6

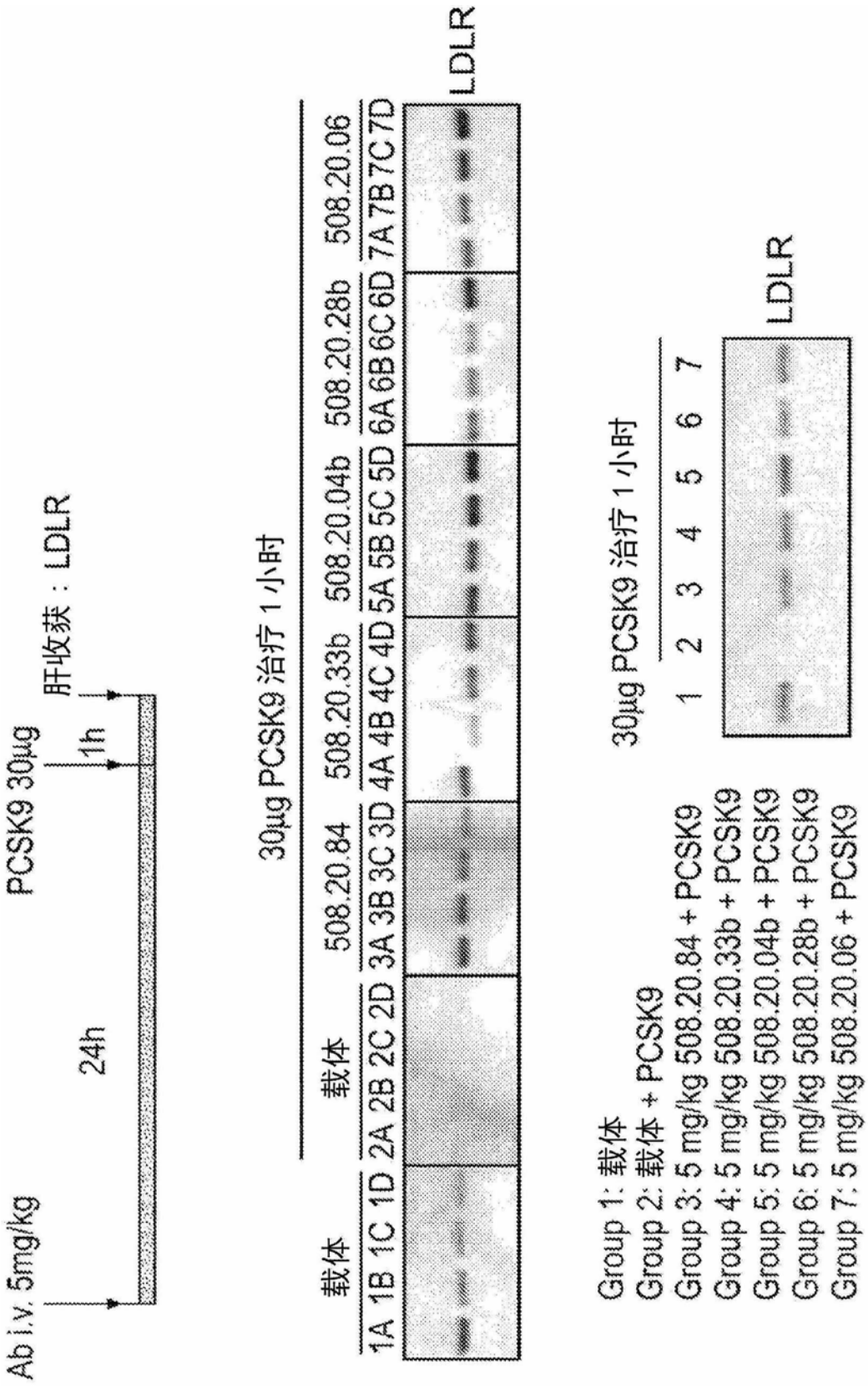

Ab i.v. 5mg/kg
24h
PCSK9 30μg
1h
肝收获：LDLR
30μg PCSK9 治疗 1 小时
载体
1A 1B 1C 1D
载体
2A 2B 2C 2D
508.20.84
3A 3B 3C 3D
508.20.33b
4A 4B 4C 4D
508.20.04b
5A 5B 5C 5D
508.20.28b
6A 6B 6C 6D
508.20.06
7A 7B 7C 7D
LDLR
30μg PCSK9 治疗 1 小时
1 2 3 4 5 6 7
LDLR
Group 1: 载体
Group 2: 载体 + PCSK9
Group 3: 5 mg/kg 508.20.84 + PCSK9
Group 4: 5 mg/kg 508.20.33b + PCSK9
Group 5: 5 mg/kg 508.20.04b + PCSK9
Group 6: 5 mg/kg 508.20.28b + PCSK9
Group 7: 5 mg/kg 508.20.06 + PCSK9

图 7

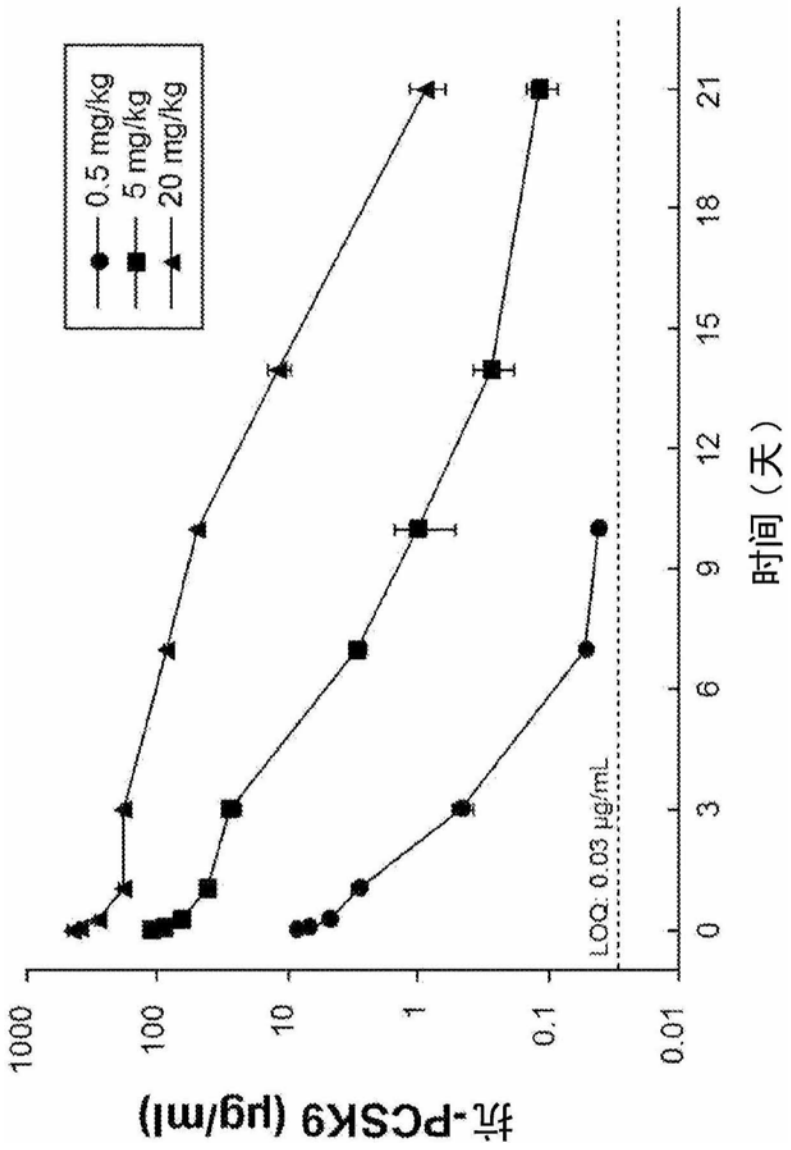

0.5 mg/kg
5 mg/kg
20 mg/kg
LOQ: 0.03 µg/mL
游离-PCSK9 (µg/ml)
1000
100
10
1
0.1
0.01
0
3
6
9
12
15
18
21
时间（天）

图 8

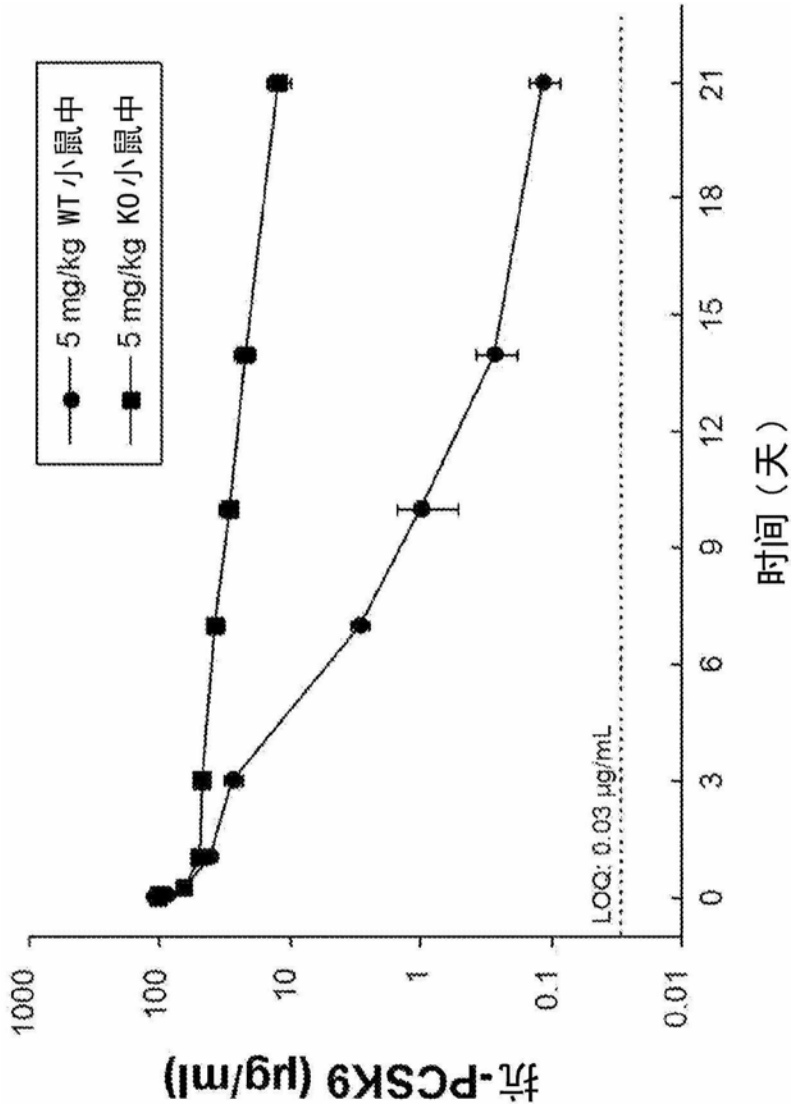

5 mg/kg WT小鼠中
5 mg/kg KO小鼠中
抗-PCSK9 (μg/ml)
1000
100
10
1
0.1
0.01
LOQ: 0.03 μg/mL
0
3
6
9
12
15
18
21
时间（天）

图 9

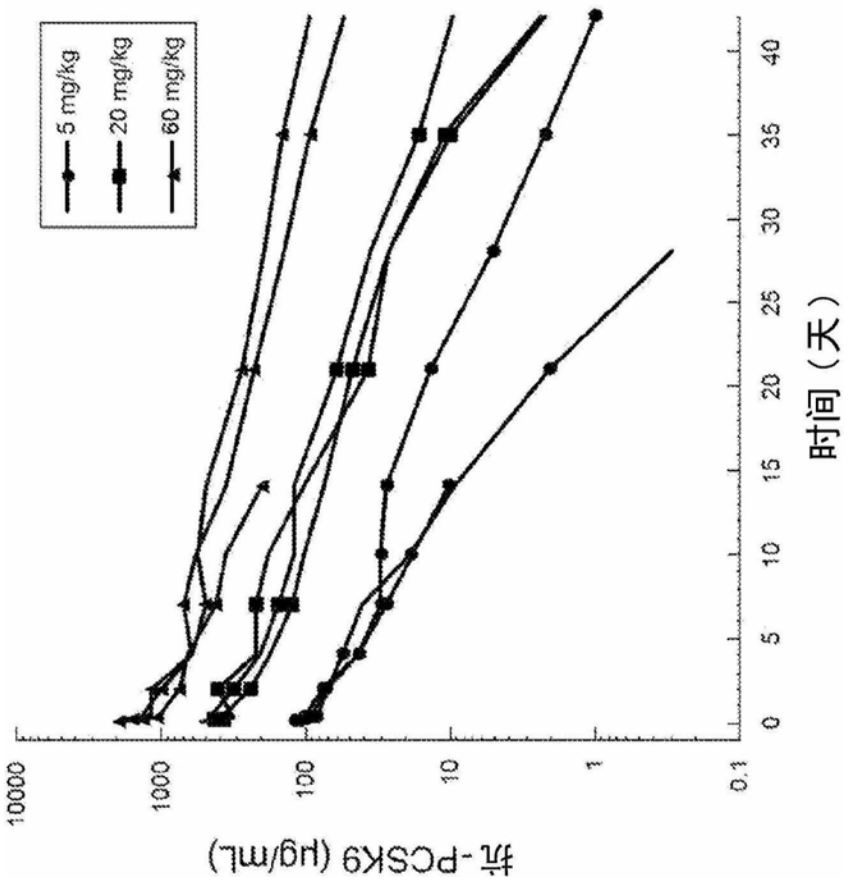

5 mg/kg
20 mg/kg
60 mg/kg
抗-PCSK9 (μg/mL)
10000
1000
100
10
1
0.1
0
5
10
15
20
25
30
35
40
时间（天）

图 10

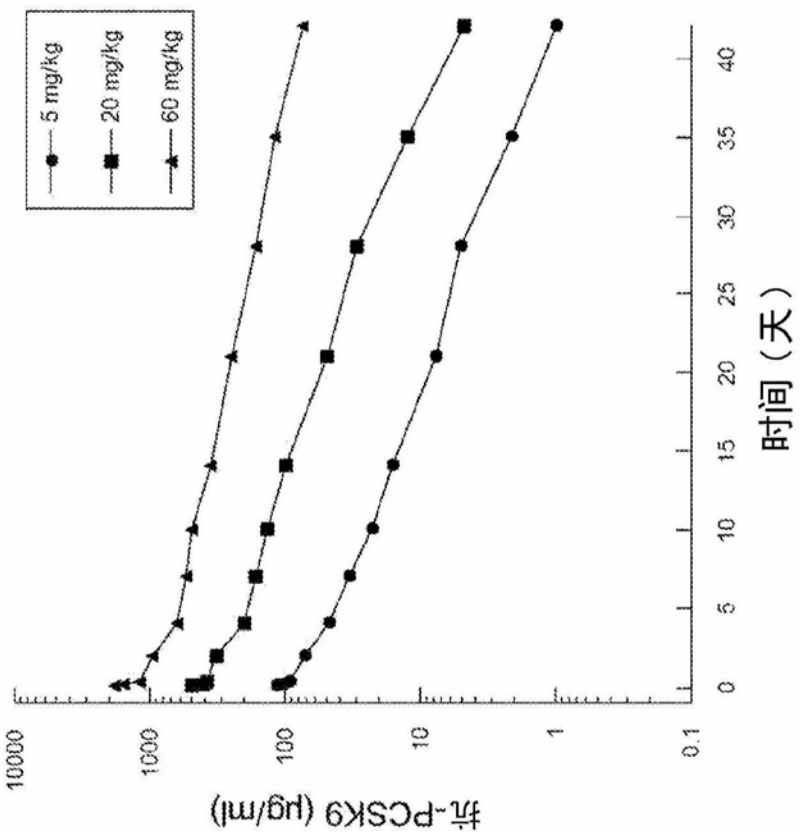

5 mg/kg
20 mg/kg
60 mg/kg
抗-PCSK9 (μg/ml)
10000
1000
100
10
1
0.1
0
5
10
15
20
25
30
35
40
时间（天）

图 11

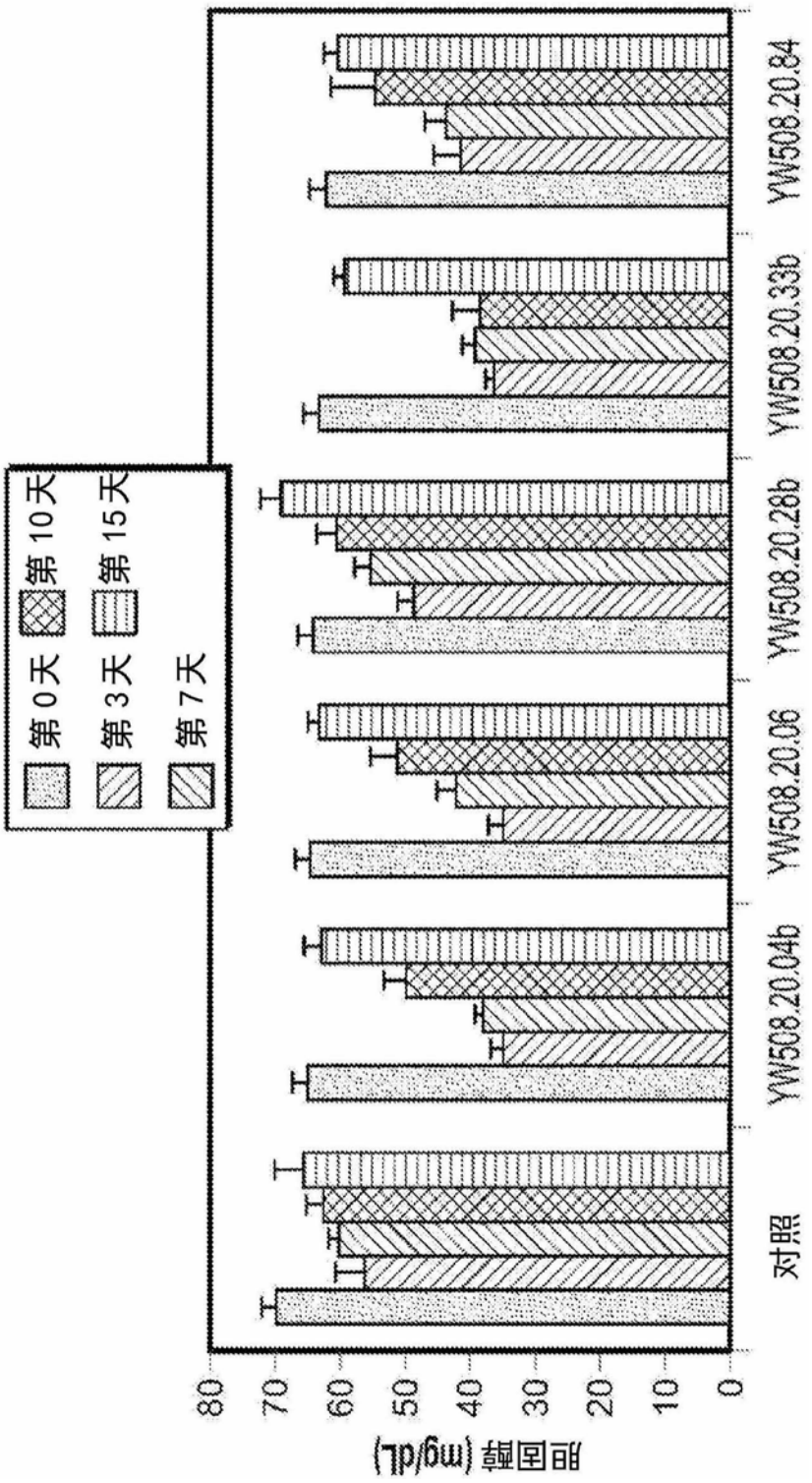

第 0 天
第 3 天
第 7 天
第 10 天
第 15 天
胆固醇 (mg/dL)
0
10
20
30
40
50
60
70
80
对照
YW508.20.04b
YW508.20.06
YW508.20.28b
YW508.20.33b
YW508.20.84

图 12

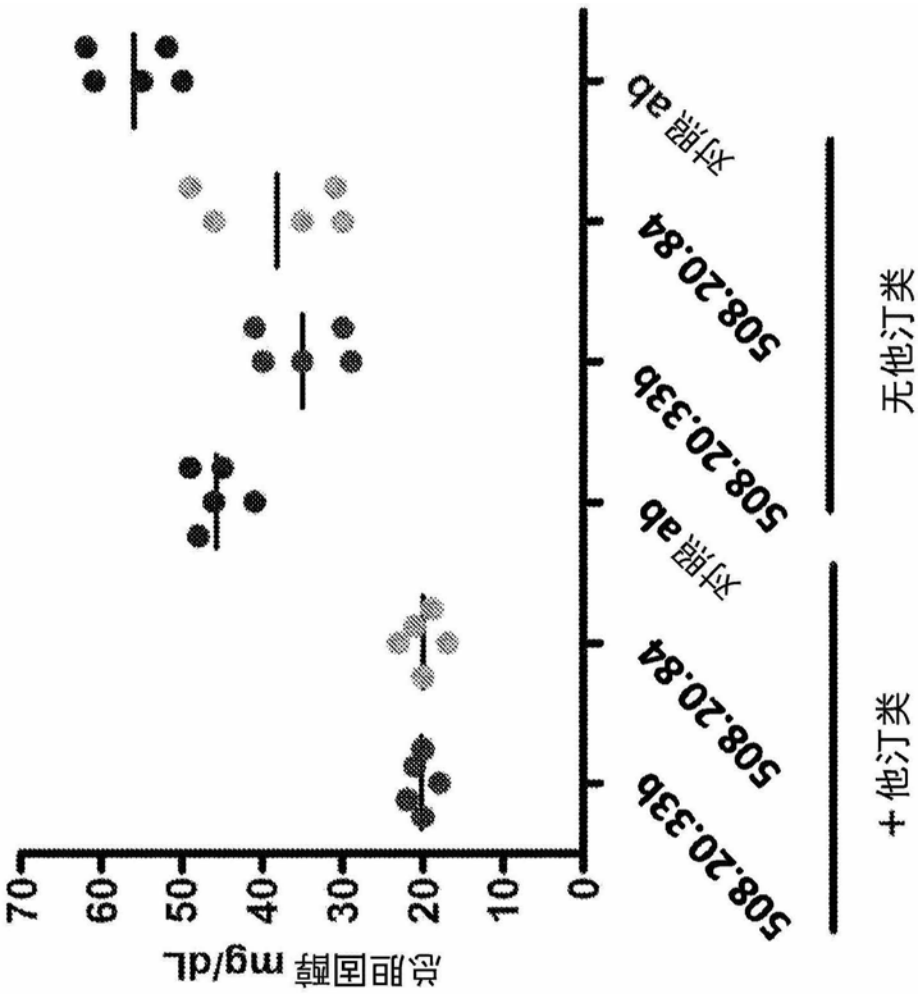

总胆固醇 mg/dL
70
60
50
40
30
20
10
0
对照 ab
508.20.84
508.20.336
对照 ab
508.20.84
508.20.336
无他汀类
+ 他汀类

图 13

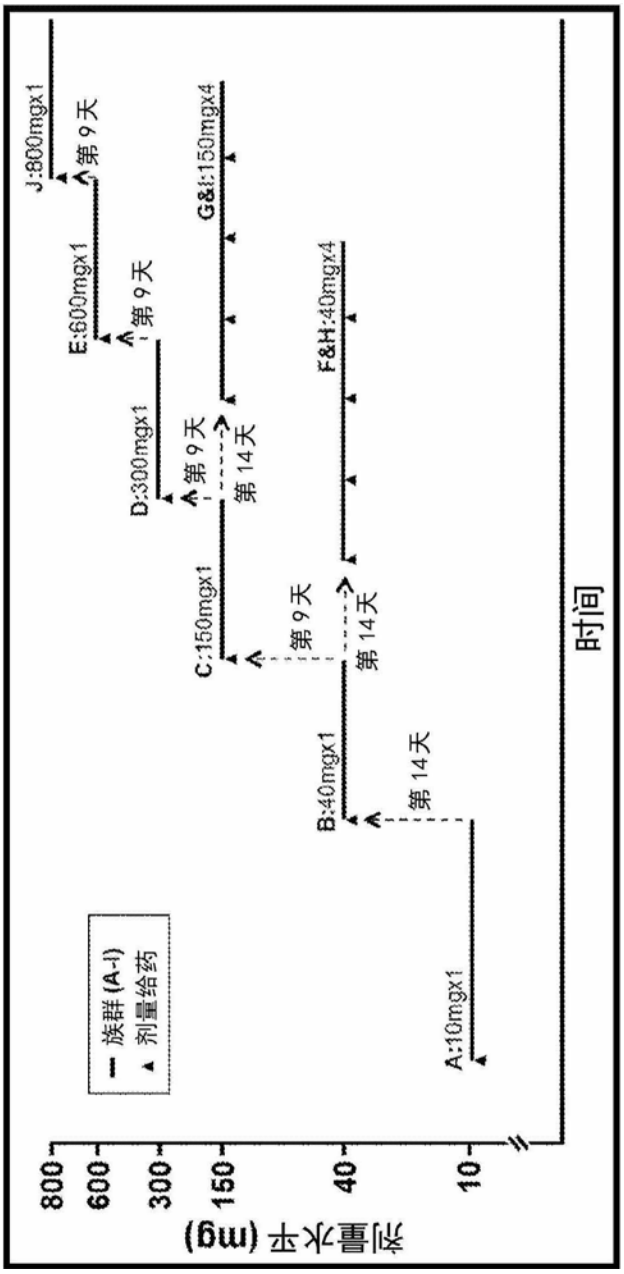

剂量水平 (mg)
800
600
300
150
40
10
时间
族群 (A-I)
剂量给药
A:10mgx1
B:40mgx1
第 14 天
C:150mgx1
第 9 天
第 14 天
D:300mgx1
第 9 天
第 14 天
E:600mgx1
第 9 天
J:800mgx1
F&H:40mgx4
G&I:150mgx4

图 14

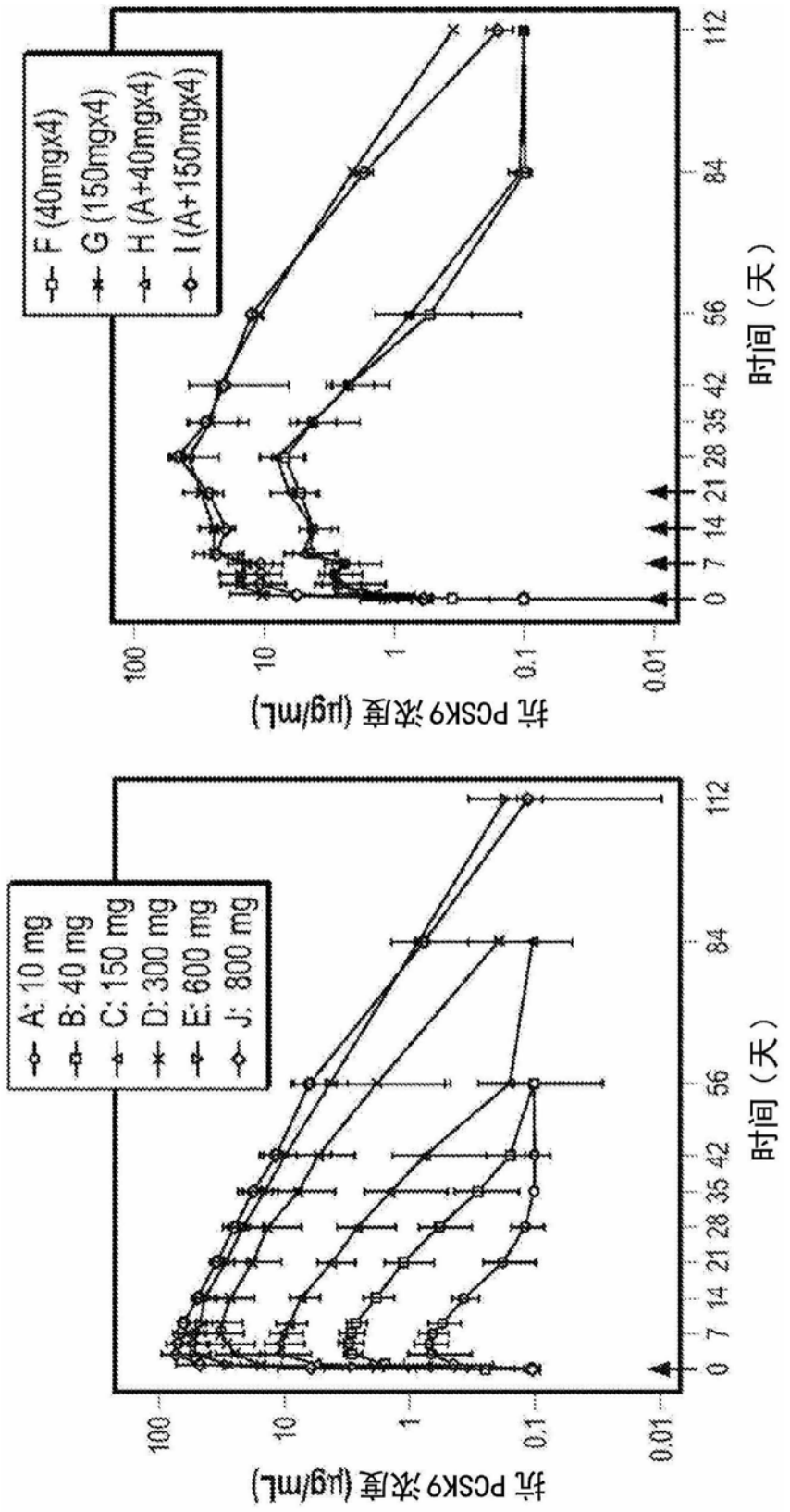

F (40mgx4)
G (150mgx4)
H (A+40mgx4)
I (A+150mgx4)
抗 PCSK9 浓度 (μg/mL)
100
10
1
0.1
0.01
0 7 14 21 28 35 42 56 84 112
时间（天）
A: 10 mg
B: 40 mg
C: 150 mg
D: 300 mg
E: 600 mg
J: 800 mg
抗 PCSK9 浓度 (μg/mL)
100
10
1
0.1
0.01
0 7 14 21 28 35 42 56 84 112
时间（天）

图 15

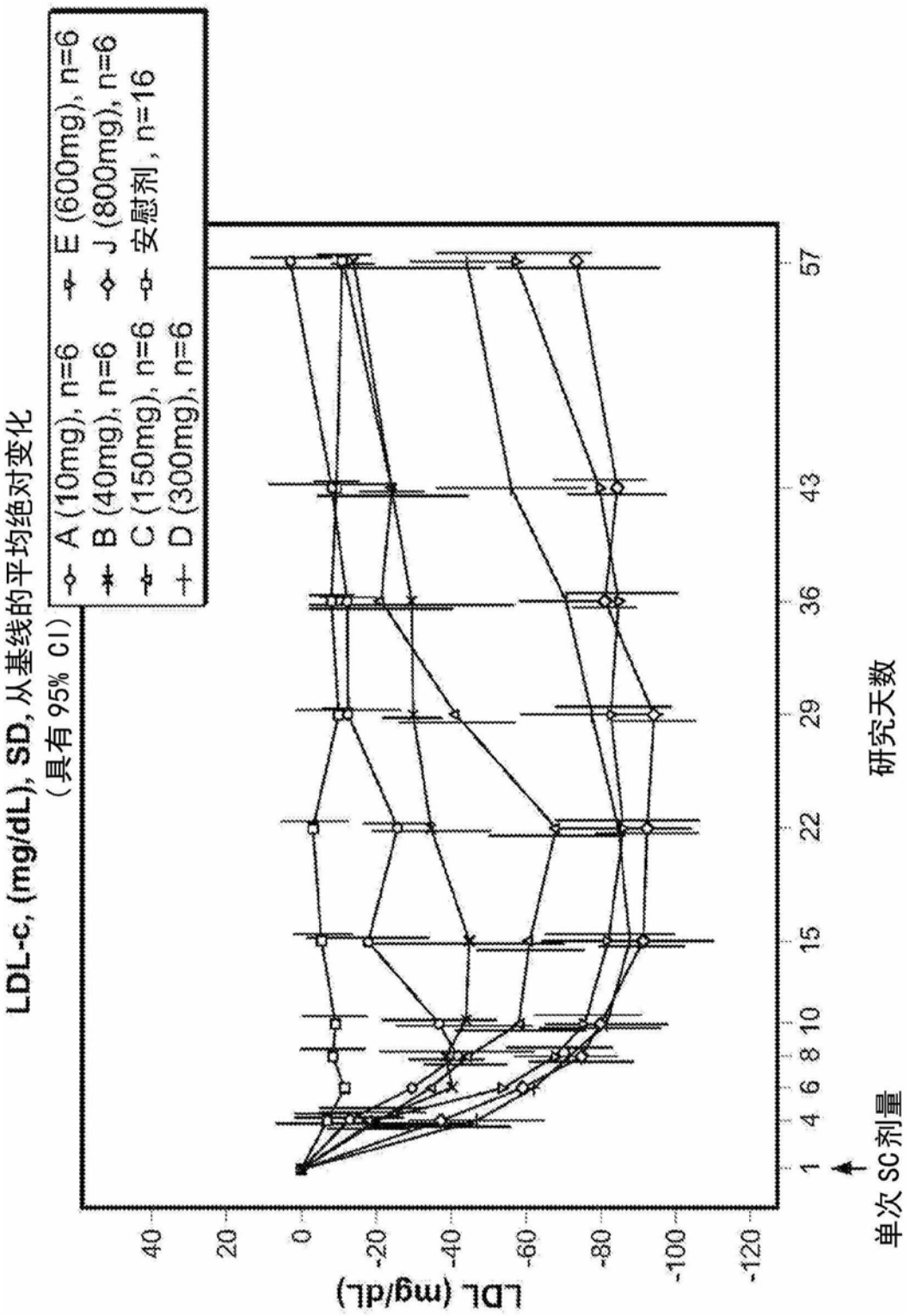

LDL-c, (mg/dL), SD, 从基线的平均绝对变化
（具有 95% CI）
A (10mg), n=6
B (40mg), n=6
C (150mg), n=6
D (300mg), n=6
E (600mg), n=6
J (800mg), n=6
安慰剂, n=16
LDL (mg/dL)
40
20
0
-20
-40
-60
-80
-100
-120
1
4
6
8
10
15
22
29
36
43
57
单次 SC 剂量
研究天数

图 16

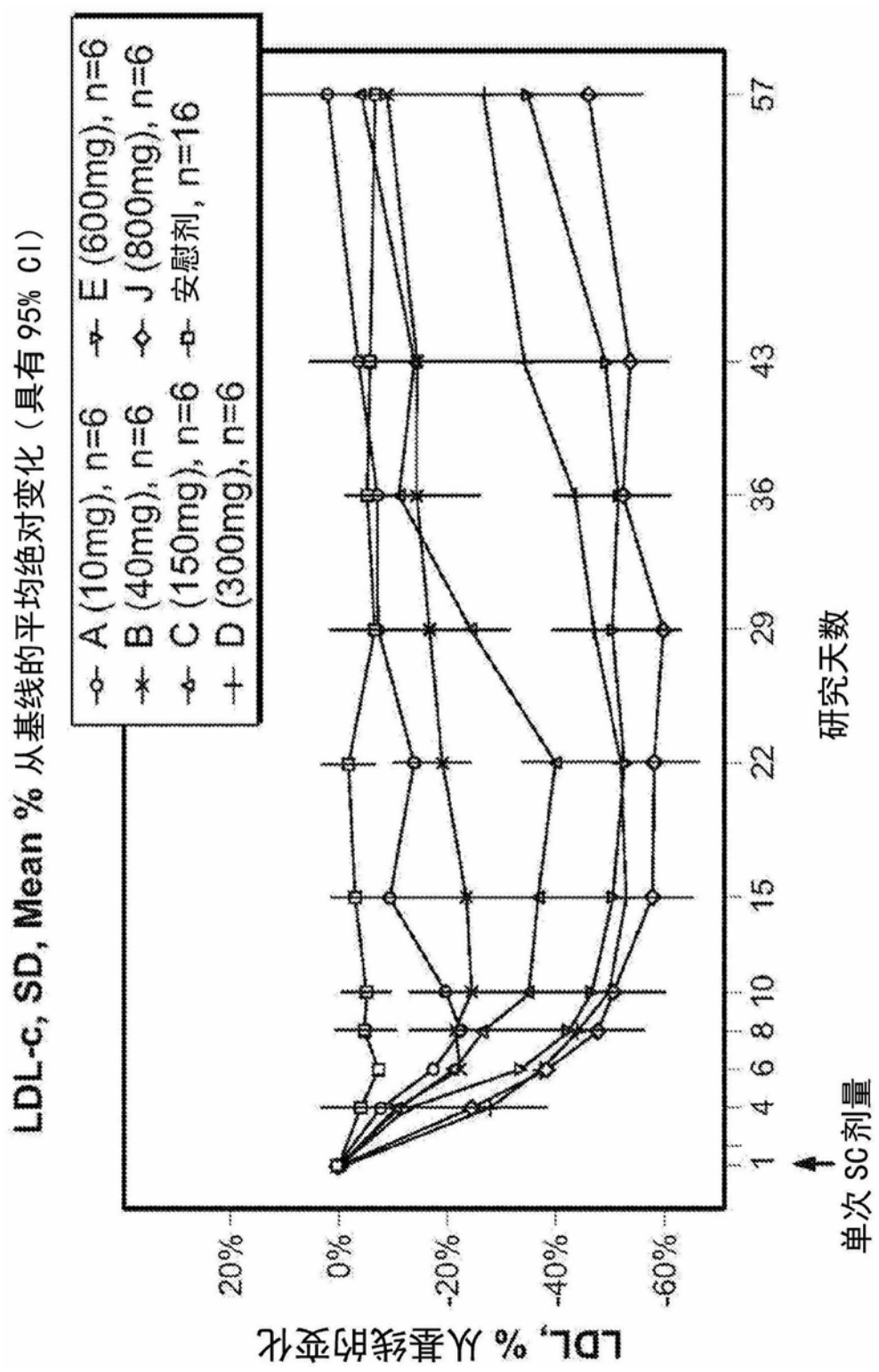

LDL-c, SD, Mean % 从基线的平均绝对变化（具有 95% CI）
A (10mg), n=6
B (40mg), n=6
C (150mg), n=6
D (300mg), n=6
E (600mg), n=6
J (800mg), n=6
安慰剂, n=16
LDL, % 从基线的变化
20%
0%
-20%
-40%
-60%
1 4 6 8 10 15 22 29 36 43 57
研究天数
单次 SC剂量

图 17

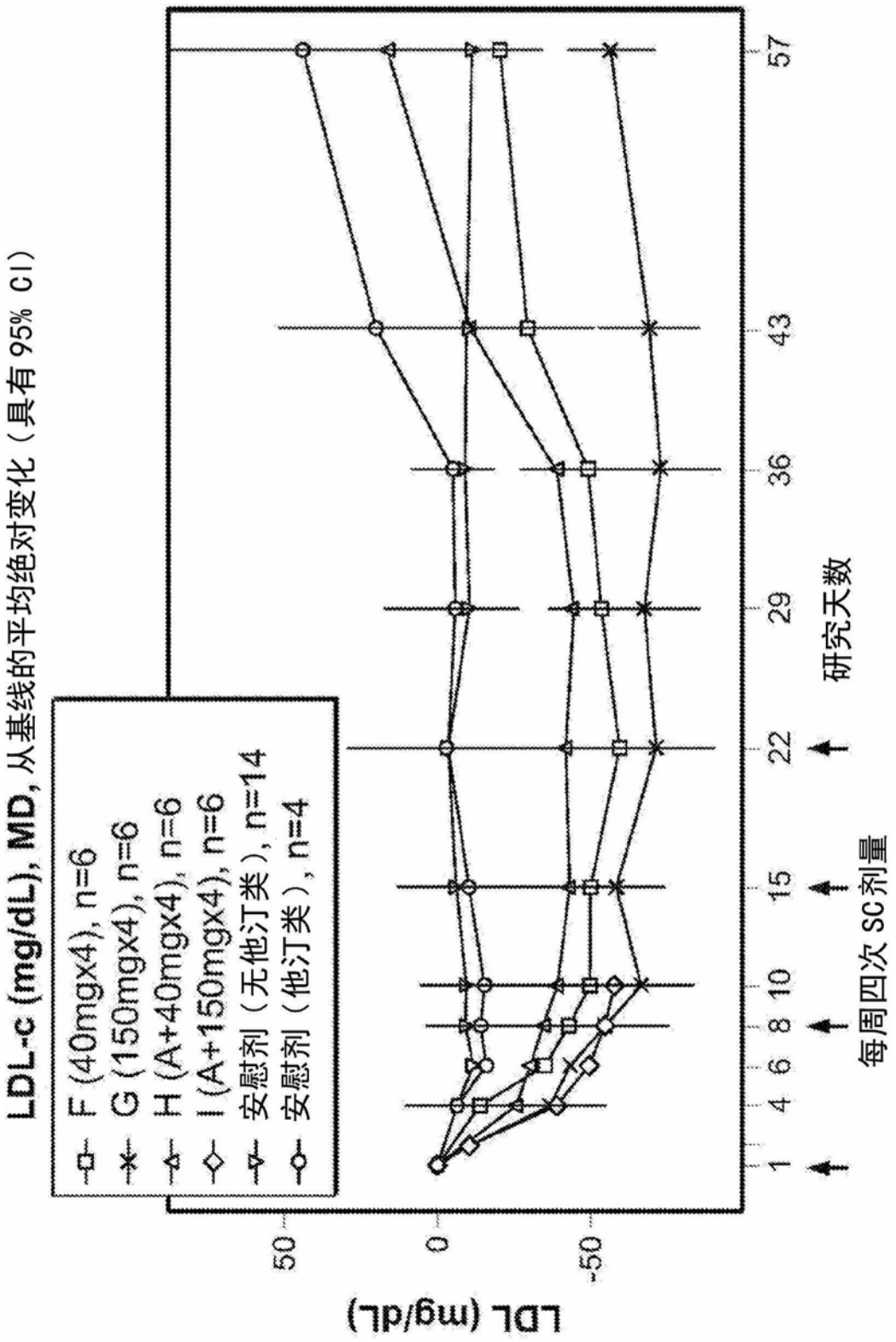

LDL-c (mg/dL), MD, 从基线的平均绝对变化（具有 95% CI）
F (40mgx4), n=6
G (150mgx4), n=6
H (A+40mgx4), n=6
I (A+150mgx4), n=6
安慰剂（无他汀类）, n=14
安慰剂（他汀类）, n=4
LDL (mg/dL)
50
0
-50
1
4
6
8
10
15
22
29
36
43
57
研究天数
每周四次 SC剂量

图 18

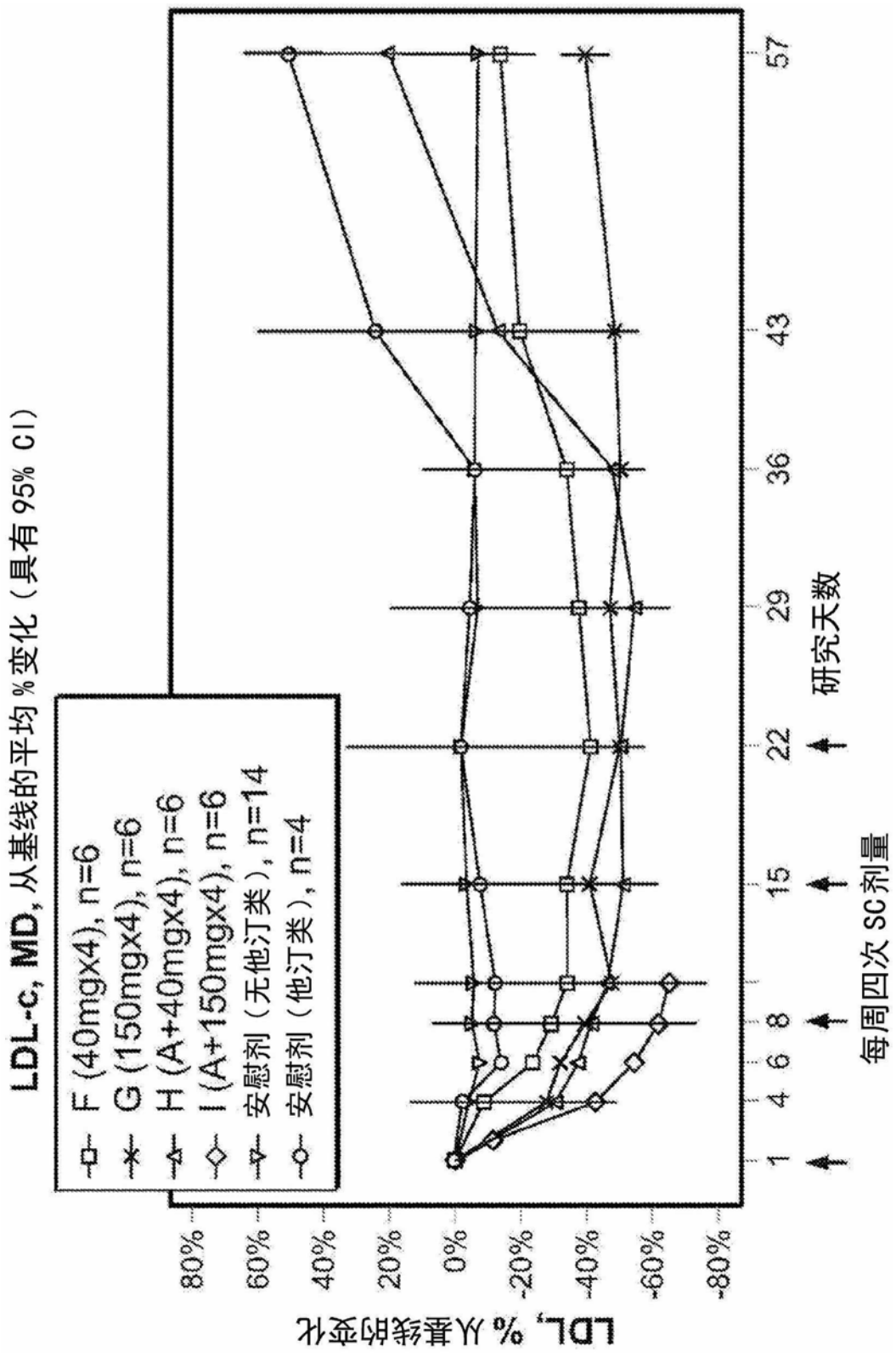

LDL-c, MD, 从基线的平均%变化（具有95% CI）
F (40mgx4), n=6
G (150mgx4), n=6
H (A+40mgx4), n=6
I (A+150mgx4), n=6
安慰剂（无他汀类）, n=14
安慰剂（他汀类）, n=4
LDL, % 从基线的变化
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
1
4
6
8
15
22
29
36
43
57
研究天数
每周四次 SC剂量

图 19

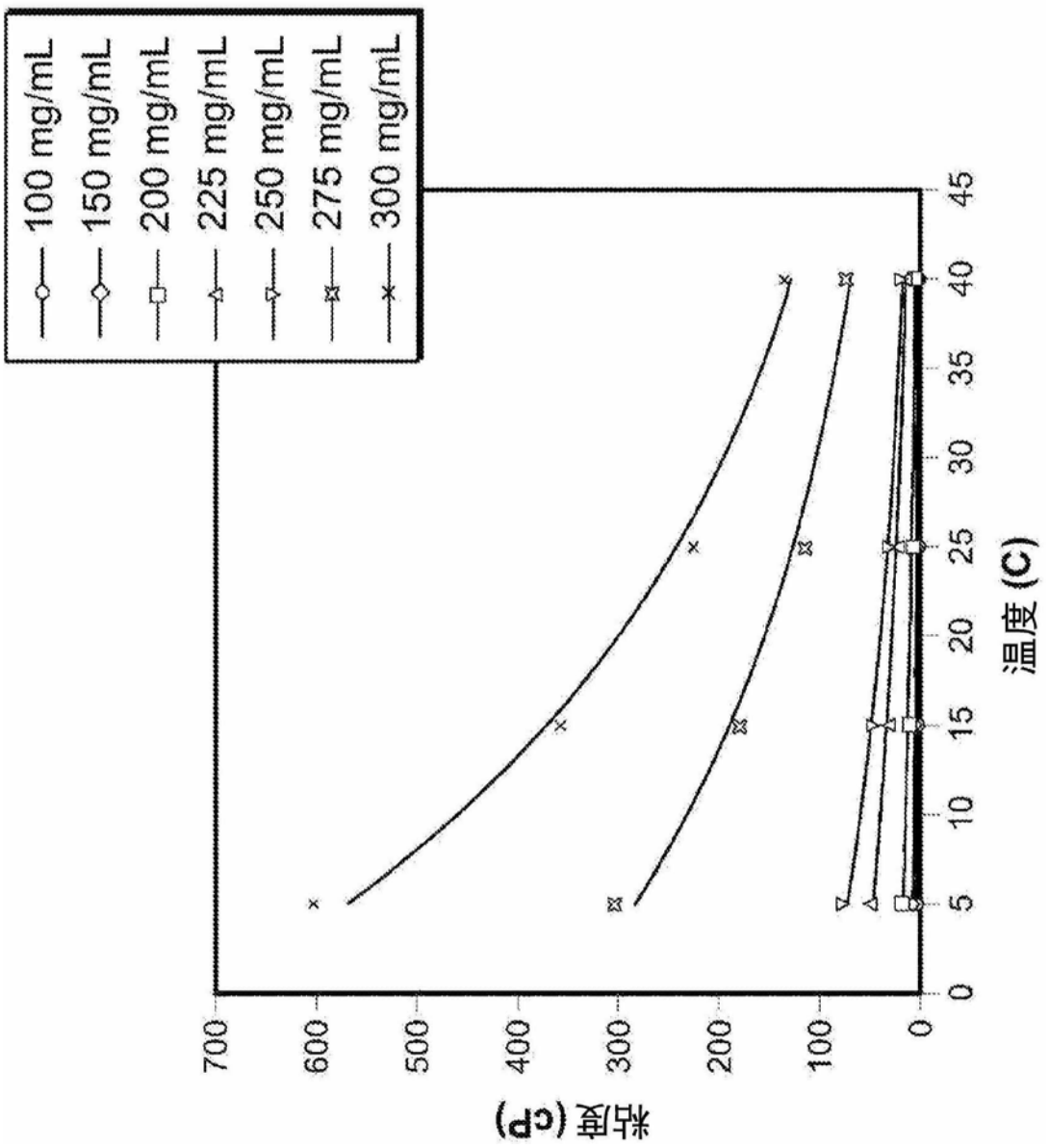

100 mg/mL
150 mg/mL
200 mg/mL
225 mg/mL
250 mg/mL
275 mg/mL
300 mg/mL
粘度 (cP)
0
100
200
300
400
500
600
700
温度 (C)
0
5
10
15
20
25
30
35
40
45

图 20

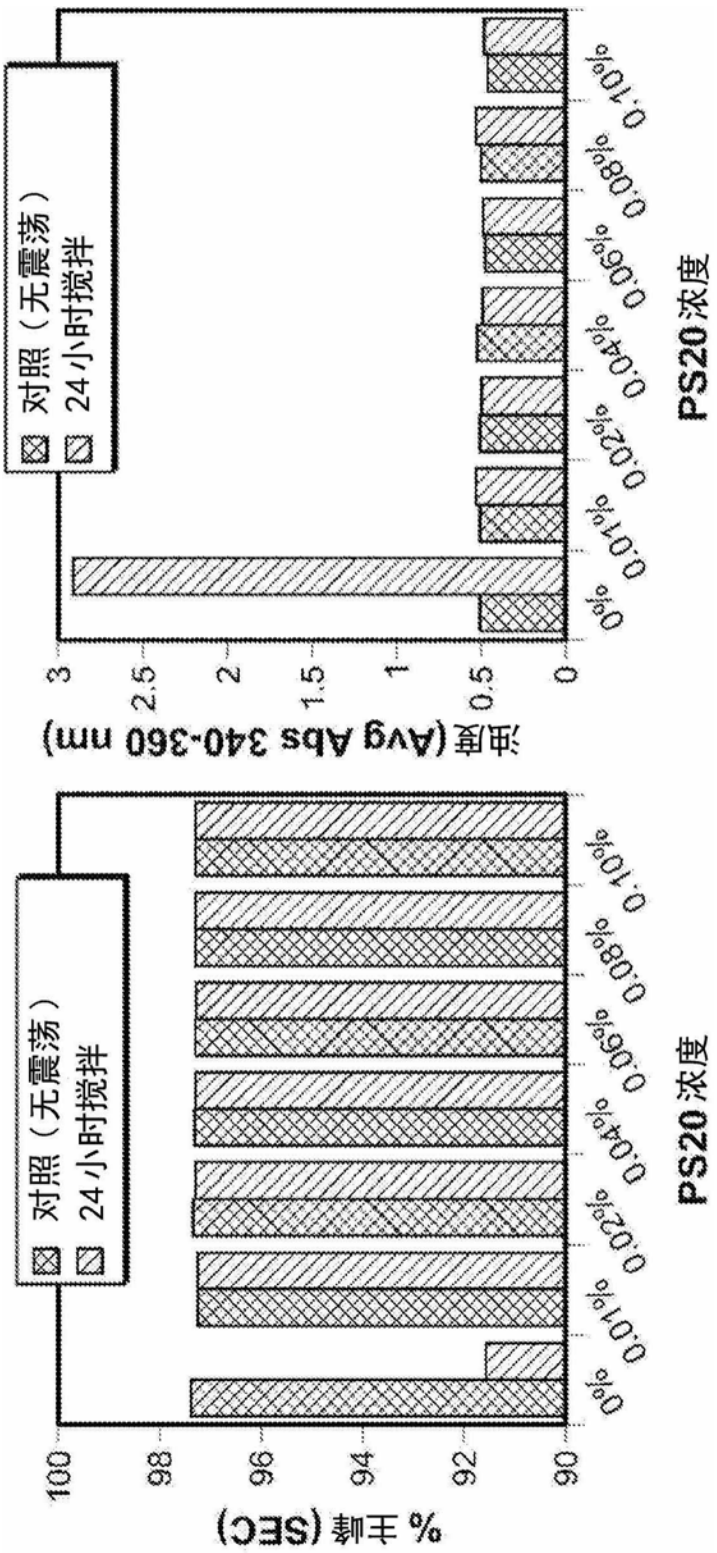

对照（无震荡）
24小时搅拌
0%
0.01%
0.02%
0.04%
0.06%
0.08%
0.10%
PS20浓度
浊度(Avg Abs 340-360 nm)
3
2.5
2
1.5
1
0.5
0
对照（无震荡）
24小时搅拌
0%
0.01%
0.02%
0.04%
0.06%
0.08%
0.10%
PS20浓度
% 主峰(SEC)
100
98
96
94
92
90

图 21

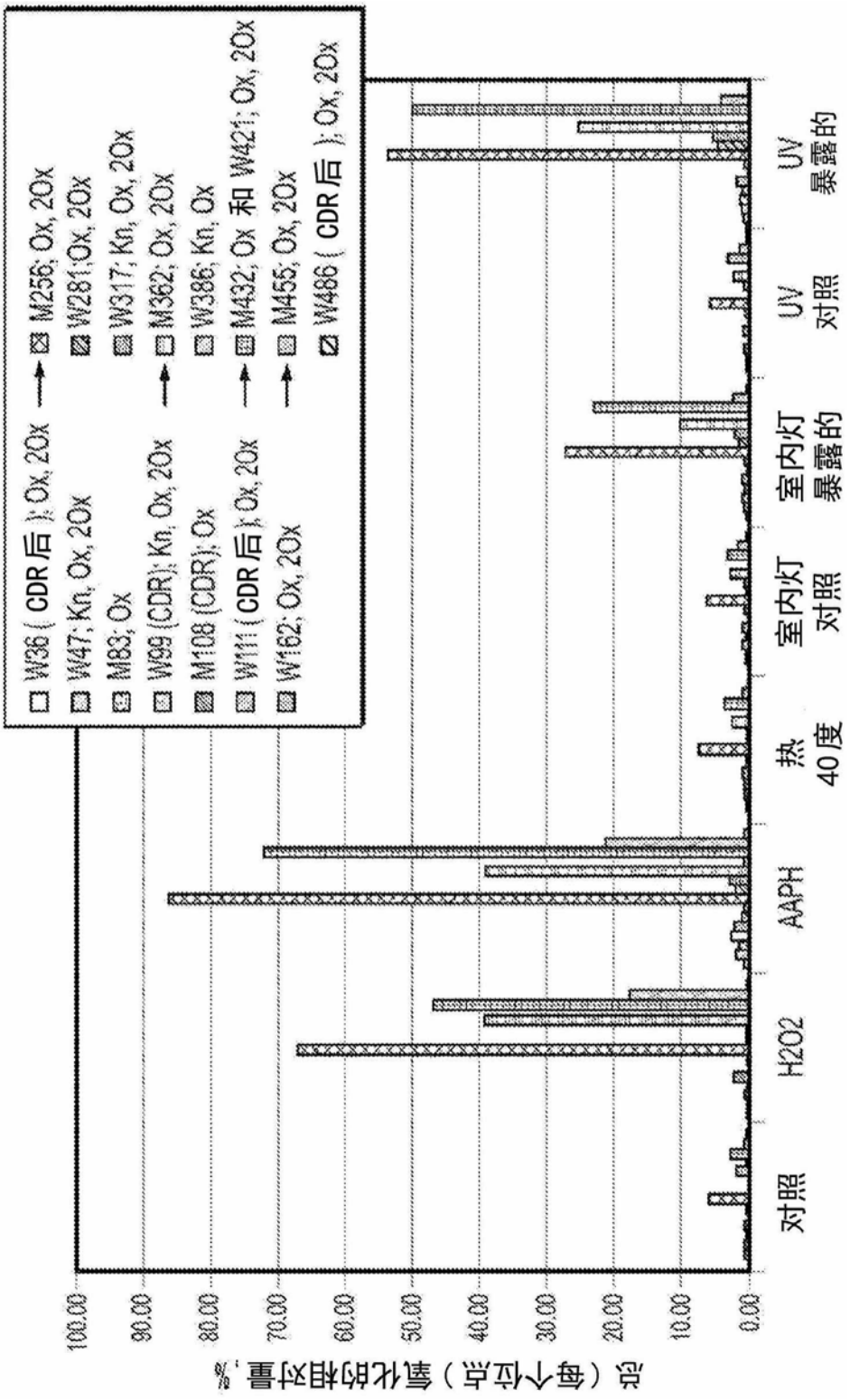

W36 (CDR后); Ox, 2Ox
W47; Kn, Ox, 2Ox
M83; Ox
W99 (CDR); Kn, Ox, 2Ox
M108 (CDR); Ox
W111 (CDR后); Ox, 2Ox
W162; Ox, 2Ox
M256; Ox, 2Ox
W281; Ox, 2Ox
W317; Kn, Ox, 2Ox
M362; Ox, 2Ox
W386; Kn, Ox
M432; Ox 和 W421; Ox, 2Ox
M455; Ox, 2Ox
W486 (CDR后); Ox, 2Ox
总（每个位点）氧化的相对量，%
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
对照
H2O2
AAPH
热 40度
室内灯 对照
室内灯 暴露的
UV 对照
UV 暴露的

图 22

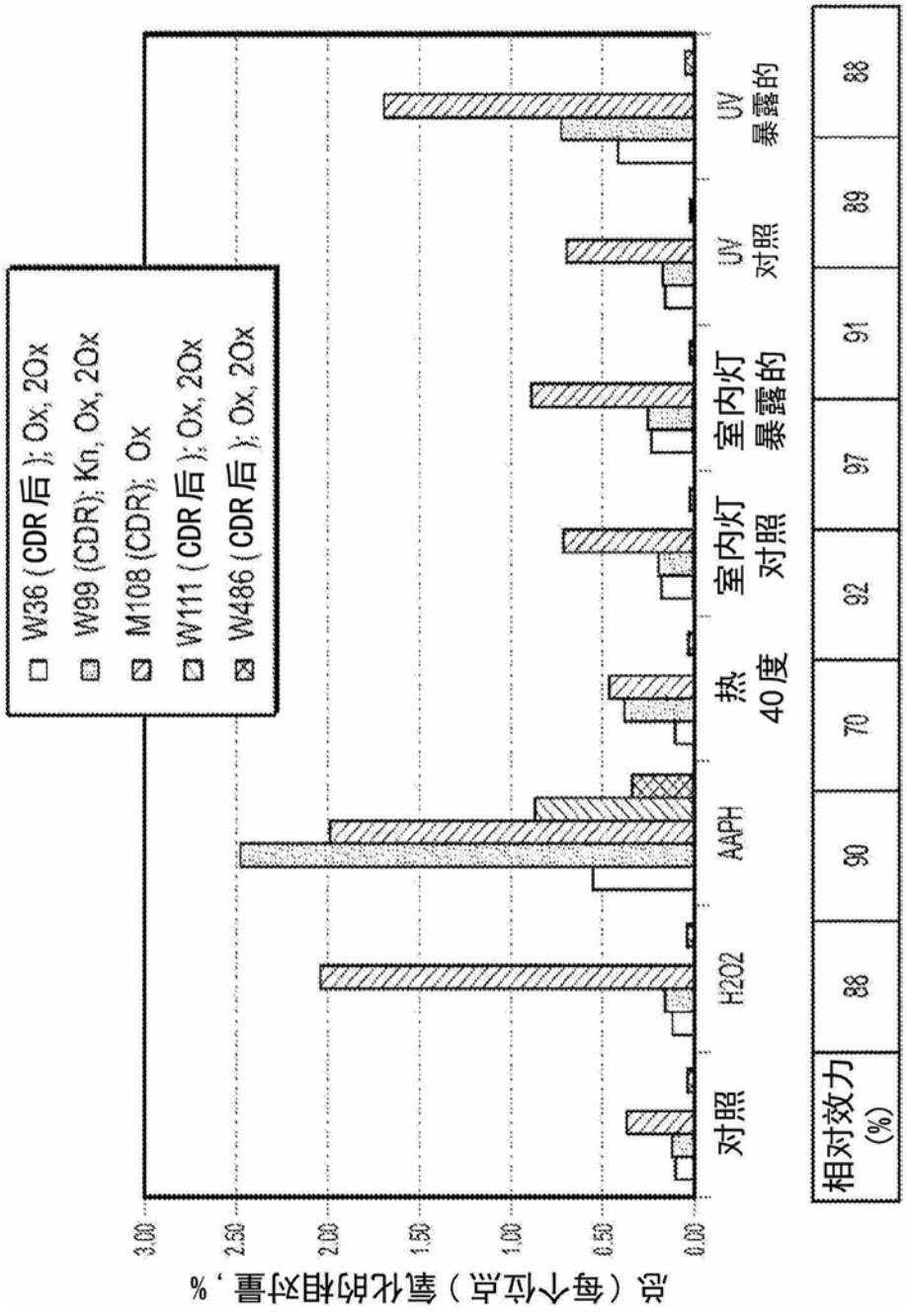

W36 (CDR后); Ox, 2Ox
W99 (CDR); Kn, Ox, 2Ox
M108 (CDR); Ox
W111 (CDR后); Ox, 2Ox
W486 (CDR后); Ox, 2Ox
总（每个位点）氧化的相对量，%
3.00
2.50
2.00
1.50
1.00
0.50
0.00
对照
H2O2
AAPH
热 40度
室内灯 对照
室内灯 暴露的
UV 对照
UV 暴露的
相对效力 (%)
88
90
70
92
97
91
89
88

图 23

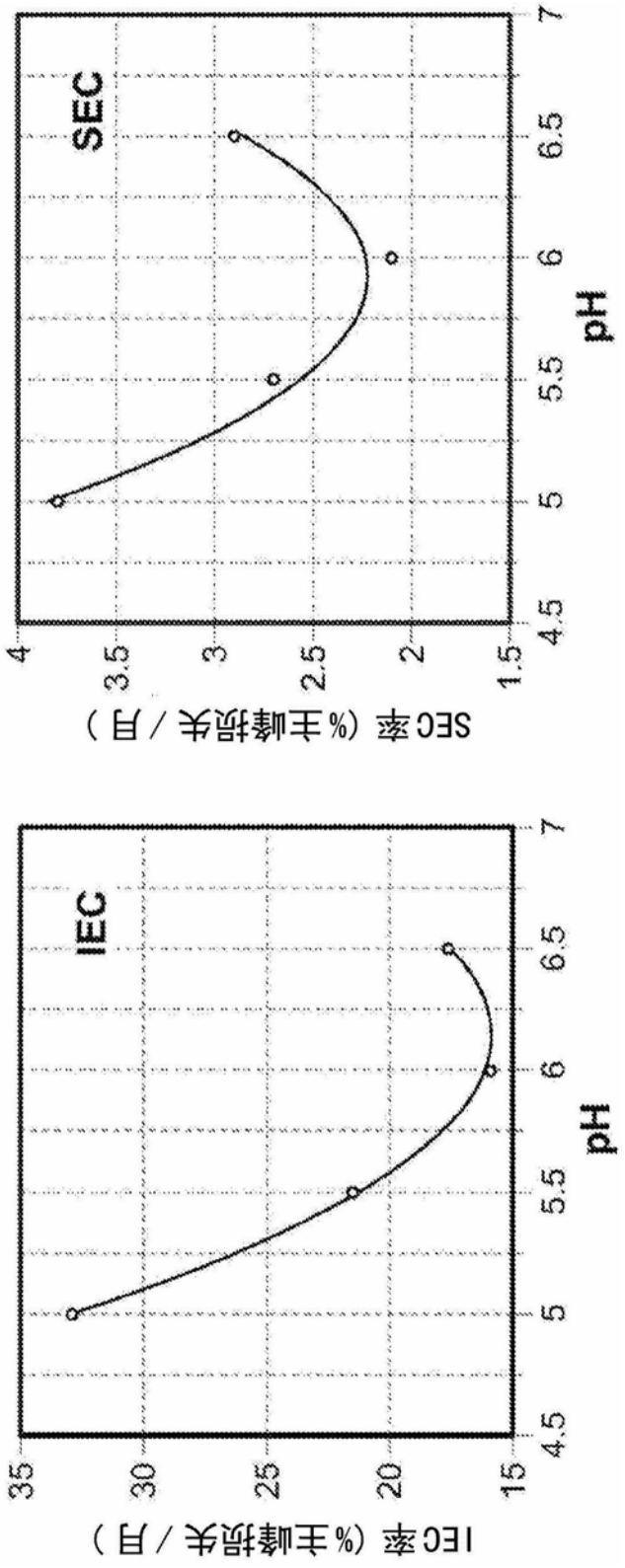


图 24

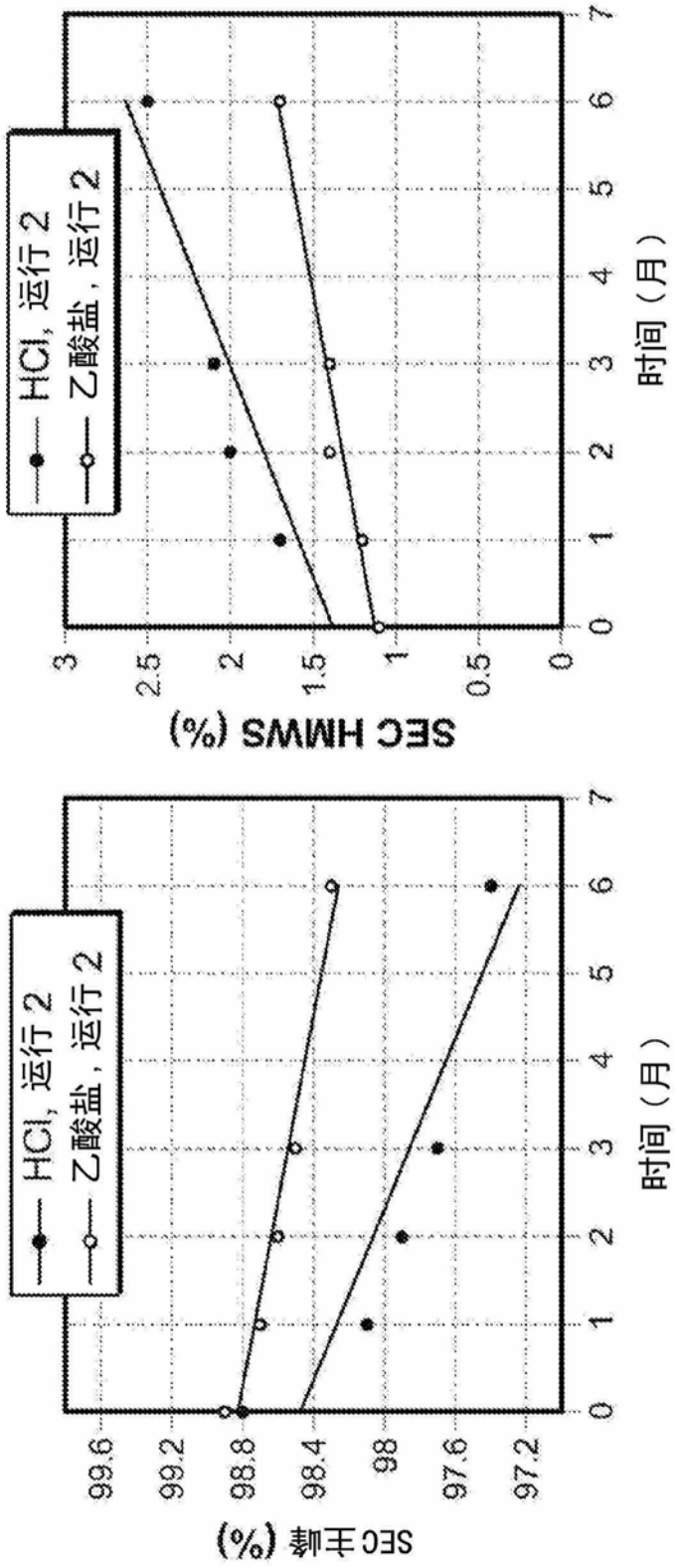

SEC HMWS (%)
3
2.5
2
1.5
1
0.5
0
HCl, 运行 2
乙酸盐，运行 2
0
1
2
3
4
5
6
7
时间（月）
SEC 主峰 (%)
99.6
99.2
98.8
98.4
98
97.6
97.2
HCl, 运行 2
乙酸盐，运行 2
0
1
2
3
4
5
6
7
时间（月）

图 25

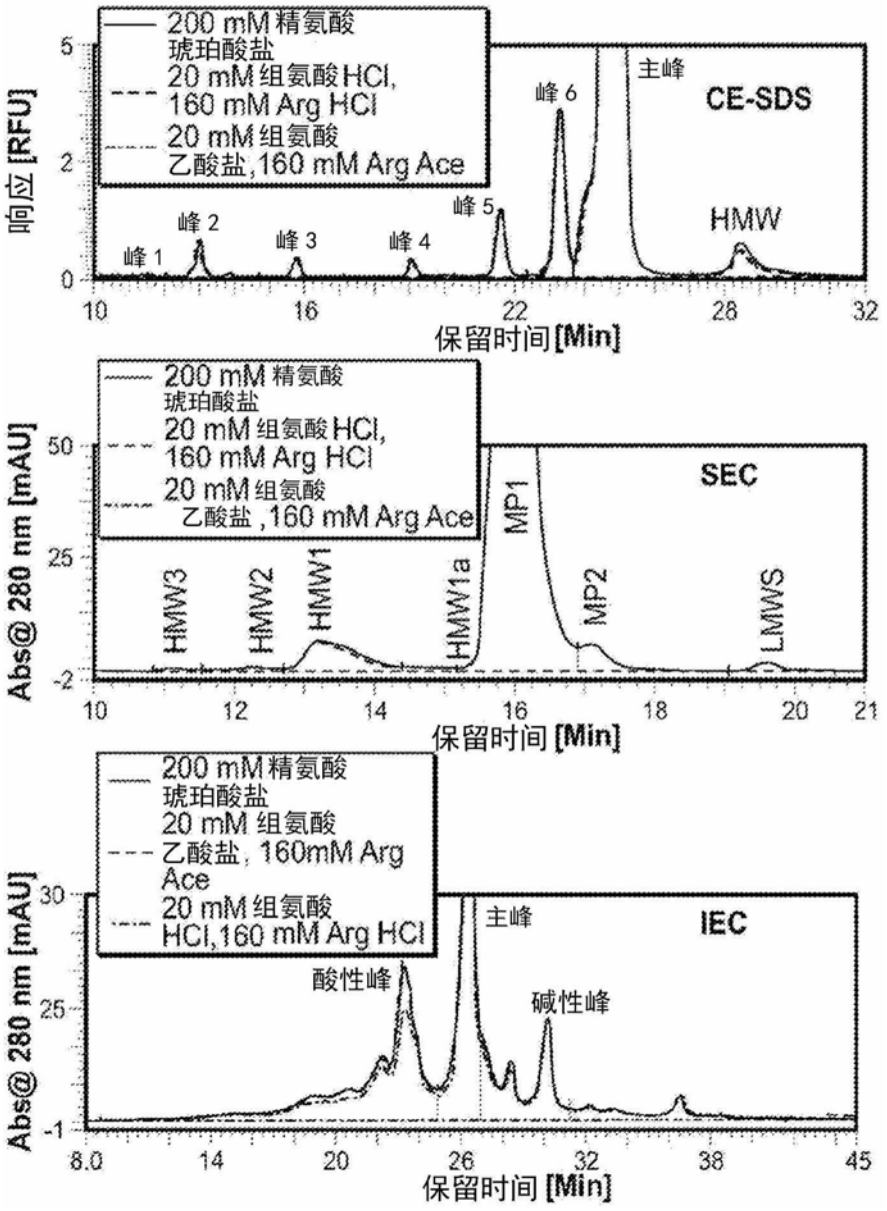

200 mM 精氨酸 琥珀酸盐
20 mM 组氨酸 HCl, 160 mM Arg HCl
20 mM 组氨酸 乙酸盐, 160 mM Arg Ace
响应 [RFU]
峰 1
峰 2
峰 3
峰 4
峰 5
峰 6
主峰
CE-SDS
HMW
保留时间 [Min]
Abs@ 280 nm [mAU]
HMW3
HMW2
HMW1
HMW1a
MP1
MP2
LMWS
SEC
保留时间 [Min]
酸性峰
主峰
碱性峰
IEC
保留时间 [Min]

图 26

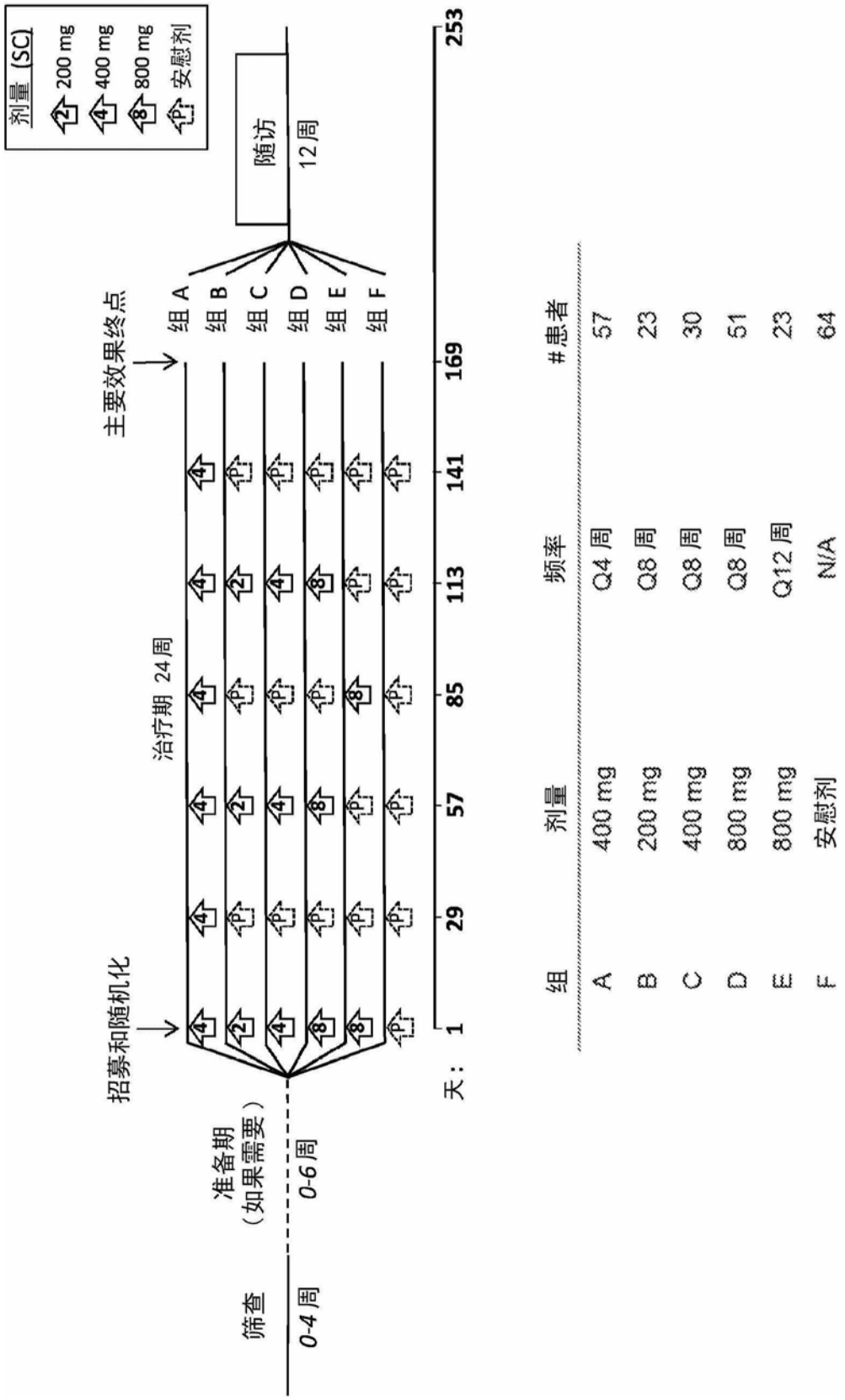

剂量 (SC)
200 mg
400 mg
800 mg
安慰剂
筛查
0-4 周
准备期
（如果需要）
0-6 周
招募和随机化
治疗期 24 周
主要效果终点
随访
12 周
组 A
组 B
组 C
组 D
组 E
组 F
天：
1
29
57
85
113
141
169
253
组 剂量 频率 #患者
A 400 mg Q4 周 57
B 200 mg Q8 周 23
C 400 mg Q8 周 30
D 800 mg Q8 周 51
E 800 mg Q12 周 23
F 安慰剂 N/A 64

图 27

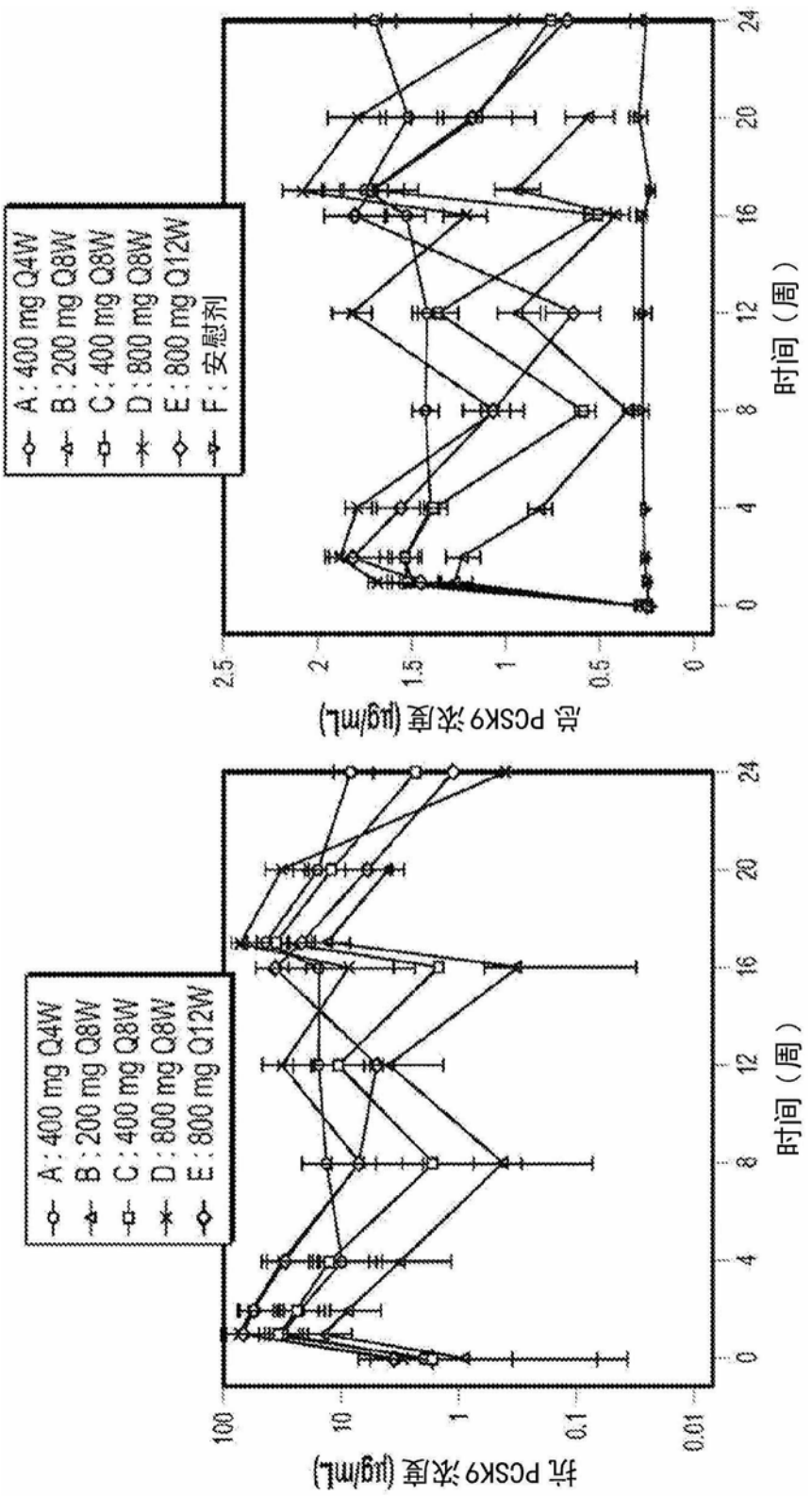
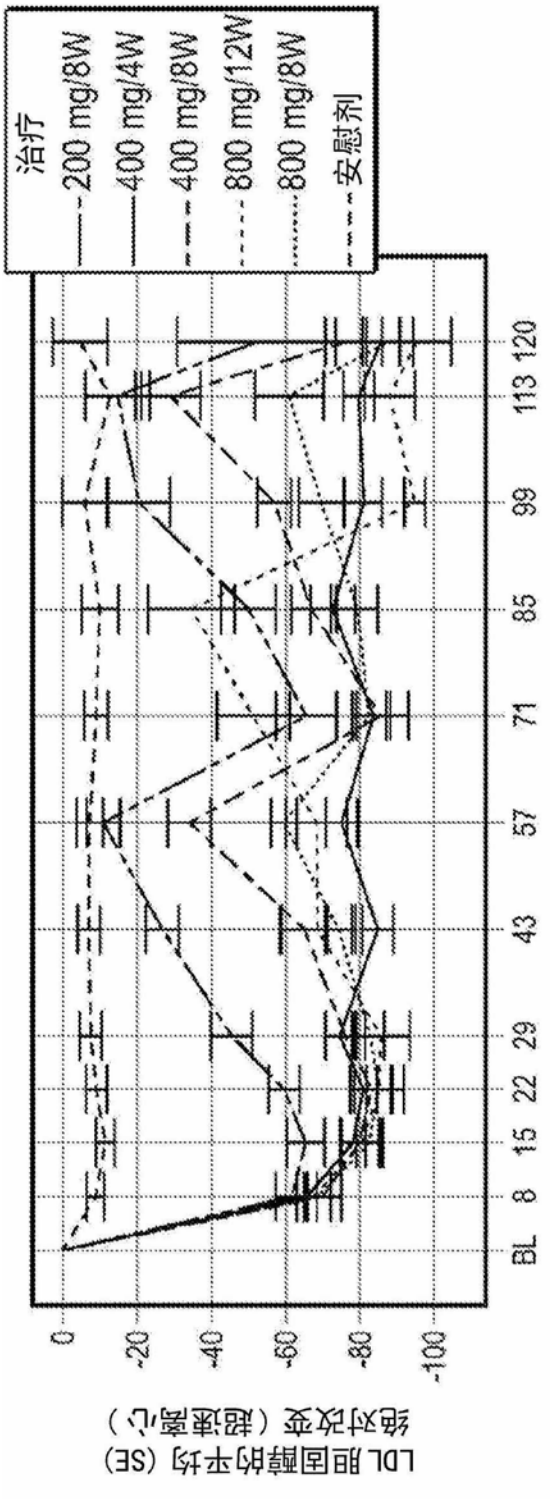

A：400 mg Q4W
B：200 mg Q8W
C：400 mg Q8W
D：800 mg Q8W
E：800 mg Q12W
F：安慰剂
总 PCSK9 浓度 (μg/mL)
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
24
时间（周）
A：400 mg Q4W
B：200 mg Q8W
C：400 mg Q8W
D：800 mg Q8W
E：800 mg Q12W
抗 PCSK9 浓度 (μg/mL)
100
10
1
0.1
0.01
0
4
8
12
16
20
24
时间（周）

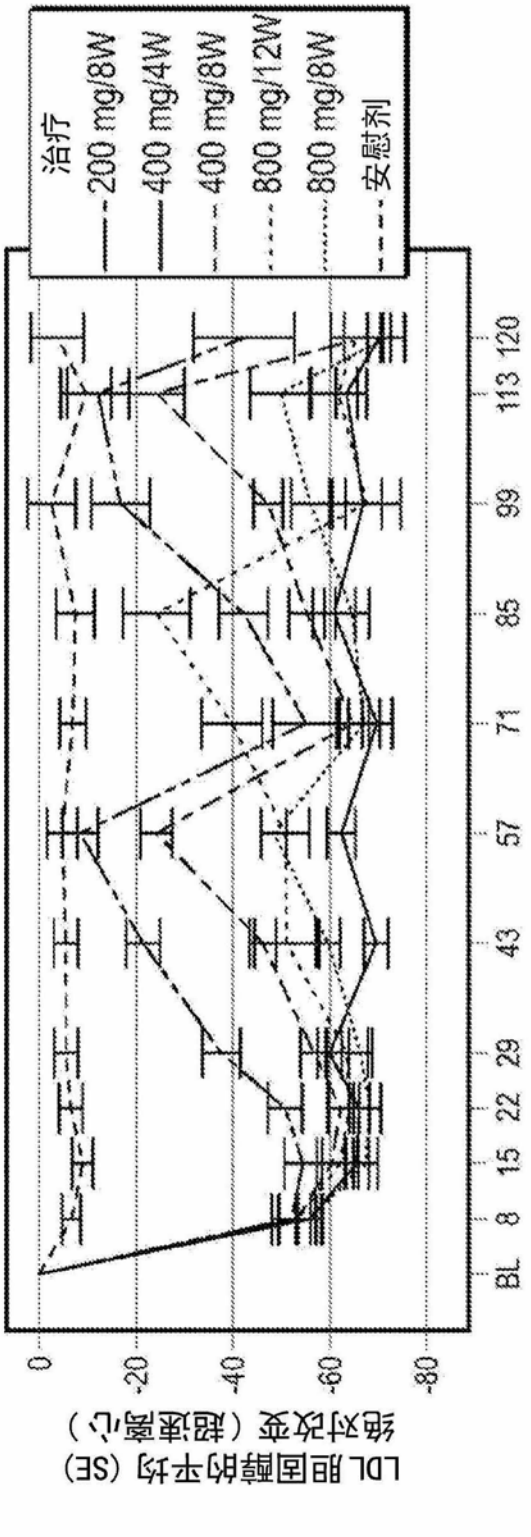
图 28



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (mg/dL)	-72.7	-50.0	-66.9	-79.3	-34.6	-10.0
从安慰剂的降低	**65.9**	**42.3**	**56.0**	**67.7**	**25.1**	
95% 置信区间	52.8, 78.9	23.7, 60.8	40.4, 71.7	54.1, 81.2	4.7, 45.5	
P- 值	<.0001	<.0001	<.0001	<.0001	0.0166	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P- 值关于基线 LDL-c(<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

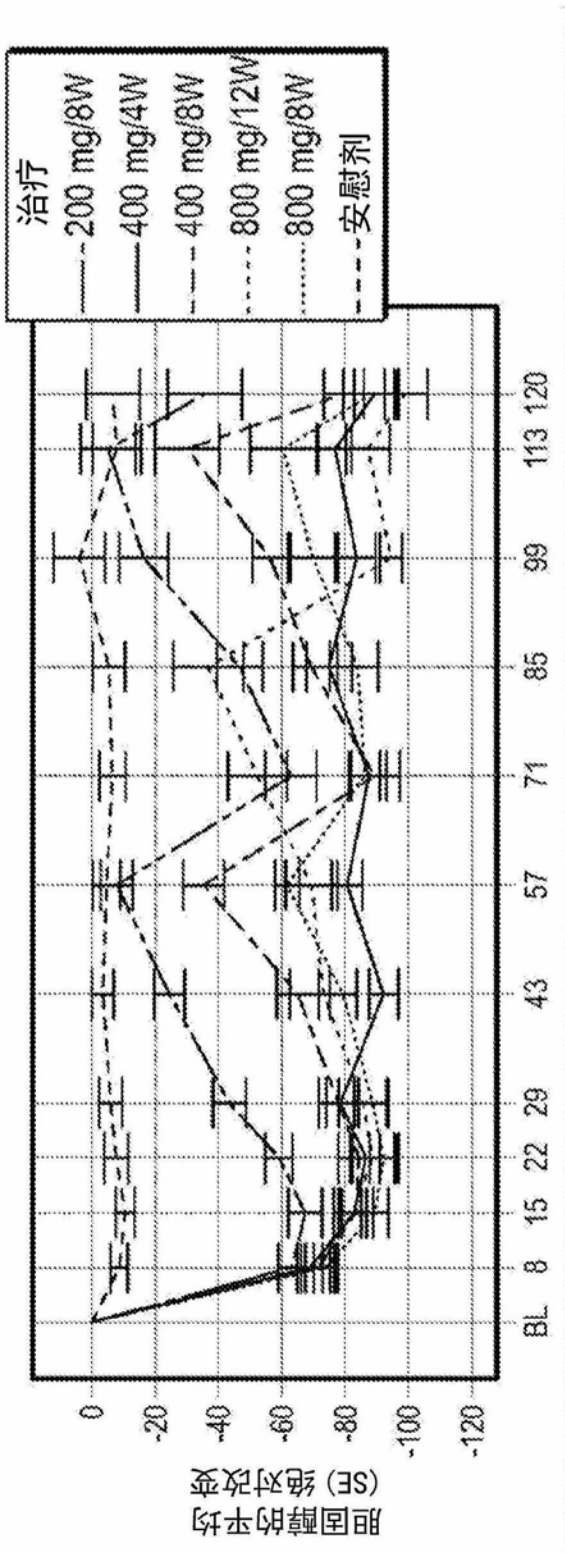
图 29



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (%)	-61.0	-42.2	-55.3	-64.7	-24.3	-7.5
从安慰剂的降低	**54.1**	**35.2**	**48.0**	**56.9**	**17.5**	
95% 置信区间	43.7, 64.5	20.4, 50.0	35.5, 60.5	46.1, 67.7	1.2, 33.8	
P- 值	<.0001	<.0001	<.0001	<.0001	0.0356	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P- 值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

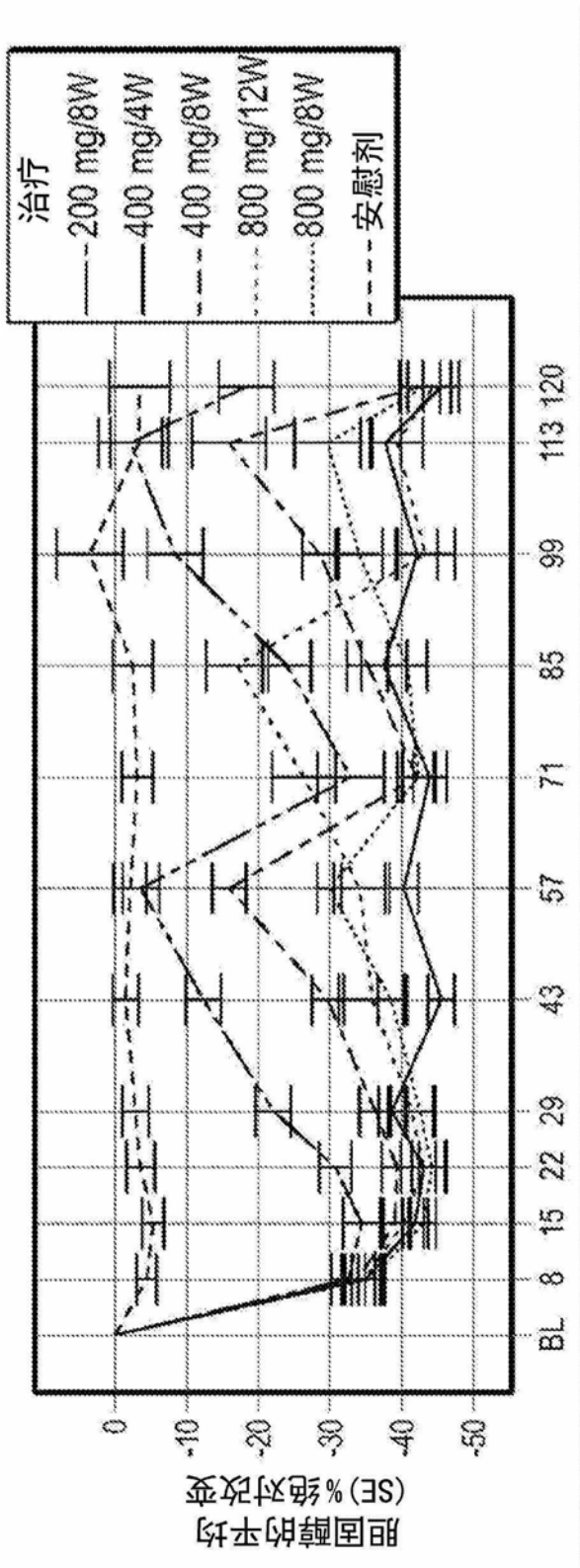
图 30



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (mg/dL)	-74.9	-46.7	-69.3	-84.0	-36.8	-5.4
从安慰剂的降低	**72.9**	**43.8**	**63.7**	**77.5**	**32.5**	
95% 置信区间	57.6, 88.1	22.0, 65.7	45.4, 82.0	61.6, 93.3	8.5, 56.6	
P-值	<.0001	0.0001	<.0001	<.0001	0.0084	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P-值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

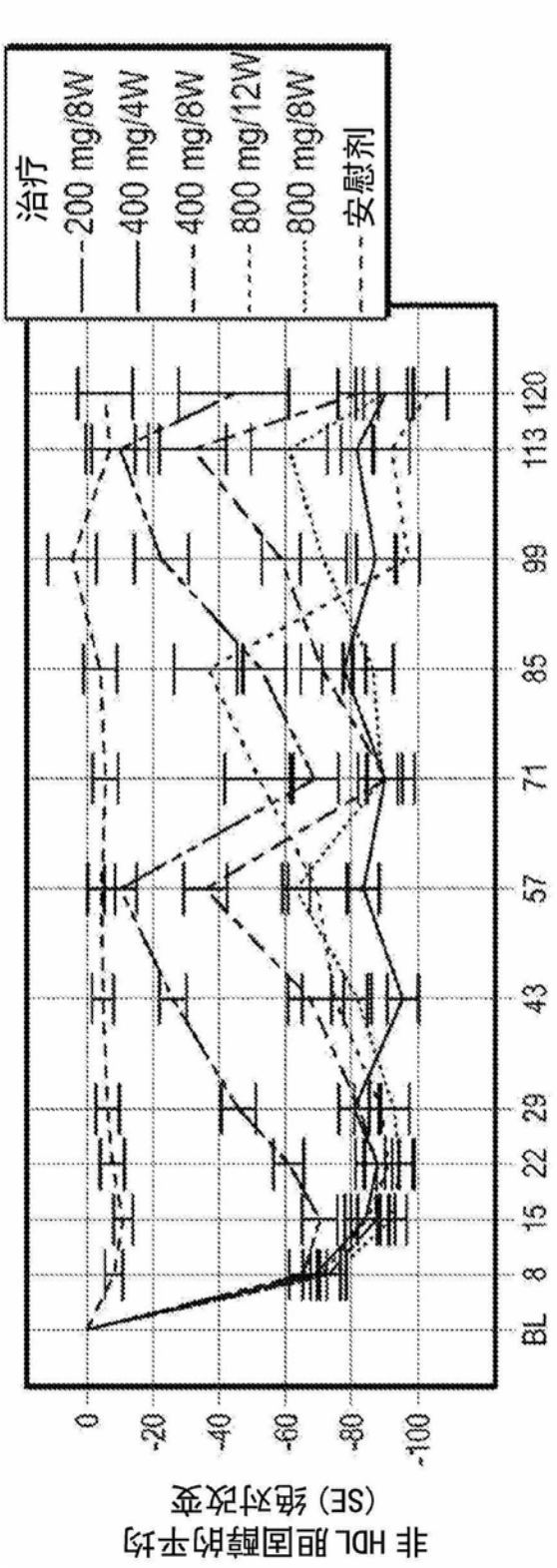
图 31



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85的平均变化(%)	-37.5	-23.9	-35.2	-40.7	-17.0	-2.4
从安慰剂的降低	**35.9**	**22.1**	**32.9**	**38.0**	**15.1**	
95%置信区间	28.4, 43.4	11.4, 32.8	23.9, 41.8	30.2, 45.7	3.4, 26.9	
P-值	<.0001	<.0001	<.0001	<.0001	0.0122	

注意：来自ANCOVA模型的与安慰剂的差异、95% CLs和P-值关于基线LDL-c(<120, >=120)和糖尿病状态（是，否）调整。对于多个测试不调整P值并且应该被谨慎解释。

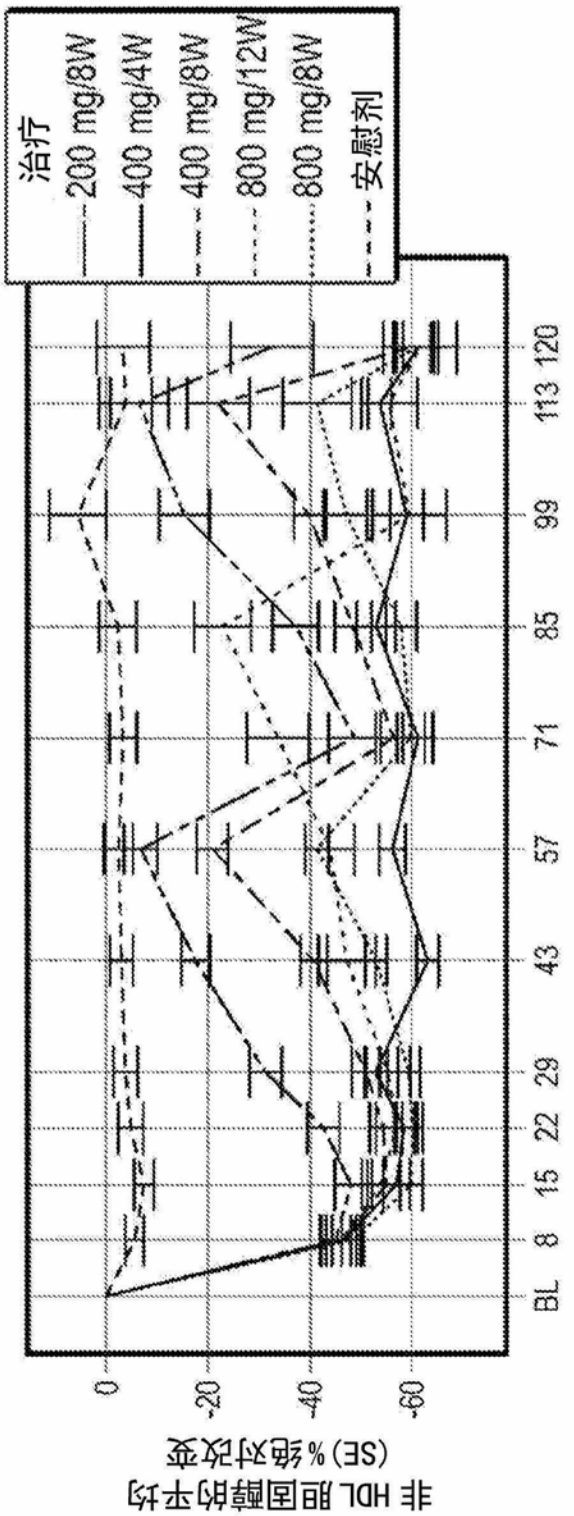
图 32



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (mg/dL)	-77.7	-52.7	-71.1	-86.6	-36.8	-3.9
从安慰剂的降低	**77.0**	**51.2**	**66.8**	**81.6**	**33.6**	
95% 置信区间	62.2, 91.7	30.1, 72.3	49.1, 84.5	66.3, 97.0	10.5, 56.8	
P-值	<.0001	<.0001	<.0001	<.0001	0.0049	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P-值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

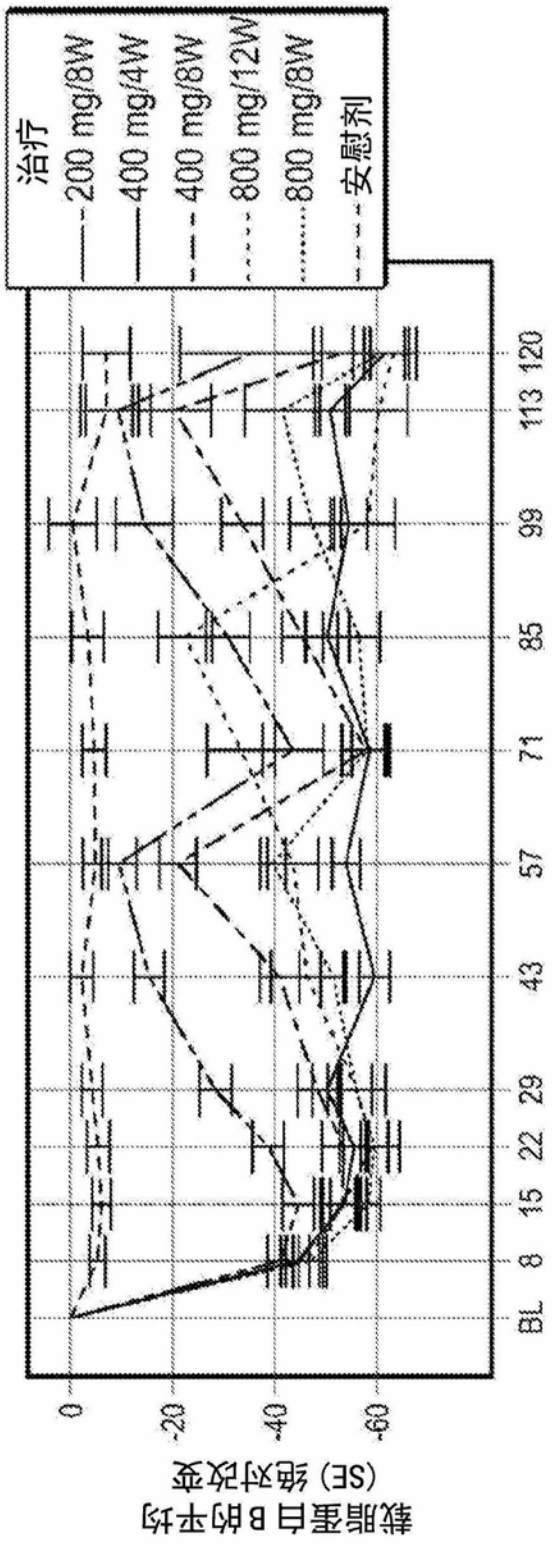
图 33



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (%)	-53.0	-37.2	-48.4	-57.9	-22.9	-2.4
从安慰剂的降低	**51.0**	**35.1**	**46.2**	**55.4**	**21.0**	
95% 置信区间	41.8, 60.3	21.9, 48.4	35.1, 57.3	45.8, 65.0	6.5, 35.6	
P-值	<.0001	<.0001	<.0001	<.0001	0.0050	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P-值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

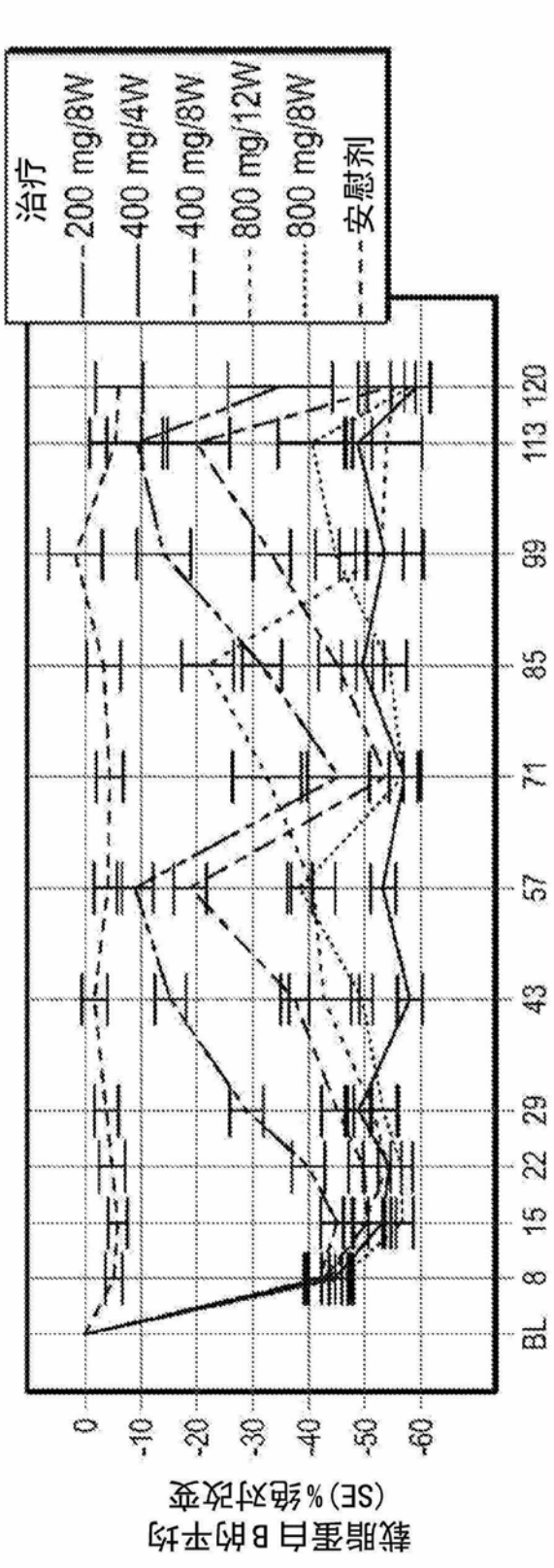
图 34



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (mg/dL)	-50.3	-30.8	-45.4	-56.5	-22.4	-3.4
从安慰剂的降低	**48.7**	**28.7**	**41.8**	**52.5**	**19.5**	
95% 置信区间	39.0, 58.4	14.8, 42.6	30.2, 53.5	42.4, 62.6	4.3, 34.8	
P-值	<.0001	<.0001	<.0001	<.0001	0.0127	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P-值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

图 35



	400 mg/4W (n=30)	200 mg/8W (n=9)	400 mg/8W (n=15)	800 mg/8W (n=25)	800 mg/12W (n=7)	安慰剂 (n=24)
D85 的平均变化 (%)	-49.6	-31.6	-45.1	-54.4	-21.9	-3.3
从安慰剂的降低	**46.5**	**28.5**	**41.9**	**51.0**	**19.0**	
95% 置信区间	37.8, 55.3	15.9, 41.0	31.4, 52.4	41.8, 60.1	5.2, 32.8	
P-值	<.0001	<.0001	<.0001	<.0001	0.0073	

注意：来自 ANCOVA 模型的与安慰剂的差异、95% CLs 和 P-值关于基线 LDL-c (<120, >=120) 和糖尿病状态（是，否）调整。对于多个测试不调整 P 值并且应该被谨慎解释。

图 36

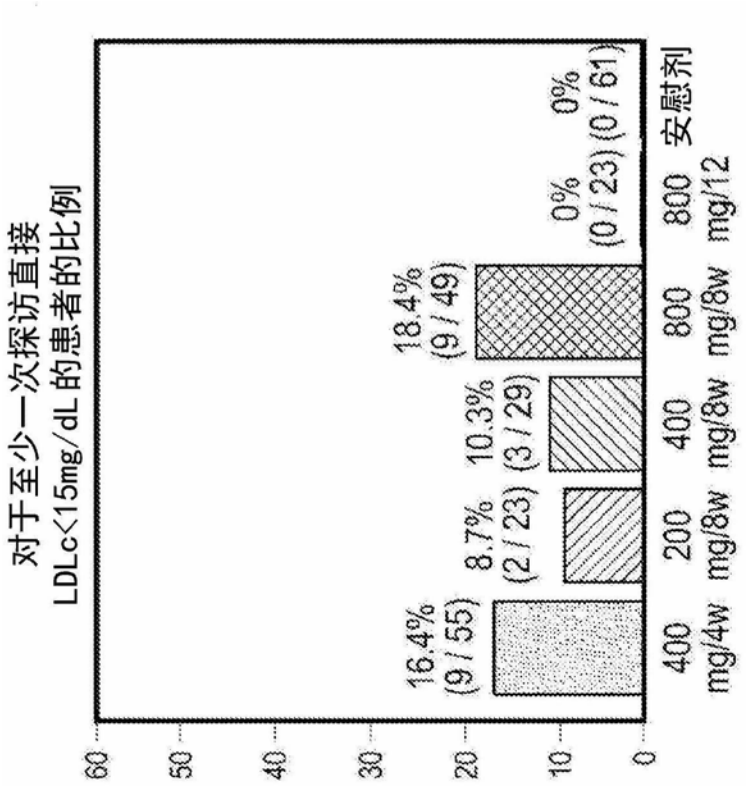

对于至少一次探访直接
LDLc<15mg/dL 的患者的比例
60
50
40
30
20
10
0
16.4%
(9 / 55)
8.7%
(2 / 23)
10.3%
(3 / 29)
18.4%
(9 / 49)
0%
(0 / 23)
0%
(0 / 61)
400 mg/4w
200 mg/8w
400 mg/8w
800 mg/8w
800 mg/12
安慰剂

图 37A

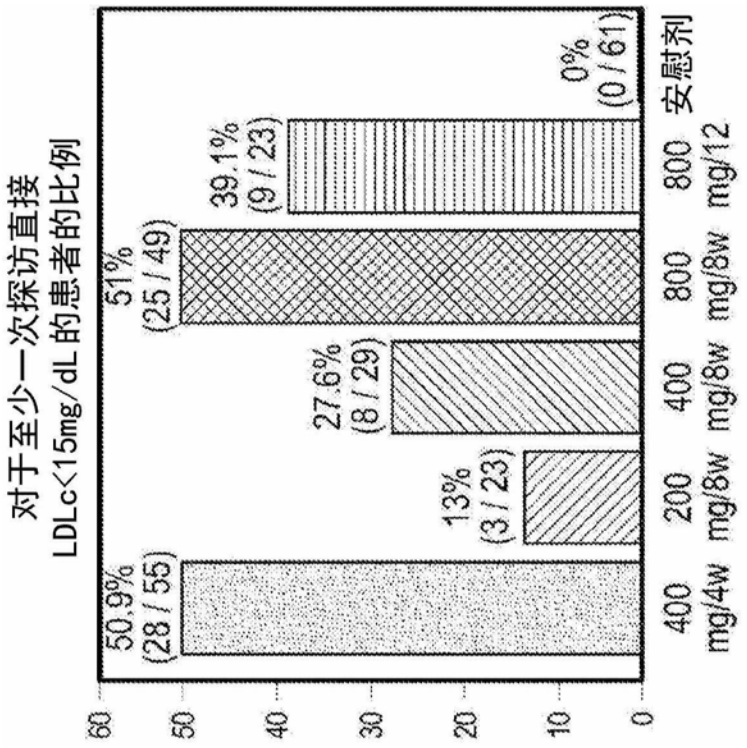

对于至少一次探访直接
LDLc<15mg/dL 的患者的比例
60
50
40
30
20
10
0
50.9%
(28 / 55)
13%
(3 / 23)
27.6%
(8 / 29)
51%
(25 / 49)
39.1%
(9 / 23)
0%
(0 / 61)
400 mg/4w
200 mg/8w
400 mg/8w
800 mg/8w
800 mg/12
安慰剂

图 37B