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(54) **Title:** HIGH AFFINITY ANTIBODIES AGAINST PCSK9

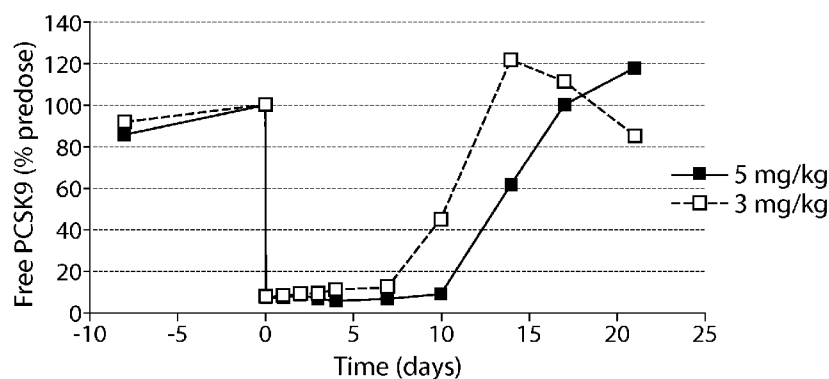


Fig. 7

(57) **Abstract:** Antibodies that target PCSK9, e.g., the prodomain of PCSK9, are provided. Also provided herein are compositions comprising the antibodies and methods of treatment, e.g., of cholesterol-related disorders, comprising administering the antibodies to a subject in need of such treatment.

HIGH AFFINITY ANTIBODIES AGAINST PCSK9

RELATED APPLICATIONS

This application claims priority to U.S. Application No. 62/016394, filed on June 24,
5 2014, the entire contents of which are hereby incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said
10 ASCII copy, created on June 17, 2015, is named D2046-7060WO_SL.txt and is 25,718 bytes in size.

FIELD OF THE INVENTION

The field of the invention relates to cholesterol metabolism. More particularly, the field
15 relates to regulation of PCSK9.

BACKGROUND

Despite advances in treating high cholesterol, there is a significant unmet need for treatments for diseases associated with high cholesterol. Conventional treatments such as statins
20 do not work for all patients. For example, some people are statin-intolerant, are not well controlled on statins, or have familial hypercholesterolemia. PCSK9 has been identified as a possible target for treating high cholesterol. However, molecules targeting PCSK9 have exhibited poor pharmacokinetics and therefore require frequent dosing and large volumes.

Proprotein convertase subtilisin-like/kexin type 9 (PCSK9, neural apoptosis-regulated
25 convertase 1, NARC-1) is a member of the serine protease family that is primarily expressed in hepatocytes. PCSK9 is initially expressed as a proprotein of about 75 kD that undergoes autocatalytic cleavage to produce mature PCSK9. The mature PCSK9 is secreted and binds to LDL receptor at the cell surface creating a bound complex (PCSK9/LDLR). PCSK9/LDLR is directed to the lysosome where it is degraded. This mechanism plays a role in regulating serum
30 LDL-c levels.

SUMMARY

The invention relates to the discovery of effective inhibitors of PCSK9 (e.g., human PCSK9). The PCSK9 inhibitors can be antibodies that target a PCSK9 (e.g., human PCSK9), or the prodomain of PCSK9, or antigen binding fragments or derivatives of such antibodies.

Typically, the PCSK9 inhibitor is an antibody (e.g., a humanized murine antibody), antibody fragment, or derivative thereof that can specifically bind to the prodomain of a human PCSK9. In embodiments, the PCSK9 inhibitor binds to PCSK9, e.g., to human PCSK9, e.g., to the prodomain of human PCSK9, with relatively high affinity. In embodiments, the PCSK9 inhibitor binds to human and/or rhesus PCSK9, e.g., with a K_d , K_{on} , or K_{off} as described herein.

In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a K_d of less than 200 pM, less than 175 pM, less than 150 pM, less than 125 pM, less than 100 pM, less than 75 pM, less than 50 pM, less than 25 pM, or less than 10 pM. In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a K_d of 1-200, 10-200, 10-150, 10-100, 10-75, 10-50, 10-30, or 10-20 pM. In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a K_d of ≤ 0.2 nM, e.g., with a K_d of 10 to 200 pM.

In embodiments, the K_d is determined as described herein. In embodiments, the K_d is determined using biolayer interferometry, ELISA, FACS, or surface plasmon resonance assay. In embodiments, the K_d is determined using biolayer interferometry, FACS, or ELISA. In embodiments, the K_d is determined using biolayer interferometry. In embodiments, the K_d is determined using ELISA. In embodiments, the K_d is determined using FACS.

In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a dissociation rate (k_{off}) of 1×10^{-5} to $1 \times 10^{-3} s^{-1}$, e.g., as determined using SPR. In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a dissociation rate (k_{off}) of less than $1 \times 10^{-3} s^{-1}$, less $0.5 \times 10^{-4} s^{-1}$, or less than $0.4 \times 10^{-4} s^{-1}$, e.g., as determined using surface plasmon resonance (SPR). In embodiments, the PCSK9 inhibitor has a dissociation rate of about $0.36 \times 10^{-4} s^{-1}$, or a dissociation rate of about $0.36 \times 10^{-4} s^{-1}$ or less, e.g., as determined using SPR.

In embodiments, the PCSK9 inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a dissociation rate (k_{off}) of 1×10^{-6} to $1 \times 10^{-3} s^{-1}$, 1×10^{-6} to $1 \times 10^{-4} s^{-1}$, or 1×10^{-6} to $1 \times 10^{-5} s^{-1}$, e.g., as determined using biolayer interferometry. In embodiments, the PCSK9

inhibitor binds to PCSK9 (e.g., human and/or rhesus PCSK9) with a dissociation rate (k_{off}) of less than $1 \times 10^{-5} \text{ s}^{-1}$ or less than $1 \times 10^{-4} \text{ s}^{-1}$, e.g., as determined using biolayer interferometry. In embodiments, the PCSK9 inhibitor has a dissociation rate of about $6 \times 10^{-6} \text{ s}^{-1}$, or a dissociation rate of about $6 \times 10^{-6} \text{ s}^{-1}$ or less, e.g., as determined using biolayer interferometry.

5 In some embodiments, the PCSK9 inhibitor exhibits pH-dependent binding to PCSK9.

Specific examples of a PCSK9 inhibitor include a polypeptide that includes at least one of SEQ ID NO:5, 6, or 7 and at least one of SEQ ID NO:8 or 9 and can specifically bind to PCSK9. In some embodiments, the PCSK9 inhibitor includes at least two sequences selected from SEQ ID NOs: 5, 6, 7, 8, 9, and 10.

10 In embodiments, the PCSK9 inhibitor (e.g., the PCSK9 antibody or antigen binding fragment) comprises an immunoglobulin chain, e.g., a heavy chain sequence, e.g., a heavy chain variable domain, comprising one or more sequences disclosed herein, e.g., three heavy chain CDR sequences disclosed herein, e.g., SEQ ID NO:5, 6, and 7.

In embodiments, the PCSK9 inhibitor (e.g., the PCSK9 antibody or antigen binding
15 fragment) comprises an immunoglobulin chain, e.g., a light chain sequence, e.g., a light chain variable domain, comprising one or more sequences disclosed herein, e.g., three light chain CDR sequences disclosed herein, e.g., SEQ ID NO:8, 9, and 10.

In embodiments, the PCSK9 inhibitor (e.g., the PCSK9 antibody or antigen binding
20 fragment) comprises (e.g., consists essentially of, or consists of) a heavy chain sequence and/or a light chain sequence comprising (e.g., consisting essentially of, or consisting of) one or more sequences described herein. In embodiments, the PCSK9 inhibitor comprises heavy chain CDR sequences (e.g., SEQ ID NO: 5, 6, and 7) and/or light chain CDR sequences (e.g., SEQ ID NO:8, 9, and 10) as disclosed herein.

In embodiments, the PCSK9 inhibitor comprises a heavy chain sequence comprising
25 heavy chain CDR sequences disclosed herein (e.g., SEQ ID NO: 5, 6, and 7) and/or a light chain sequence comprising light chain CDR sequences disclosed herein (e.g., SEQ ID NO:8, 9, and 10), or comprises CDRs that differ by at most 1, 2, 3, 4, or 5 amino acids (e.g., collectively comprise at most 1, 2, 3, 4, or 5 amino acid substitutions) relative to the heavy and/or light chain CDR sequences disclosed herein.

30 In embodiments, the PCSK9 inhibitor comprises a heavy chain variable domain sequence disclosed herein (e.g., SEQ ID NO:17) and/or a light chain variable domain sequence disclosed

herein (e.g., SEQ ID NO:18). In embodiments, the PCSK9 inhibitor comprises (i) a heavy chain variable domain sequence that is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a heavy chain variable domain sequence disclosed herein (e.g., SEQ ID NO:17) and/or (ii) a light chain variable domain sequence that is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a light chain variable domain sequence disclosed herein (e.g., SEQ ID NO:18). In embodiments, the PCSK9 inhibitor comprises (i) a heavy chain variable domain sequence that differs by at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., comprises at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions) relative to a heavy chain variable domain sequence disclosed herein (e.g., SEQ ID NO:17) and/or (ii) a light chain variable domain sequence that differs by at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., comprises at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions) relative to a light chain variable domain sequence disclosed herein (e.g., SEQ ID NO:18).

In embodiments, the PCSK9 inhibitor comprises a heavy chain sequence (e.g., SEQ ID NO:19) and/or a light chain sequence (e.g., SEQ ID NO:20) disclosed herein. In embodiments, the PCSK9 inhibitor comprises (e.g., consists of) (i) a heavy chain sequence that is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a heavy chain sequence disclosed herein (e.g., SEQ ID NO:19) and/or (ii) a light chain sequence that is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to a light chain sequence disclosed herein (e.g., SEQ ID NO:20). In embodiments, the PCSK9 inhibitor comprises (e.g., consists of) (i) a heavy chain sequence that differs by at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., comprises at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions) relative to a heavy chain sequence disclosed herein (e.g., SEQ ID NO:19) and/or (ii) a light chain sequence that differs by at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., comprises at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions) relative to a light chain sequence disclosed herein (e.g., SEQ ID NO:20).

In certain embodiments, the PCSK9 inhibitor (e.g., antibody, fragment, or derivative) can bind (e.g., specifically bind) to S127 and D129 of a human PCSK9 (e.g., SEQ ID NO:1)

In embodiments, the PCSK9 inhibitor is an antigen binding fragment, e.g., a Fab, Fab', Fab'-SH, Fd, Fv, F(ab')₂, scFv, dsFv, or other antigen binding fragment (e.g., as disclosed herein or known in the art).

In embodiments, the PCSK9 inhibitor (e.g., the PCSK9 antibody) comprises a human Fc region sequence, e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region sequence.

Also provided herein is a nucleic acid sequence encoding a PCSK9 inhibitor (e.g., a PCSK9 antibody or antigen-binding fragment) disclosed herein. Also provided herein is a vector comprising the nucleic acid sequence. Also provided herein is a host cell comprising the nucleic acid sequence or vector.

5 Also provided herein is a pharmaceutical composition comprising a PCSK9 inhibitor (e.g., a PCSK9 antibody or antigen-binding fragment) disclosed herein and optionally a pharmaceutically acceptable excipient (e.g., a pharmaceutically acceptable carrier).

Also provided herein is a kit comprising a PCSK9 inhibitor disclosed herein and optionally, instructions for use. The kit can include other features or elements disclosed herein.

10 In another embodiment, the invention relates to a method of inhibiting PCSK9. The method includes contacting a sample comprising a PCSK9 with a PCSK9 inhibitor disclosed herein (e.g., an antibody, fragment, or derivative thereof) that can bind, e.g., specifically bind, to a human PCSK9, e.g., to the prodomain of human PCSK9.

A further embodiment includes a method of detecting a PCSK9 polypeptide in a sample.
15 The method includes providing a sample (e.g., a biological sample), contacting the sample with a PCSK9 inhibitor described herein (e.g., under conditions suitable for specific binding of a PCSK9 polypeptide), and detecting a complex comprising the PCSK9 inhibitor.

Another embodiment includes a method of treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a PCSK9 inhibitor
20 disclosed herein (e.g., a PCSK9 antibody, fragment, or derivative thereof that can bind, e.g., specifically bind, to the prodomain of a human PCSK9). In embodiments, the method further comprises administering a second agent (e.g., a conventional treatment, e.g., a statin) to the subject.

In embodiments, the second agent is administered concurrently with the PCSK9 inhibitor.
25 In one embodiment, the second agent is part of the same composition as the PCSK9 inhibitor. In another embodiment, the second agent is in a separate composition. In embodiments, the second agent is administered before or after the PCSK9 inhibitor.

The second agent can be one or more (e.g., a combination of) agents (e.g., lipid lowering agents or other agents known to be useful in treating a cholesterol-related disorder) disclosed
30 herein or known in the art.

In one embodiment, the second agent is a second PCSK9 inhibitor. In embodiments, the second PCSK9 inhibitor is a protein or peptide (e.g., a PCSK9 antibody or antigen binding fragment) that binds to PCSK9. In embodiments, the second PCSK9 inhibitor binds to a different epitope of PCSK9 (e.g., the second PCSK9 inhibitor does not bind to the prodomain of PCSK9 or binds to a different epitope of PCSK9 (e.g. human PCSK9) than does the PCSK9 inhibitor). In one embodiment, the second PCSK9 inhibitor binds to the catalytic domain of PCSK9, e.g., human PCSK9. In one embodiment, the second PCSK9 inhibitor binds to the C terminal domain of PCSK9, e.g., human PCSK9.

Lipid-lowering agents are described, e.g., in Pahan, K. (2006) Cell Mol Life Sci, 63(10): 1165-1178. In embodiments, the second agent is a statin (statins include, e.g., lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, pitavastatin, and mevastatin), a fibrate (fibrates include, e.g., clofibrate, ciprofibrate, bezafibrate, fenofibrate, gemfibrozil, and ciprofibrate), ezetimibe, cholestyramine, colestipol, colesevelam, torcetrapib, avasimibe, implitapide, or niacin. In embodiments, the subject is at risk for or has been diagnosed with a cholesterol-related disorder. In embodiments, the subject is at risk for or has been diagnosed with hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease, atherosclerosis, or a dyslipidemia. In embodiments, the dyslipidemia is elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL.

In embodiments, the subject is a subject having an elevated level of circulating free PCSK9, triglycerides, and/or LDL (e.g., LDL-c).

In embodiments, the subject has shown intolerance to or an inadequate response to a prior treatment with a lipid-lowering agent. In embodiments, method further comprises selecting a subject for treatment with the PCSK9 inhibitor based on intolerance to or an inadequate response to a prior treatment with a lipid-lowering agent. In embodiments, the subject has suffered an adverse effect during a prior treatment with a lipid-lowering agent. For example, adverse effects associated with statin use include increased concentrations of liver enzymes, muscle problems (e.g., muscle pain, myopathy, or rhabdomyolysis), diabetes or increased risk of diabetes, cognitive loss, neuropathy, pancreatic dysfunction, hepatic dysfunction, and sexual dysfunction. Adverse effects associated with fibrate use include, e.g., peroxisome proliferation, hepatomegaly, and hepatic tumors.

In embodiments, the treatment with the PCSK9 inhibitor disclosed herein is not associated with an adverse effect that is associated with another lipid-lowering agent.

In embodiments, the method comprises identifying a subject in need of treatment. In embodiments, the method comprises obtaining a sample from the subject prior to treatment (e.g.,
5 prior to treatment with the PCSK9 inhibitor or prior to treatment with a statin) and/or determining (e.g., analyzing the sample to determine that) that a subject is in need of treatment. In embodiments, the subject in need of treatment has an elevated level of circulating free PCSK9, triglycerides, and/or LDL. In embodiments, the subject has elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL),
10 and/or low HDL.

In embodiments, the method is effective to reduce the level of circulating free PCSK9 in the subject (e.g., in the blood, plasma, or serum of the subject). In embodiments, the level of free PCSK9 (e.g., the blood, plasma, or serum level) is reduced relative to the level of free PCSK9 prior to administration of the PCSK9 inhibitor.

15 In embodiments, the method is effective to reduce the level of triglycerides and/or LDL (e.g., LDL-c) in the subject. In embodiments, the level of triglycerides and/or LDL (e.g., LDL-c) is reduced relative to the respective level of triglycerides and/or LDL (e.g., LDL-c) prior to administration of the PCSK9 inhibitor.

In embodiments, the method comprises obtaining a sample from the subject (e.g., a blood
20 sample, plasma sample, or serum sample) before and/or after treatment with the PCSK9 inhibitor to identify a subject in need of treatment and/or to determine or monitor the efficacy of the treatment. In embodiments, the method comprises analyzing the sample, e.g., determining the level of a variable of interest, e.g., a variable disclosed herein (e.g., (e.g., LDL-c), triglycerides, free PCSK9, serum cholesterol, LDL, VLDL, and/or HDL .

25 In embodiments, the method is effective to improve the level of the variable, e.g., to reduce the level of free PCSK9, triglycerides, and/or LDL (e.g., LDL-c). In embodiments, the method is effective to reduce total serum cholesterol level, reduce LDL level, reduce the level of triglycerides, reduce VLDL level, and/or increase HDL level.

In some aspects, the PCSK9 inhibitor is an antibody, fragment, or variant thereof that
30 exhibits pH-dependent binding to a PCSK9. In certain aspects, the subject is also treated with a second agent, e.g., a second agent disclosed herein, e.g., a statin.

The invention also relates to a PCSK9 inhibitor as disclosed herein (e.g., a PCSK9 antibody, fragment, or derivative thereof) for use in treating a PCSK9-associated disease in a subject. In embodiments, the PCSK9 inhibitor can specifically bind to the prodomain of a human PCSK9. In some embodiments, the PCSK9 inhibitor is an antibody, fragment, or variant thereof that exhibits pH-dependent binding to a PCSK9. In some embodiments, the PCSK9 inhibitor is used in combination with second agent, e.g., a second agent disclosed herein, e.g., a statin.

The invention further relates to use of a PCSK9 inhibitor (e.g., a PCSK9 antibody, fragment, or derivative thereof) in the manufacture of a medicament for the treatment of a PCSK9-associated disease in a subject. In embodiments, the PCSK9 inhibitor can specifically bind to the prodomain of a human PCSK9. In some embodiments, the PCSK9 inhibitor is an antibody, fragment, or derivative that exhibits pH-dependent binding to a PCSK9. In some embodiments, the PCSK9 inhibitor is used in combination with a second agent, e.g., a second agent disclosed herein, e.g., a statin.

The entire disclosure of each patent document and scientific article referred to herein, and those patent documents and scientific articles cited thereby, is expressly incorporated by reference herein for all purposes. Unless otherwise defined, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the disclosed invention belongs.

Additional embodiments include the following:

Embodiment 1: An antibody, antibody fragment, or derivative thereof comprising at least one of SEQ ID NO:5, 6, 7, 8, or 9 that can specifically bind to the prodomain of a human PCSK9.

Embodiment 2: The antibody of Embodiment 1, wherein the antibody can inhibit an activity of a PCSK9.

Embodiment 4: The antibody, fragment, or derivative thereof of Embodiment 1 comprising SEQ ID NO:5, 6, 7, 8, 9, and 10.

Embodiment 5: The antibody or fragment thereof of Embodiment 1, comprising sequences that are at least 95% identical to SEQ ID NO:5, 6, 7, 8, 9, or 10.

Embodiment 6: The antibody, fragment, or derivative thereof of Embodiment 1, wherein the antibody, fragment, or derivative thereof can specifically bind to S127, D129, or S127 and D129 of SEQ ID NO:1.

Embodiment 7: The antibody, fragment, or derivative thereof of Embodiment 1, wherein
5 the antibody, fragment, or derivative thereof binds to PCSK9 with a K_d equal to or less than in a cell-based assay.

Embodiment 8: The antibody, fragment, or derivative thereof of Embodiment 1 comprising SEQ ID NOs: 5, 6, 7, 8, 9, and 10.

Embodiment 9: A method of inhibiting PCSK9, the method comprising contacting a
10 sample comprising a PCSK9 with a PCSK9 antibody, fragment, or derivative thereof that can specifically bind to the prodomain of human PCSK9 and comprises SEQ ID NOs: 5, 6, 7, 8, 9, and 10.

Embodiment 10. A method of detecting a PCSK9 polypeptide in a sample, the method comprising (a) providing a sample, (b) contacting the sample with a PCSK9 antibody, fragment,
15 or derivative thereof of Embodiment 1 under conditions suitable for specific binding of a PCSK9 polypeptide, and (c) detecting a complex comprising the PCSK9 antibody.

Embodiment 11. A method of treating a subject in need thereof, the method comprising (a) identifying a subject in need of treatment with a PCSK9 inhibitor; and (b) administering a therapeutically effective amount of a PCSK9 antibody, fragment, or derivative thereof to the
20 subject, wherein the PCSK9 antibody can specifically bind to the prodomain of human PCSK9.

Embodiment 12: The method of Embodiment 11, further comprising testing the subject's plasma for free PCSK9 before and after treatment, wherein the subject has decreased free PCSK9 in their serum after treatment with the PCSK9 inhibitor compared to the level of free PCSK9 after treatment.

Embodiment 13: The method of Embodiment 11, further comprising testing the subject's plasma for free PCSK9 after treatment and comparing it to a reference.

Embodiment 14: The method of Embodiment 11, wherein the subject is at risk for or has been diagnosed with a cholesterol-related disorder.

Embodiment 15: The method of Embodiment 11, wherein the subject is at risk for or has
30 been diagnosed with hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease, atherosclerosis, or a

dyslipidemia, e.g., elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL.

Embodiment 16: The method of Embodiment 11, further comprising treating the subject with a statin.

5 Embodiment 17: A vector comprising a nucleic acid sequence encoding an antibody, fragment, or derivative thereof of Embodiment 1.

Embodiment 18. A host cell comprising a nucleic acid sequence encoding an antibody, fragment, or derivative thereof of Embodiment 1.

10 Embodiment 19. A pharmaceutical composition comprising the antibody, fragment, or derivative thereof of Embodiment 1 and a pharmaceutically acceptable excipient.

Embodiment 20. A kit comprising an antibody, fragment, or derivative thereof of Embodiment 1 and instructions for use of the antibody, fragment, or derivative thereof.

15 Additional features and advantages of the invention are more particularly described below.

DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph depicting the results of ELISA experiments determining the binding affinity of antibodies for human PCSK9.

20 Fig. 2 is a graph depicting the results of a yeast binding titration assay determining binding affinity of PCSK9 antibodies.

Fig. 3 is a graph depicting plasma LDL-c levels in monkeys treated with PCSK9 antibodies.

Fig. 4 is a graph depicting triglyceride levels in monkeys treated with PCSK9 antibodies.

25 Fig. 5 is a graph depicting cholesterol levels in monkeys treated with PCSK9 antibodies.

Fig. 6 is a graph depicting the results of PCSK9 antibody PK assays in monkeys.

Fig. 7 is a graph depicting the results of assays determining the amount of unbound PCSK9 in monkeys treated with PCSK9 antibody.

DETAILED DESCRIPTION

It has been found that a PCSK9 inhibitor (e.g., an antibody) can be targeted to, for example, the prodomain of PCSK9 and is effective in inhibiting PCSK9 activity. For example, PCSK9 inhibitors disclosed herein were found to be effective in decreasing LDL (e.g., LDL-c), triglycerides, and circulating levels of free PCSK9. PCSK9 inhibitors disclosed herein can be used, e.g., to treat cholesterol-related disorders, either alone or in combination with a second agent, e.g., another treatment for a cholesterol-related disorder (e.g., a statin or a fibrate). PCSK9 inhibitors disclosed herein can also be used to improve pharmacokinetics and/or improve dosing of a second agent. For example, administration of a PCSK9 inhibitor disclosed herein may allow less frequent dosing and/or administration of a lower dose of a second agent, e.g., a second agent described herein. The second agent can be another PCSK9 inhibitor (e.g., a protein or peptide (e.g., an antibody or antigen binding fragment) that binds a different epitope of PCSK9 (e.g., human PCSK9), e.g., an epitope in the catalytic domain or the C terminal domain of PCSK9). The second agent can also be any known lipid-lowering agent (e.g., a statin, a fibrate, ezetimibe, cholestyramine, colestipol, colesevelam, torcetrapib, avasimibe, implitapide, or niacin). PCSK9 inhibitors disclosed herein generally have high affinity for PCSK9. Typically, the inhibitors disclosed herein are not targeted to the catalytic domain or the C terminal domain of PCSK9 but instead bind to the prodomain of PCSK9 (e.g., the prodomain of human PCSK9).

As used herein, singular terms such as “a” or “an” include the plural unless the context clearly indicates otherwise.

As used herein, the terms “treat”, “treating,” “treatment” and the like refer to a measurable or clinically observable therapeutic effect. Typically, the effect is an improvement in a patient’s condition, e.g., as indicated by prevention or reduction of at least one sign or symptom of the disease, a reduction in the severity of at least one sign or symptom of the disease, or inhibition of the progression of at least one sign or symptom of the disease.

PCSK9 Inhibitors

Affinity

In general, a molecule that is a PCSK9 inhibitor (e.g., an antibody, fragment, or derivative thereof) can bind to PCSK9. Typically, the PCSK9 inhibitor can specifically bind to a PCSK9 and does not significantly bind to molecules other than PCSK9, unless the molecule also contains a distinct binding portion that binds to a molecule other than a PCSK9, (for example as in some bispecific or bifunctional molecules that have multiple functions, at least one of which is to specifically bind PCSK9). A molecule as described herein that can specifically bind a PCSK9 generally has a dissociation constant (K_d) of <0.4 nM, e.g., <0.3 nM, <0.2 nM, <0.1 nM, ≤ 0.1 nM when assayed using human PCSK9, e.g., in an ELISA or Octet[®] assay. In some embodiments, the K_d is between about 0.01 and 100 nM, 0.1 nM and 100 nM, between about 0.1 nM and 50 nM, between about 0.1 and 20 nM, between about 0.2 nM and 10 nM, between about 0.2 nM and 1 nM, between about 1 nM and 10 nM, between about 0.01 and 10 nM, between about 0.01 and 1 nM, 10 and 100 pM), or between 10 and 20 pM for binding to a PCSK9, e.g., a human PCSK9. In some embodiments, the K_d is between 0.1 nM and 100 nM, between 0.1 nM and 50 nM, between 0.1 and 20 nM, between 0.2 nM and 10 nM, between 0.2 and 1 nM, between 1 nM and 10 nM for binding to a PCSK9, e.g., a human PCSK9. In some cases, a PCSK9 antibody can specifically bind to an epitope, e.g., an epitope in the prodomain, of PCSK9 that is conserved among PCSK9 from different species, for example, at least between human and one or more of a primate (e.g., rhesus monkey, African green monkey, or cynomolgous monkey), rodent (e.g., rat, mouse, guinea pig), or other mammal (e.g., canine, bovine, ovine, equine, or camelid). An antibody that can specifically bind to a PCSK9 or fragment thereof is referred to herein as a “PCSK9 antibody.” In some embodiments, a PCSK9 inhibitor (e.g., an antibody) is an inhibitor (e.g., an antibody) that can specifically bind to a PCSK9 and can inhibit at least one activity of PCSK9. “Inhibition” includes reduction of a PCSK9 activity. In some embodiments, the PCSK9 inhibitor inhibits a PCSK9 activity, e.g., with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , or 10^{-10} M. In some embodiments, the PCSK9 inhibitor has an IC_{50} of less than 100 nM, 10 nM, or 1 nM.

Affinity of a PCSK9 inhibitor can be determined using methods known in the art. For example, K_d can be measured using a radiolabeled antigen binding assay (RIA) performed with the Fab derived from a test PCSK9 antibody and PCSK9. In one such as assay, solution binding

affinity of a Fab for PCSK9 is measured by equilibrating the Fab with a minimal concentration of (¹²⁵I)-labeled PCSK9 in the presence of a titration series of unlabeled PCSK9, then capturing bound antigen with an anti-Fab antibody-coated plate. Methods of performing RIAs are known in the art.

5 In some cases, K_d is determined using a surface plasmon resonance assay with a BIACORE[®]-2000 or a BIACORE[®]-3000 (BIAcore, Inc., Piscataway, N.J.) with PCSK9 immobilized on carboxymethylated dextran biosensor chips. Methods are generally those recommended by the manufacturer. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE[®] Evaluation
10 Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} (e.g., Chen et al., J Mol Biol 293:865-881, 1999). In some cases, the on-rate can be determined by using a fluorescent quenching technique that measures a change in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass). Such measurements are made using a
15 spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments, Lakewood Township, NJ).

In some cases, K_d is determined by biolayer interferometry using an Octet[®] RED96 System (FortéBIO, Menlo Park, CA). Antibodies are captured on anti-human-IgG-Fc coated tips then incubated in target antigen at a range of concentrations. Optical thickness is measured over
20 time to assess association. Tips are then dipped into buffer and antigen dissociation measured over time. Association and dissociation rates are fit to a 1:1 reversible biomolecular interaction model to determine k_{on} , k_{off} , and K_d . *Antibodies*

In some embodiments, the invention is an antibody that can specifically bind a PCSK9. The term "antibody" as used herein refers to antibody molecules including, but not limited to
25 monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit specific binding to PCSK9. 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 without limitation Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; triabodies,
30 tetrabodies, linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments. Such fragments are further discussed *infra*. In

general, throughout this disclosure, the term “antibody” should be read as “antibody, fragment, or variant thereof” except when, as will be apparent to those in the art, the term exclusively refers to an antibody. Examples of CDRs from PCSK9 antibodies that are PCSK9 inhibitors are in Table 1.

5

Table 1

Designation	Heavy chain CDRs	Light chain CDRs
PCSK91a	GY <u>H</u> FT <u>N</u> YPID (SEQ ID NO:5; CDR1), NFHPYNDD <u>A</u> N (SEQ ID NO:6; CDR2), <u>R</u> <u>M</u> EL <u>S</u> <u>V</u> FAY (SEQ ID NO:7; CDR3)	RTS <u>D</u> NVY <u>R</u> YLT (SEQ ID NO:8; CDR1), <u>Y</u> AKTLAE (SEQ ID NO:9; CDR2), QHGYGTPYT (SEQ ID NO:10; CDR3)

The invention described herein also encompasses antibodies with a shared epitope with an antibody described herein. Methods of determining whether a first antibody has a shared epitope with a second antibody are known in the art. For example, an antibody with a shared epitope can be identified by the ability of the antibody to block binding of a reference antibody (e.g., a PCSK9 antibody described herein) in a competition assay by at least 50%. Another method for identifying such an antibody is by determining whether a reference antibody described herein can block binding of the antibody to PCSK9 by at least 50%. Another method for determining competition between two antibodies is a bridging assay in which a first antibody is captured on an ELISA plate or SPR chip, PCSK9 is then bound to the first antibody followed by incubation with the second antibody to permit binding. If the second antibody is captured on the plate or chip through the bound PCSK9 then it is non-competitive with the first antibody.

Antibodies having a shared epitope and therefore encompassed by the invention include antibodies that have a shared epitope with an antibody described herein as determined using mutation studies, crystallography, or other methods known in the art.

In some embodiments, a PCSK9 inhibitor increases the availability of LDL receptors when administered to an animal, e.g., a mammal such as a human, non-human primate, mouse, rat, guinea pig, pig, sheep, cow, dog, cat, or horse.

In embodiments, the PCSK9 inhibitor is an antibody, fragment, or derivative thereof that can specifically bind to an epitope within a fragment of PCSK9 as described herein. In some cases, the antibody, fragment, or derivative thereof binds to an epitope in a fragment of a human PCSK9 polypeptide, the amino acid sequence of which is depicted in SEQ ID NO:1.

5

1 mgtvssrrsw wplplllll lllgpagara qededgdyee lvlalrseed glaeapehgt
 61 tatfhrcakd pwrlpgtyvv vlkeethlsq sertarrlqa qaargyltk ilhvfhgllp
 121 gflvkmsgdl lelalklphv dyieedssvf aqsipwnler itppryrade yqppdggslv
 181 evylldtsiq sdhreiegrv mvtdfenvpe edgtrfhrqa skcdshgthl agvvsgrdag
 10 241 vakgasmrsl rvlncqgkgt vsgtliglef irksqlvqpvp gplvllpla ggysrvlnaa
 301 cqrlaragvv lvtaagnfrd daclyspasa pevitvgatn aqdqpvltgt lgtngfrcvd
 361 lfapgediig assdcstcfv sqsgtsqaaa hvagiaamml saepeltlae lrqrlhfsa
 421 kdvineawfp edqrvltpnl vaalppsthg agwqlfctv wsahsgptrm atavarcapd
 481 eellscssfs rsgkrrgerm eaqggklvcr ahnafggeg v yaiarccllp qancsvhtap
 15 541 paeasmgrtv hchqqghvlt gcsshweved lgthkppvlr prgqpncvqg hreasihasc
 601 chapgleckv kehqipapqe qvtvaceegw tltgcsalpg tshvlgayav dntcvvrsrd
 661 vsttgstseg avtavaiccr srhlaqasqe lq (SEQ ID NO:1)

In certain embodiments, the functional and/or structural epitope of an antibody according to this invention is between residues 31 and 152 (SEQ ID NO:12), inclusive (prodomain) of human PCSK9 (as depicted in SEQ ID NO:1).

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qededgdyee lvlalrseed glaeapehgt tatfhrcakd pwrlpgtyvv vlkeethlsq
 sertarrlqa qaargyltk ilhvfhgllp gflvkmsgdl lelalklphv dyieedssvf aq (SEQ ID NO:12)

In embodiments, the epitope comprises SEQ ID NO:12.

In some embodiments, the epitope is between residues 61 and 152, inclusive, of human PCSK9 (e.g., of SEQ ID NO:1), for example,

30

tatfhrcakd pwrlpgtyvv vlkeethlsq sertarrlqa qaargyltk ilhvfhgllp

gflvkmsgdl lelalklphv dyieedssvf aq (SEQ ID NO:13).

In embodiments, the epitope comprises SEQ ID NO:13.

- 5 In some embodiments, the epitope includes at least residues 31 to 151, inclusive of SEQ ID NO:1 (SEQ ID NO:14)

qededgdyee lvlaalseed glaeapehgt tatfhrcakd pwrllpgtyvv vlkeethlsq
sertarrlqa qaarrgyltk ilhvfhgllp gflvkmsgdl lelalklphv dyieedssvf a (SEQ ID NO:14)

10

In embodiments, the epitope comprises SEQ ID NO:14.

In some cases, the epitope includes at least one residue of human PCSK9 selected from the group consisting of S127 and D129.

- 15 In certain embodiments, the functional and/or structural epitope of an antibody, fragment, or derivative according to this invention is not in the catalytic domain of PCSK9, for example the epitope is not between residues 153 and 447, inclusive, of human PCSK9 (SEQ ID NO:15).

sipwnler itppryrade yqppdggsly evylldtsiq sdhreiegrv mvtdfenvpe
edgtrfhrqa skcdshgthl agvvsgrdag vakgasmrsl rvlncqgkgt vsqgtliglef
20 irksqlvqpvl gplvvllpla ggysrvlnaa cqlraragvv lvtagnfrd daclyspasa
pevitvgatn aqdqpvltgt lgtngfrcvd lfapgediig assdcstcfv sqsgtsqaaa
hvagiaamml saepeltlae lrqlrhfsa kdvineawfp edqrvltpln vaalpps (SEQ ID NO:15)

In embodiments, the epitope does not comprise SEQ ID NO:15.

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In some cases, the epitope does not include D192, R194, D238, D374, or S376 of human PCSK9. In some embodiments, the epitope does not include at least two, three, four, or five of these residues.

- 30 In certain embodiments, the functional and/or structural epitope of an antibody according to this invention is not in the C-terminal domain of PCSK9, for example the epitope is not within residues 453 to 662 of SEQ ID NO:1, inclusive, e.g., within SEQ ID NO:16, inclusive.

wqlfctv wsahsgptrm atavarcapd eellscssfs rsgkrrgerm eaqggklvcr
 ahnafggegv yaiairccllp qancsvhtap paeasmgrtv hchqqghvlt gcsshweved
 lgthkppvlr prgqpncvlg hreasihasc chapgleckv kehgapapqe qvtvaceegw
 5 tltgcsalpg tshvlgayav dntcvvrsrd vs (SEQ ID NO:16)

In embodiments, the epitope does not comprise SEQ ID NO:16.

10 In some embodiments, the epitope of a PCSK9 antibody does not include one or more of residues R194, E195, D238, A239, A341, Q342, E366, D367, I369, S376, T377, C378, F379, S381, H391 of SEQ ID NO:1, e.g., the epitope does not include two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen of the residues. In some cases, the epitope of a PCSK9 antibody does not extend from residues 153 to 205 of SEQ ID NO:1, e.g., residues 194-199 of SEQ ID NO:1.

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Chimeric and Humanized Antibodies

Antibodies described herein can include chimeric antibodies. Chimeric antibodies are known in the art, for example, see U.S. Pat. No. 4,816,567; and Morrison et al. (Proc Natl Acad Sci USA, 81:6851-6855, 1984). In some cases, a chimeric antibody comprises a non-human
 20 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 to differ from that of the parent antibody. Chimeric antibodies include antigen-binding fragments of a parent antibody or variants of such fragments.

25 A chimeric antibody can be a humanized antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs or portions of CDRs are derived from a non-human antibody, and framework regions or portions of framework regions are derived from human antibody sequences. Optionally, a humanized antibody also comprises at least a portion of a human constant region. In some embodiments, selected framework residues are substituted
 30 with corresponding residues from a non-human antibody (e.g., the antibody from which the CDRs are derived), e.g., to restore or improve antibody specificity, affinity, or pH dependence.

Humanized antibodies and methods of making them are known in the art, for example, Kontermann and Dübel (Antibody Engineering, 2010, Springer), Riechmann et al. (Nature 332:323-329, 1988); Queen et al. (Proc Nat Acad Sci USA 86:10029-10033, 1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al. (Methods 36:25-34, 2005, specificity determining residue (SDR) grafting); Padlan (Mol Immunol 28:489-498, 1991, resurfacing); Dall'Acqua et al. (Methods 36:43-60, 2005, framework shuffling); and Osbourn et al. (Methods 36:61-68, 2005) and Klimka et al. (Br J Cancer, 83:252-260, 2000, guided selection approach to framework shuffling).

Human framework regions that can be used for humanization include but are not limited to framework regions selected using a best-fit method (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 (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 (e.g., Almagro and Fransson, Front Biosci 13:1619-1633, 2008); and framework regions derived from screening framework libraries (e.g., Baca et al., J Biol Chem 272:10678-10684, 1997; and Rosok et al., J Biol Chem 271:22611-22618, 1996)).

A PCSK9 antibody as described herein can be a human antibody. Such antibodies can be generated using methods known in the art, for example, in Lonberg (Curr Opin Immunol 20:450-459, 2008). In some techniques, a human antibody is generated by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions. Examples of such methods are found in Lonberg, (Nat Biotech 23:1117-1125, 2005); U.S. Pat. Nos. 6,075,181 and 6,150,584; U.S. Pat. No. 5,770,429; U.S. Pat. No. 7,041,870, and U.S. Patent Application Publication No. 2007/0061900. Human variable regions from intact antibodies generated using such animals can be further modified, for example, via combination with other human constant regions.

Human antibodies can also be made by hybridoma-based methods. Such methods are known in the art, and are described, for example, in 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 using human B-cell hybridoma technology can be used (for example, see Li et al., Proc Natl Acad Sci USA, 103:3557-3562, 2006).

Human antibodies can also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. The selected variable domain sequences
5 can be combined with a selected human constant domain.

PCSK9 antibodies 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 (e.g., Hoogenboom et al. In: Methods in Molecular Biology
10 178:1-37, O'Brien et al., ed., Human Press, Totowa, NJ, 2001; 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, N.J., 2003); Sidhu et al., J Mol Biol 338:299-310, 2004; Lee et al., J Mol Biol 3405:1073-1093, 2004); Fellouse, Proc Nat Acad Sci USA 10:12467-12472, 2004; and Lee et
15 al., J Immunol Methods 284:119-132, 2004). In some embodiments, methods of identifying PCSK9 inhibitors (e.g., antibodies) are those described in the Examples.

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:
20 433-455, 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
25 et al., EMBO J, 12: 725-734 (1993). Naive libraries can 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: U.S. Pat. No. 5,750,373, and
30 US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

In some embodiments, yeast display methods such as those known in the art can be used to identify a PCSK9 inhibitor, e.g., antibody, for example, see Feldhaus et al. (Nature Biotechnology, 21:163-170, 2003) and Chao et al. (Nature Protocols, 1:755-768, 2006).

A human antibody or fragment that is isolated from a human antibody library is a human
5 antibody or fragment.

Antibody variants

As used herein, the term “antibody variants” refers to variants and derivatives of an antibody described herein. In certain embodiments, amino acid sequence variants of the
10 antibodies described 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
15 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. In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitution mutagenesis include the HVRs and framework
20 sequences. Examples of such substitutions are provided in Table 2. 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. Substitutions may be conservative or non-conservative.

Table 2

Starting residue	Substitution (examples)
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala

Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

In another example of a substitution to create a variant PCSK9 antibody, one or more hypervariable region residues of a parent antibody are substituted. In general, variants are then selected based on improvements in desired properties compared to a parent antibody, for example, increased affinity, reduced affinity, reduced immunogenicity, increased pH dependence of binding. For example, an affinity matured variant antibody can be generated, e.g., using phage display-based affinity maturation techniques such as those described herein and known in the art.

Substitutions can be made in hypervariable regions (HVR) of a parent PCSK9 antibody to generate variants having desirable properties (e.g., as in Chowdhury, *Methods Mol Biol* 207:179-196, 2008), and/or in SDRs. The variants generated using such methods are generally selected by testing 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., Humana Press, Totowa, N.J., 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. Substitutions can be in one, two, three, four, or more
5 sites within a parent antibody sequence.

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
10 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.

In some cases, a PCSK9 antibody is modified such that its glycosylation pattern is altered compared to a parent antibody. Addition or deletion of glycosylation sites to an antibody can be accomplished by altering the amino acid sequence such that one or more glycosylation sites is
15 created or removed. Methods of engineering antibodies to modify glycosylation sites are known in the art.

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
20 IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

In certain embodiments, the invention relates to 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
25 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 FcγRIII only, whereas monocytes express FcγRI, FcγRII
30 and FcγRIII. 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. Pat. No. 5,500,362 (see, e.g. Hellstrom et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063, 1986) and Hellstrom et al., Proc Nat'l Acad Sci USA 82:1499-1502, 1985; U.S. Pat. No. 5,821,337 (see Bruggemann et al., J. Exp. Med. 166:1351-1361, 1987). Alternatively, non-radioactive assays methods may be employed (see, for example, 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 Acad Sci USA 95:652-656, 1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity (e.g., WO 2006/029879 and WO 2005/100402). A CDC assay can be performed to evaluate complement activity (for example, Gazzano-Santoro et al., J Immunol Meth 202:163, 1996; Cragg et al., Blood 101:1045-1052, 2003; and Cragg and Glennie, Blood 103:2738-2743, 2004). FcRn binding and *in vivo* clearance/half-life determinations can be performed using methods known in the art (e.g., Petkova et al., Int Immunol 18:1759-1769, 2006).

Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn) are described in, for example, US2005/0014934A1. Such antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. The 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 (U.S. Pat. No. 7,371,826). Other examples of Fc region variants are known in the art, for example, Duncan and Winter (Nature 322:738-740, 1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351.

Derivatives

In certain embodiments, a PCSK9 antibody provided herein is further modified to contain one or more non-protein moieties that are known in the art. The moieties include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, 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 (homopolymers or

random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary. In embodiments in which more than one polymer is attached, the polymers can be the same or different molecules.

Expression of recombinant PCSK9 antibodies

PCSK9 antibodies described herein can be produced using recombinant methods and compositions as are known in the art, e.g., as described in U.S. Pat. No. 4,816,567. In one embodiment, isolated nucleic acid encoding a PCSK9 antibody described herein is provided. Such nucleic acid can 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 a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In another embodiment, a host cell comprising a nucleic acid encoding all or a fragment of a PCSK9 antibody is provided. In one such embodiment, a host cell comprises 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 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 some embodiments, the host cell comprises nucleic acid sequences encoding a VH and a VL of a PCSK9 antibody and, optionally a vector or portion of a vector. In some embodiments, the VH and VL encoding sequence is integrated into a chromosome of the host cell.

In some embodiments, a method of making a 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 or variant thereof, and optionally recovering the antibody from the host cell or from host cell culture medium.

For recombinant production of a PCSK9 antibody or variant thereof, nucleic acid encoding an antibody or variant thereof, 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 a nucleic acid can be isolated and sequenced using methods known in the art.

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies or variants thereof may
5 be produced in bacteria, in particular when glycosylation and Fc effector function are not needed or are undesirable. Non-limiting examples of such methods are described in, e.g., U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523; and Charlton (Methods in Molecular Biology, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003, pp. 245-254). After expression, the antibody can be isolated from the bacteria and further purified.

10 Eukaryotes such as filamentous fungi or yeast can also be used as cloning and/or expression hosts for vectors comprising nucleic acid sequences encoding PCSK9 antibodies or fragments, including fungi and yeast strains whose glycosylation pathways have been humanized, and therefore can express an antibody with a partially or fully human glycosylation pattern (e.g., Gerngross, Nat Biotech 22:1409-1414, 2004), and Li et al., Nat Biotech 24:210-
15 215, 2006).

Suitable host cells for the expression of an antibody include those of invertebrate origin. Baculovirus strains are known in the art and can be used as vectors in conjunction with insect cells.

Plant cells can be utilized as hosts (e.g., U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548,
20 7,125,978, and 6,417,429, and plantibody technology for producing antibodies in transgenic plants).

Cells derived from a vertebrate, e.g., a mammal can be used as hosts. Examples of mammalian host cell lines include monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells); baby hamster kidney cells (BHK); mouse
25 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); TR1 cells (e.g., in Mather et al., Annals N.Y. Acad Sci 383:44-68, 1982); MRC 5 cells; FS4 cells; Chinese hamster
30 ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., Proc Nat Acad Sci USA 77:4216, 1980); and myeloma cell lines such as YO, NSO and Sp20. Methods for using such cells are

known in the art, for example, Yazaki and Wu (Methods in Molecular Biology, Vol. 248, B.K.C. Lo, ed., Humana Press, Totowa, NJ, pp. 255-268, 2003).

Assays

5 PCSK9 antibodies as described herein can be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art. Non-limiting methods are known in the art and some are described *infra* and in the Examples.

In some embodiments the PCSK9 binding activity of a PCSK9 antibody is assayed.

Methods for conducting such assays are known in the art, for example, using ELISA, Western

10 blot, or related methods. A competitive binding assay can be used to determine whether a selected PCSK9 antibody competes with another antibody. In an example, competition can be assayed using 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 I¹²⁵ label (see, e.g., Morel et al., Molec Immunol 25:7-15, 1988); solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., Virology 176:546-552, 1990); and direct labeled RIA (Moldenhauer et al., Scand J Immunol. 32:77-82, 20 1990). Typically in such assays, purified antigen is bound to a solid surface or cells bearing an unlabeled 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. In general, the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing 25 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. In general, when a competing antigen binding protein is present in excess, it will inhibit (e.g., reduce) specific binding of a reference antigen binding 30 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.

In certain embodiments, a competition assay is used to identify an antibody that can compete with a selected PCSK9 antibody described herein. In some cases, the competing
5 antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by a PCSK9 antibody described herein. Methods for mapping an antibody epitope are known in the art are described in, for example, Morris (1996, "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). In one example, immobilized PCSK9 is incubated in a solution that contains a first labeled antibody that can bind to PCSK9 (e.g., a
10 PCSK9 antibody described herein) 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 can optionally 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 that permit binding of the first antibody to PCSK9, excess unbound
15 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 (e.g., Harlow and Lane, 1988, Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

20 An activity assay can be used to identify a PCSK9 antibody having a biological activity. Biological activity of a PCSK9 antibody can include, e.g., blocking, antagonizing, suppressing, interfering, modulating and/or reducing one or more biological activities of PCSK9. Such an assay can be an *in vitro* assay, for example an ELISA assay measuring the ability of the antibody to block PCSK9-LDLR binding, or a cell-based LDL uptake assay. In some cases, the assay is an
25 *in vivo* assay.

In certain embodiments, a PCSK9 antibody can specifically bind human PCSK9 and can prevent interaction of PCSK9 with the LDLR. In some embodiments, a PCSK9 antibody as provided herein can inhibit binding of human PCSK9 to LDLR by at least about 20%-40%, 40-60%, 60-80%, 80-85%, or more (as determined, for example, by measuring binding in an *in vitro*
30 competitive binding assay), for example by at least 20%, at least 40%, at least 60%, at least 80%, at least 85%, at least 90%, or at least 95%. In certain embodiments, the invention provides

isolated PCSK9 antibodies that specifically bind to PCSK9 and which antagonize the PCSK9-mediated effect on LDLR levels when measured *in vitro* using an LDLR down regulation assay in HepG2 cells.

5 *Immunoconjugates*

In some embodiments, a PCSK9 antibody is conjugated (e.g., by a covalent attachment (e.g., via a peptide bond, e.g., via a peptide linker) or non-covalent attachment) to at least one heterologous agent, creating an immunoconjugate. Such agents can be, e.g., cytotoxic agents, including a chemotherapeutic agent or drug, a growth inhibitory agent, or a toxin (e.g., a protein
 10 toxin, toxic enzyme, or radioactive isotope). In one embodiment, the PCSK9 antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF; U.S. Pat. Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (U.S. Pat. Nos.
 15 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 (Kratz et al., Curr Med Chem 13:477-523, 2006); Jeffrey et al., Bioorganic Med Chem Lett 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);
 20 Dubowchik et al., Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et al., J. Med. Chem. 45:4336-4343 (2002); and U.S. Pat. No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody (e.g., an antibody or antigen binding fragment thereof) as described herein conjugated to an enzymatically active
 25 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, curcun, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin,
 30 and the tricothecenes.

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 At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for
 5 detection, it can include a radioactive atom for scintigraphic studies, or a spin label for nuclear magnetic resonance (NMR) imaging, e.g., iodine¹²³, iodine¹³¹, indium¹¹¹, fluorine¹⁹, carbon¹³, nitrogen¹⁵, oxygen¹⁷, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent can be made using methods known in the art, typically using bifunctional protein coupling agents such as N-succinimidyl-3-(2-
 10 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
 15 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"
 20 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. Pat. No. 5,208,020) may be used.

The immunoconjugates herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS,
 25 GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SLAB, 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, Ill., U.S.A).

Detection of PCSK9 and Diagnostics

A PCSK9 antibody as described herein is useful for detecting the presence of PCSK9, for example, in a biological sample. "Detecting" as used herein encompasses quantitative or qualitative detection. A biological sample can be blood, serum, plasma, CSF, or other samples of biological origin including a cell or a tissue.

Provided herein is a PCSK9 antibody for use in a method of diagnosis or detection, for example, in a method of detecting the presence of PCSK9 in a biological sample. 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 a PCSK9 antibody as described herein under conditions permissive for binding of the PCSK9 antibody to PCSK9, and detecting whether a complex is formed between the PCSK9 antibody and PCSK9. Such method may be an *in vitro* or *in vivo* method. In one embodiment, a PCSK9 antibody is used to select subjects eligible for therapy with a PCSK9 antibody, e.g. where PCSK9 or LDL-cholesterol is a biomarker for selection of patients.

Disorders that can be diagnosed using a PCSK9 inhibitor as described herein or for which assay of PCSK9 is otherwise useful include without limitation, cholesterol-related disorders such as hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's 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. An embodiment includes a method for treating or preventing hypercholesterolemia, and/or at least one symptom of dyslipidemia, atherosclerosis, cardiovascular disease (CVD) or coronary heart disease, in a subject by administering to the subject a therapeutically effective amount of a PCSK9 inhibitor. In certain embodiments, a therapeutically effective amount of an PCSK9 inhibitor is for use in treating or preventing a cholesterol-related disorder (e.g., hypercholesterolemia, dyslipidemia, atherosclerosis, CVD or coronary heart disease), in a subject. The invention further provides the use of an effective amount of a PCSK9 inhibitor (e.g., an inhibitor that decreases the level of extracellular or circulating PCSK9) in the manufacture of a medicament for treating or preventing a cholesterol-related disorder (e.g., hypercholesterolemia, dyslipidemia, atherosclerosis, CVD or coronary heart disease) in a subject.

Labeled PCSK9 inhibitors (e.g., PCSK9 antibodies, fragments, and derivatives thereof) are also provided. Labels include, but are not limited to, labels or moieties that can be directly detected (e.g., 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. Examples of labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase, luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, f3-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.

Articles of Manufacture

In certain embodiments, an article of manufacture or kit comprising a PCSK9 inhibitor disclosed herein (e.g., a PCSK9 antibody or antigen binding fragment) is provided. The article of manufacture may further contain materials useful for the treatment, prevention and/or diagnosis of a condition described herein. The article of manufacture comprises a container containing a composition comprising the PCSK9 inhibitor. Typically, a label or package insert is 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. The container 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 a PCSK9 inhibitor (e.g., a PCSK9 antibody or antigen binding fragment) as described herein. The label or package insert indicates that the composition is used for treating the condition of choice. In an embodiment, the article of manufacture comprises (a) a first container with a composition that includes a PCSK9 inhibitor (e.g., a PCSK9 antibody or antigen

binding fragment) disclosed herein; and optionally (b) a second container with a composition containing a second agent disclosed herein. Alternatively, the composition in the first container may further comprise a second agent.

In embodiments, the second agent is a statin, a fibrate, ezetimibe, cholestyramine, colestipol, colesevelam, torcetrapib, avasimibe, implitapide, or niacin. In embodiments, the statin is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, pitavastatin, and mevastatin. In embodiments, the fibrate is selected from the group consisting of clofibrate, ciprofibrate, bezafibrate, fenofibrate, gemfibrozil, and ciprofibrate.

In certain embodiments, the second agent is a lipid-lowering or cholesterol-lowering drug, e.g., 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®.

The article of manufacture in this embodiment can also include a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture can include a second or third container containing a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can further include additional useful materials, including other buffers, diluents, filters, needles, and syringes.

An article of manufacture as described herein can include an immunoconjugate of the invention in place of or in addition to a PCSK9 inhibitor (e.g., the PCSK9 antibody or antigen binding fragment).

Inhibitor molecules derived from antibodies

A PCSK9 antibody of the invention can be a fragment of an antibody disclosed herein that binds, e.g., specifically binds, to PCSK9, e.g., to the prodomain of PCSK9, provided that the fragment comprises an antigen binding site. Antibody fragments of the invention are generally obtained starting with a reference (parent) antibody molecule such as those comprising one or

sequences disclosed herein, e.g., one or more CDRs as disclosed herein, e.g., SEQ ID NOs: 5, 6, 7, 8, 9, and 10 and derivatives thereof. Antibody fragments can be generated using methods known in the art such as recombinant DNA, enzymatic cleavage (for example, using pepsin or papain), chemical cleavage of an antibody (for example, chemical reduction of disulfide bridges). Antibody fragments that comprise an antibody antigen-binding site include, but are not limited to, molecules such as Fab, Fab', Fab'-SH, scFv, Fv, dAb, Fd and disulfide stabilized variable region (dsFv). Various other antibody molecules including one or more antibody antigen-binding sites can be engineered, including for example F(ab')₂, F(ab)₃, diabodies, triabodies, tetrabodies, and minibodies. Examples of antibody molecules and methods for their construction and use are described in Holliger and Hudson, 2005, Nat Biotechnol. 23:1126-1136. Non-limiting examples of binding fragments are a Fab fragment composed of VL, VH, constant light chain domain (CL) and constant heavy chain domain 1 (CH1) domains; an Fd fragment composed of VH and CH1 domains; an Fv fragment composed of the VL and VH domains of a single antibody; a dAb fragment composed of a VH or a VL domain; isolated CDR regions; an F(ab')₂ fragment, a bivalent fragment comprising two linked Fab fragments; a single chain Fv molecule (scFv), in which a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site; a bispecific single chain Fv dimer (for example as disclosed in WO 1993/011161) and a diabody, which is a multivalent or multispecific fragment constructed using gene fusion (for example as disclosed in WO94/13804 and [50]). Fv, scFv, or diabody molecules can be stabilized by the incorporation of disulfide bridges linking the VH and VL domains. Minibodies comprising an scFv joined to a CH3 domain can also be used as a PCSK9 inhibitor. Other fragments and derivatives of an antibody that can be used as a PCSK9 inhibitor include a Fab', which differs from a Fab fragment by the addition of a few amino acid residues at the carboxyl terminus of the heavy chain CH1 domain, including one or more cysteines from the antibody hinge region, and Fab'-SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group.

In some cases, a PCSK9 inhibitor that is an antibody fragment has been chemically modified to improve or introduce a desirable property, for example PEGylation to increase half-life or incorporation.

A dAb (domain antibody) is a small monomeric antigen-binding fragment of an antibody (the variable region of an antibody heavy or light chain). VH dAbs occur naturally in camelids

(e.g., camels and llamas) and can be produced by immunizing a camelid with a target antigen, isolating antigen-specific B cells and directly cloning dAb genes from individual B cells. A PCSK9 inhibitor of the present invention can be a dAb comprising a VH or VL domain substantially as set out herein, or a VH or VL domain comprising a set of CDRs substantially as
5 set out herein.

Disease Treatment

PCSK9 inhibitors are useful for treatment of diseases in which a reduction in PCSK9 activity is desirable (PCSK9 related diseases). The term 'disease' as used herein is intended to
10 include conditions that cause or could cause discomfort, dysfunction, distress, and/or death to the person affected, and includes, for example, injuries, disabilities, disorders, syndromes, infections, isolated symptoms, and atypical (not within the norm) variations in function. Examples of such PCSK9 related diseases include hypercholesterolemia, coronary heart disease, metabolic syndrome, acute coronary syndrome and related conditions, for example, diabetes, stroke,
15 cardiovascular diseases, Alzheimer's disease and dyslipidemias exhibiting elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL. Non-limiting additional examples of primary and secondary dyslipidemias that can be treated using a PCSK9 inhibitor include familial combined hyperlipidemia, familial hypertriglyceridemia, familial hypercholesterolemias, including heterozygous hypercholesterolemia, homozygous
20 hypercholesterolemia, familial defective apolipoprotein B-100; polygenic hypercholesterolemia; remnant removal disease, hepatic lipase deficiency; dyslipidemia. Dyslipidemia may be the result of, for example diet, hypothyroidism, drugs such as estrogen and progestin, beta-blockers, thiazide diuretics; nephrotic syndrome, chronic renal failure, Cushing's syndrome, primary biliary cirrhosis, glycogen storage diseases, hepatoma, cholestasis,
25 acromegaly, insulinoma, isolated growth hormone deficiency, and alcohol-induced hypertriglyceridemia.

PCSK9 inhibitors as described herein can also be useful in preventing or treating atherosclerotic diseases, such as coronary heart disease, coronary artery disease, peripheral arterial disease, stroke (ischaemic or hemorrhagic), angina pectoris, or cerebrovascular disease
30 and acute coronary syndrome, myocardial infarction. Prophylactic uses of PCSK9 inhibitors include reducing the risk of heart attack, fatal and non-fatal stroke, in certain heart surgeries,

hospitalization for heart failure, chest pain in patients with heart disease, and/or cardiovascular events due to prior conditions such as heart attack, heart surgery, and/or chest pain. In certain embodiments, the PCSK9 antibodies and methods described herein can be used to reduce the risk of recurrent cardiovascular events.

5

Treatment and Dosage

Subjects in need of treatment with a PCSK9 inhibitor are treated with a therapeutically effective amount of the inhibitor, e.g., an amount that is effective at dosages and administration regimes sufficient to achieve a desired result as a therapeutic or prophylactic. For example, a therapeutically effective amount of a PCSK9 inhibitor decreases serum cholesterol levels in a subject, e.g., reduces serum LDL-cholesterol levels in a subject compared to levels in the subject before initiating treatment with the PCSK9 inhibitor. In some cases, a therapeutically effective amount is sufficient to decrease serum cholesterol levels in a subject to below a predetermined level.

15 PCSK9 inhibitors described herein are useful for reducing at least one activity of PCSK9 such as binding of PCSK9 to LDLR and/or reducing or ameliorating at least one sign or symptom of a PCSK9-associated disease. Inhibition of one or more of PCSK9-associated functional properties can be determined using methods known to the art (e.g., Barak and Webb, 1981 J Cell Biol 90:595-604; Stephan and Yurachek, 1993 J Lipid Res 34:325330; and
20 McNamara et al., 2006 Clin Chim Acta 369:158-167) including those described herein. Inhibition of PCSK9 activity is typically assayed relative to activity observed in the absence of the inhibitor. In general, a PCSK9-inhibitor as described herein inhibits a PCSK9 activity by at least 10% compared to a control, for example, decreasing activity by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95% compared to a control. As used herein, the term “control”
25 when applied to assays of PCSK9 activity includes an assay in which PCSK9 activity is compared to a sample that does not contain a component of the assay, typically, the PCSK9 inhibitor, or refers to an assay in which activity of a PCSK9 inhibitor is compared to a predetermined reference activity level.

A PCSK9 inhibitor as described herein can be used in a method that includes contacting a
30 cell, population of cells, or a tissue that expresses or contains an LDL receptor with a PCSK9 inhibitor disclosed herein under conditions that allow the PCSK9 inhibitor to bind to PCSK9. In

general, the binding of PCSK9 by the PCSK9 inhibitor results in increased uptake of cellular LDL. The cell, population of cells or tissue can be *in vitro* or *in vivo* and are generally mammalian, e.g., rodents (including rats, mice, rabbits, and guinea pigs), canines (including dogs), bovines (including cattle), ovines, porcine, cats, equines, and camelids.

5 PCSK9 inhibitors described herein can be used in methods for inhibiting PCSK9 in a subject in need of such methods by administering to the subject a therapeutically effective amount of a PCSK9 inhibitor described herein. In general, the method is for treating a disease associated with PCSK9 activity. The PCSK9 inhibitor is useful for the preparation of a medicament for treating a PCSK9 associated disease, for example, an undesirably high LDL
10 level, e.g., hypercholesterolemia, coronary heart disease, metabolic syndrome, acute coronary syndrome, or related conditions. Treatment of a subject can be prophylactic or therapeutic.

The terms "therapeutically effective" or "prophylactically effective" refers to the amount necessary at the intended dosage to achieve the desired therapeutic or prophylactic effect for the period of time desired. The desired effect can be, for example, amelioration of at least one
15 symptom associated with condition being treated. Those skilled in the art will appreciate that the amount of PCSK9 inhibitor administered will vary according to various factors, including but not limited to, the disease state, age, sex and weight of the individual. The response can be assessed using methods known in the art including assay of circulating LDL levels and/or amelioration of other symptoms of disease or predisposition to disease.

20 A PCSK9 inhibitor is generally administered as a pharmaceutical composition. Such compositions include a PCSK9 inhibitor and, generally, a pharmaceutically acceptable carrier that can include an excipient, diluent, stabilizer, buffer, or other component that facilitates administration or storage of the inhibitor.

Pharmaceutical compositions can be formulated using methods known in the art, e.g.,
25 McGoff and Scher, 2000 *Solution Formulation of Proteins/Peptides*, In: McNally, E. J., ed. Protein Formulation and Delivery, New York, NY, Marcel Dekker; pp. 139-158; Akers and Defilippis, 2000, *Peptides and Proteins as Parenteral Solutions*, In: Pharmaceutical Formulation Development of Peptides and Proteins, Philadelphia, PA, Taylor and Francis, pp. 145-177; Akers et al., 2002, *Pharm Biotechnol* 14:47-127.

30 The composition can be in liquid or solid form. Solid formulations can be produced using methods known in the art including, but not limited to, lyophilization, spray drying, or drying by

supercritical fluid technology. An oral formulation can be in, for example, a tablet, capsule, or powder. A solid formulation can be a lyophilized composition and is made into a solution prior to administration for either single or multiple dosing according to methods known to the skilled artisan. Compositions are generally formulated within a biologically relevant pH range and can be buffered to maintain an acceptable pH during storage. Both liquid and solid formulations can require storage at lower temperatures (e.g., 2-8°C). Compositions may contain a bacteriostat to prevent or minimize proteolysis during storage, including but not limited to effective concentrations. Examples include benzyl alcohol, phenol, m-cresol, chlorobutanol, methylparaben, and/or propylparaben. A bacteriostat may be contraindicated for some patients.

Therefore, a lyophilized formulation may be reconstituted in a solution either containing or not containing such a component. Additional components may be added to either a buffered liquid or solid inhibitor formulation, including but not limited to a cryoprotectant (including but not limited to polyhydroxy hydrocarbons such as sorbitol, mannitol, glycerol, and dulcitol and/or disaccharides such as sucrose, lactose, maltose, or trehalose) and, a salt (including but not limited to NaCl, KCl, or LiCl). Osmolarity of certain formulations is selected to support long term stability at temperatures of, for example, 2-8°C or higher, and parenteral injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included in a formulation. A formulation can contain a divalent cation (including but not limited to MgCl₂, CaCl₂, and MnCl₂); and/or a non-ionic surfactant (including but not limited to polysorbate-80 (Tween® 80), polysorbate-60 (Tween® 60), polysorbate-40 (Tween® 40), and polysorbate-20 (Tween 20), polyoxyethylene alkyl ethers, including but not limited to Brij™ 58, Brij™ 3, Triton® X-100, Triton® X-114, NP40, Span 85 and the Pluronic® series of non-ionic surfactants (e.g., Pluronic® 121)).

Pharmaceutical compositions in liquid format can include a liquid carrier, e.g., water, petroleum, animal oil, vegetable oil, mineral oil, or synthetic oil. The liquid format may also include physiological saline solution, dextrose or other saccharide solution or glycols, such as ethylene glycol, propylene glycol or polyethylene glycol.

Pharmaceutical compositions can be formulated for administration after dilution in isotonic vehicles such as sodium chloride, Ringer's solution, or lactated Ringer's solution.

A PCSK9 pharmaceutical composition can include 50 to 200 mg/mL of a PCSK9-specific inhibitor described herein. In addition, such a composition can include at least one of

the following; a polyhydroxy hydrocarbon (including but not limited to sorbitol, mannitol, glycerol and dulcitol) and/or a disaccharide (including but not limited to sucrose, lactose, maltose and trehalose) at 1% to 6% weight per volume ("w/v") of the formulation; 5 mM to about 200 mM of histidine, imidazole, phosphate or acetic acid, 5 mM to about 200 mM of arginine, proline, phenylalanine, alanine, glycine, lysine, glutamic acid, aspartic acid or methionine; 0.01M to about 0.1M of hydrochloric acid ("HCl") in an amount sufficient to achieve a pH in the range of about 5.5 to about 7.5; and a carrier including but not limited to sterile water, petroleum, animal oil, vegetable oil, mineral oil, synthetic oil, physiological saline solution, dextrose or other saccharide solution or glycols, such as ethylene glycol, propylene glycol or polyethylene glycol; such that the pharmaceutical composition has a pH in the range of about 5.5 to about 7.5. In some cases, the pharmaceutical composition contains 0.01% to about 1% w/v of the formulation of a non-ionic surfactant. HCl can be added to a composition as free acid, histidine-HCl or arginine-HCl.

Use of the term "about" with respect to amounts disclosed in the specification means within at least 10%, e.g., within 5%, of the specified numbers provided. A range provided as, for example" in "about 50 to about 200" expressly includes as distinct embodiments each number within said range. As such in the above example, embodiments including but not limited to those having 50, 100, 125, 150 and 200 form specific embodiments herein. Pharmaceutical compositions as disclosed herein have general applicability despite the mode of administration. In specific embodiments, the disclosed pharmaceutical compositions are useful for subcutaneous administration as a liquid or upon reconstitution of a lyophilized form.

In some cases, the pharmaceutical composition comprises about 50 to about 200 mg/mL of a PCSK9 inhibitor as described herein. Optionally, the composition can include about 1% to about 6% (e.g., from about 2% to about 6%) w/v mannitol, trehalose or sucrose; about 10 mM to about 100 mM of histidine; about 25 mM to about 100 mM of arginine or proline; about 0.02 M to about 0.05M of hydrochloric acid ("HCl") in an amount sufficient to achieve a pH in the range of about 5.8 to about 7; and a liquid including but not limited to sterile water, petroleum, animal oil, vegetable oil, mineral oil, synthetic oil, physiological saline solution, dextrose or other saccharide solution or glycols, such as ethylene glycol, propylene glycol or polyethylene glycol; wherein said pharmaceutical composition has a pH in the range of about 5.8 to about 7. The pharmaceutical composition optionally can include about 0.01% to about 1% w/v of the

formulation of a non-ionic surfactant (including but not limited to Polysorbate-80, Polysorbate-60, Polysorbate-40, and Polysorbate-20, polyoxyethylene alkyl ethers, including but not limited to Brij 58, Brij35, Triton X-100, Triton X-114, NP40, Span 85 and the Pluronic series of non-ionic surfactants (e.g., Pluronic® 121)).

5 Also provided herein are pharmaceutical compositions that are lyophilized and can be reconstituted. In some cases, the PCSK9 inhibitor concentration in a lyophilized and reconstituted solution is up to 2-fold higher than in the pre-lyophilized composition. In specific embodiments, the PCSK9 inhibitor concentration in the lyophilized and/or reconstituted pharmaceutical composition is in the range of about 50 mg/mL to about 300 mg/mL. Diluents
10 useful for reconstituting the lyophilized pharmaceutical compositions include but are not limited to sterile water, bacteriostatic water for injection ("BWFI"), phosphate-buffered saline, a sterile saline solution, physiological saline solution, Ringer's solution or dextrose solution. Other constituents such as those described *supra* can be included in such solutions used for reconstitution.

15 In general, a composition comprising a PCSK9 inhibitor is stable. "Stable" as used herein refers to the property of a PCSK9 inhibitor to retain its physical or chemical stability, conformational integrity, at 2-37°C for at least 30 days, for example, for at least 3 months, at least 6 months, at least 12 months, at least 2 years, or at least 3 years when maintained in a specified temperature range, for example, 2°C-37°C, 2°C-10°C, or 2°C-15°C. Degradation of the
20 PCSK9 inhibitor within a composition is generally less than 10%, for example, less than 5%, 3%, or 2% compared with a freshly prepared composition. Stability of a formulation can be tested using methods known in the art including, but not limited to, size exclusion chromatography (SEC-HPLC), dynamic light scattering (DLS), capillary SDS-PAGE, and capillary iso-electric focusing (cIEF) or cation exchange chromatography ("CEX").

25 Determination of dose of a PCSK9 inhibitor can be determined by those skilled in the art. (e.g., Lederman et al., Int J Cancer 47:659-664, 1991; Bagshawe et al., Antibody, Immunoconjugates and Radiopharmaceuticals 4:915-922, 1991), and will vary based on a number of factors including but not limited to the particular PCSK9-specific inhibitor, the subject being treated, the condition of the subject, the tissue or system being treated, the route of
30 administration, and the desired outcome. A physician or veterinarian of skill can determine and prescribe the therapeutically effective amount of the inhibitor. Dosage ranges can be from about

0.01 to 100 mg/kg, e.g., 0.05 to 25 mg/kg, of the host body weight. For example, dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. In one non-limiting example, a dosage of 5 mg to 2.0 g is used for systemic delivery. A PCSK9 inhibitor as described herein can be used alone at appropriate dosages. Alternatively, the inhibitor is administered according to a dosing regime with other agents, for example, with a statin. For example, a PCSK9 inhibitor can be used in combination or in conjunction with other drugs (therapeutic and/or prophylactic), including but not limited to cholesterol-lowering drugs, for example, cholesterol absorption inhibitors (e.g., Zetia®) and cholesterol synthesis inhibitors (e.g., Zocor® and Vytorin®). The present invention contemplates such combinations and they form an important embodiment hereof. Accordingly, the present invention relates to methods of treatment as described above where the PCSK9-specific inhibitor is administered/delivered simultaneously with, following or prior to another drug or drugs (therapeutic and/or prophylactic), including but not limited to cholesterol-lowering drugs, cholesterol absorption inhibitors and cholesterol absorption inhibitors.

Individuals (subjects) that can be treated with a composition described herein include primates, human and non-human, and include any non-human mammal or vertebrate of commercial or domestic veterinary importance.

A PCSK9 inhibitor (e.g., antibody, fragment, or derivative) can be administered to an individual by a route of administration known in the art, including but not limited to oral administration, administration by injection (specific embodiments of which include intravenous, subcutaneous, intraperitoneal or intramuscular injection), administration by inhalation, intranasal, or topical administration, either alone or in combination with other agents designed to assist in the treatment of the individual. The PCSK9 inhibitor can also be administered using an injection device, injector pen, needleless device, or patch delivery systems. The route of administration should be determined based on a number of considerations appreciated by those of skill in the art including, but not limited to, the desired physiochemical characteristics of the treatment. Treatment may be provided on a daily, weekly, biweekly, or monthly basis, or any other regimen that delivers the appropriate amount of PCSK9 inhibitor to the individual at the prescribed times such that the desired treatment is effected and maintained. The formulations may be administered in a single dose or in more than one dose at separate times.

A PCSK9 inhibitor (e.g., antibody, fragment, or derivative thereof) described herein can be used in the manufacture of a medicament for treatment of a PCSK9-associated disease. The medicament is useful in a subject exhibiting a disease or symptoms associated with undesirable PCSK9 activity. In some embodiments, the condition is hypercholesterolemia, coronary heart
5 disease, metabolic syndrome, acute coronary syndrome or a related condition.

Accordingly, the invention also relates to use of a PCSK9 inhibitor (e.g., a PCSK9 antibody, fragment, or derivative thereof) in the manufacture of a medicament for the treatment of a PCSK9-associated disease in a subject, wherein the PCSK9 inhibitor can specifically bind to the prodomain of a human PCSK9. In some embodiments, a subject in need of treatment with a
10 PCSK9 inhibitor is identified. In some embodiments, the PCSK9 inhibitor is an antibody, fragment, or variant thereof that exhibits pH-dependent binding to a PCSK9. In some embodiments, the subject's plasma is further tested for free PCSK9 before and after treatment, wherein the subject has decreased free PCSK9 in their serum after treatment with the PCSK9 inhibitor compared to the level of free PCSK9 after treatment. In some embodiments, the
15 subject's plasma is further tested for free PCSK9 after treatment and comparing it to a reference. In some embodiments, the subject is at risk for or has been diagnosed with a cholesterol-related disorder. In some embodiments, the subject is at risk for or has been diagnosed with hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease, atherosclerosis, or a dyslipidemia, e.g.,
20 elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL. In some embodiments, the PCSK9 inhibitor is used in combination with a statin.

The invention further relates a PCSK9 inhibitor (e.g., a PCSK9 antibody, fragment, or derivative thereof) for use in treating a PCSK9-associated disease in a subject, wherein the
25 PCSK9 inhibitor can specifically bind to the prodomain of a human PCSK9. In some embodiments, a subject in need of treatment with a PCSK9 inhibitor is identified. In some embodiments, the PCSK9 inhibitor is an antibody, fragment, or variant thereof that exhibits pH-dependent binding to a PCSK9. In some embodiments, the subject's plasma is further tested for free PCSK9 before and after treatment, wherein the subject has decreased free PCSK9 in their
30 serum after treatment with the PCSK9 inhibitor compared to the level of free PCSK9 after treatment. In some embodiments, the subject's plasma is further tested for free PCSK9 after

treatment and comparing it to a reference. In some embodiments, the subject is at risk for or has been diagnosed with a cholesterol-related disorder. In some embodiments, the subject is at risk for or has been diagnosed with hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease, atherosclerosis, or a dyslipidemia, e.g., elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL. In some embodiments, the PCSK9 inhibitor is used in combination with a statin.

Genetic Therapy

Also provided herein are methods for administering a disclosed PCSK9 antibody for purposes of gene therapy. In this method, one or more cells of a subject are transformed with nucleic acid encoding a PCSK9 inhibitor (e.g., an antibody or antigen binding fragment) as described herein. Subjects treated in this manner can endogenously produce the PCSK9 antibody. An example of the method is provided by Alvarez, et al. (Clin Cancer Res 6:3081-3087, 2000) in which single-chain anti-ErbB2 nucleic acid was introduced into subjects using a gene therapy approach.

A subject can be treated for a PCSK9 disease using a nucleic acid-based therapy, e.g., a using a nucleic acid that codes for a PCSK9 inhibitor, e.g., antibody. In general, in such methods, a nucleic acid encoding a PCSK9 inhibitor is introduced into the cells of a subject using methods known in the art, for example, by incorporating sequences encoding the PCSK9 inhibitor into a viral vector. Examples of such viral vectors include those derived from lentivirus, herpes virus, adenovirus, adeno-associated virus, vaccinia virus, baculovirus, alphavirus, and influenza virus. Such vectors are commercially available, e.g., from Avigen, Inc. (Alameda, CA; AAV vectors), Cell Genesys (Foster City, CA; retroviral, adenoviral, AAV vectors, and lentiviral vectors), Clontech (retroviral and baculoviral vectors), Genovo, Inc. (Sharon Hill, PA.; adenoviral and AAV vectors), Genvec (adenoviral vectors), IntroGene (Leiden, Netherlands; adenoviral vectors), Molecular Medicine (retroviral, adenoviral, AAV, and herpes viral vectors), Norgen (adenoviral vectors), Oxford BioMedica (Oxford, United Kingdom; lentiviral vectors), and Transgene (Strasbourg, France; adenoviral, vaccinia, retroviral, and lentiviral vectors).

Methods for constructing and using viral vectors are known in the art (e.g., Miller, et al, BioTechniques 7:980-990, 1992). Typically, the vector is replication defective or attenuated in

some other way. Examples of such vectors include, for example a herpes virus vector (Kanno et al, Cancer Gen Ther 6:147-154, 1999; Kaplitt et al, J Neurosci Meth 71:125-132, 1997 and Kaplitt et al, J Neuro Onc 19:137-147, 1994); adenovirus vectors (e.g., Strafford-Perricaudet et al, J Clin Invest 90:626-630, 1992; PCT Publication Nos. WO94/26914, WO94/28938, WO94/28152, WO94/12649, WO95/02697 and WO96/22378). Retroviral vectors can also be used to introduce a sequence encoding a PCSK9 inhibitor, for example, see U.S. Patent Nos. 5,399,346, 4,650,764, 4,980,289, and 5,124,263; Mann et al, Cell 33:153, 1983; Markowitz et al, J. Virol., 62:1120, 1988; EP 453242 and EP178220.

Lentiviral vectors can be used as agents for the direct delivery and sustained expression of nucleic acids encoding a PCSK9-specific inhibitor of the invention in several tissue types, including brain, retina, muscle, liver and blood. The vectors can efficiently transduce dividing and nondividing cells in these tissues, and maintain long-term expression of the PCSK9-specific inhibitor. For a review, see Zufferey et al, J. Virol. 72:9873-80, 1998 and Kafri et al, Curr. Opin. Mol. Ther. 3:316-326, 2001. Lentiviral packaging cell lines are available and known generally in the art. They facilitate the production of high-titer lentivirus vectors for gene therapy. An example is a tetracycline-inducible VSV-G pseudotyped lentivirus packaging cell line which can generate virus particles at titers greater than 10^{sup.6} IU/ml for at least 3 to 4 days; see Kafri et al, J. Virol. 73:576-584, 1999. The vector produced by the inducible cell line can be concentrated as needed for efficiently transducing nondividing cells in vitro and in vivo.

Sindbis virus is a member of the alphavirus genus and has been studied extensively since its discovery in various parts of the world beginning in 1953. Gene transduction based on alphavirus, particularly Sindbis virus, has been well-studied in vitro (see Straus et al., Microbiol Rev., 58:491-562, 1994; Bredenbeek et al., J Virol, 67:6439-6446, 1993; Ijima et al., Int J Cancer 80:110-118, 1999 and Sawai et al, Biochim Biophys Res Comm 248:315-323, 1998). Many properties of alphavirus vectors make them a desirable alternative to other virus-derived vector systems being developed, including rapid engineering of expression constructs, production of high-titered stocks of infectious particles, infection of nondividing cells, and high levels of expression (Strauss et al, 1994 *supra*). Use of Sindbis virus for gene therapy has been described. (Wahlfors et al., Gene Ther 7:472-480, 2000 and Lundstrom, J Recep Sig Transduct Res 19:673-686, 1999).

In another embodiment, a vector can be introduced to cells by lipofection or with other transfection facilitating agents (peptides, polymers, etc.). Synthetic cationic lipids can be used to prepare liposomes for *in vivo* and *in vitro* transfection of a gene encoding a marker (Feigner et al., Proc Natl Acad Sci USA 84:7413-7417, 1987 and Wang et al., Proc Natl Acad Sci USA 84:7851-7855, 1987). Useful lipid compounds and compositions for transfer of nucleic acids are described in PCT Publication Nos. WO 95/18863 and WO 96/17823, and in U.S. Pat. No. 5,459,127.

It is also possible to introduce the vector *in vivo* as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into desired host cells by methods known in the art, e.g., electroporation, microinjection, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, e.g., Wilson, et al., J Biol Chem 267:963-967, 1992; Williams et al., Proc Natl Acad Sci USA 88:2726-2730, 1991). Other reagents commonly used for transfection of plasmids include, but are by no means limited to, FuGENE®, Lipofectin®, and Lipofectamine®. Receptor-mediated DNA delivery approaches can also be used (Wu et al., J Biol Chem 263:14621-14624, 1988). U.S. Pat. Nos. 5,580,859 and 5,589,466 disclose delivery of exogenous DNA sequences, free of transfection facilitating agents, in a mammal. Electrotransfer, a relatively low voltage, high efficiency *in vivo* DNA transfer technique can be used (Vilquin et al., Gene Ther 8:1097, 2001; Payen et al., Exp Hematol 29:295-300, 2001; Mir, Bioelectrochemistry 53:1-10, 2001; PCT Publication Nos. WO 99/01157, WO 99/01158 and WO 99/01175).

Pharmaceutical compositions suitable for such gene therapy approaches and comprising nucleic acids encoding a PCSK9 antibody or antigen binding fragment of the present invention are included within the scope of the present invention.

Research and Related Uses

In another aspect, the present invention provides a method for identifying, isolating, quantifying or antagonizing PCSK9 in a sample of interest using a PCSK9 antibody provided herein. For example, a PCSK9 antibody or variant thereof can be utilized as a research tool in immunochemical assays, such as Western blots, ELISAs, radioimmunoassay, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art (see, e.g., Immunological Techniques Laboratory Manual, ed. Goers 1993, Academic

Press) or various purification protocols. The inhibitors may have a label incorporated therein or affixed thereto to facilitate ready identification or measurement of the activities associated therewith. Various types of detectable labels (e.g., enzymes, dyes, or other suitable molecules that are either readily detectable or cause some activity/result that is readily detectable) that can
5 be useful in such protocols are known in the art.

Formulation

In some embodiments, treatment includes administering a pharmaceutical PCSK9 inhibitor composition to a patient, the composition comprising the PCSK9 inhibitor composition
10 and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is an oral dosage form. In some embodiments, the pharmaceutical compositions comprise, as the active ingredient, a PCSK9 inhibitor, e.g., PCSK9 antibody, in combination with one or more pharmaceutically acceptable carriers (excipients). In making the compositions of the invention, the agent is typically mixed with an excipient, diluted by an excipient or
15 enclosed within such a carrier in the form of, for example, a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the formulations can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing,
20 for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, the PCSK9 inhibitor composition can be milled to provide the appropriate particle size prior to combining with the other ingredients. If the PCSK9 inhibitor composition is substantially insoluble, it can be milled to a particle size of less than 200 mesh. If
25 the agent is substantially water soluble, the particle size can be adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl
30 cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents

such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

A PCSK9 inhibitor composition can be formulated in a unit dosage form, each dosage
5 containing from about 5 to about 1000 mg (1 g), more usually about 100 to about 500 mg, of the agent. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

10 A PCSK9 inhibitor composition can be effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood by those in the art that the amount of the agent actually administered is typically determined by a physician, according to the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the
15 individual patient, the severity of the patient's symptoms, and the like.

For preparing a solid PCSK9 inhibitor formulation such as a tablet, the PCSK9 inhibitor composition is mixed with a pharmaceutical excipient to form a solid composition containing a homogeneous mixture of a PCSK9 inhibitor composition. Such formulations are typically provided in unit dosage forms, for example, about 0.1 to about 1000 mg of the PCSK9 inhibitor
20 composition.

A tablet or pill comprising a PCSK9 inhibitor composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by
25 an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

30 A liquid formulation comprising a PCSK9 inhibitor composition can be prepared for oral delivery or for injection, for example, in an aqueous solutions, suitably flavored syrups, aqueous

or oil suspensions, or flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Formulations for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. In some embodiments, the formulations are administered by the oral or nasal respiratory route for local or systemic effect. Solution, suspension, or powder formulations can be administered orally or nasally from devices that deliver the formulation in an appropriate manner.

The amount and frequency of a PCSK9 inhibitor composition administered to a patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions can be administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. Effective doses will depend on the disease condition being treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the disease, the age, weight and general condition of the patient, and the like.

The therapeutic dosage of a PCSK9 inhibitor composition agents can vary according to, for example, the particular use for which the treatment is made, the manner of administration of the compound, the health and condition of the patient, and the judgment of the prescribing physician. The proportion or concentration of an agent in a pharmaceutical composition can vary depending upon a number of factors including dosage, chemical characteristics (e.g., hydrophobicity), and the route of administration. For example, the agents can be provided in an aqueous physiological buffer solution containing about 0.1 to about 10% w/v of the compound for parenteral administration. Some typical dose ranges are from about 1 $\mu\text{g/kg}$ to about 1 g/kg of body weight per day. In some embodiments, the dose range is from about 0.01 mg/kg to about 100 mg/kg of body weight per day. The dosage is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, formulation of the excipient, and its route of administration. Effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems.

EQUIVALENTS

All technical features can be individually combined in all possible combinations of such features.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

EXAMPLES

The following non-limiting examples further illustrate embodiments of the inventions described herein.

Example 1: Humanization of a PCSK9 antibody

A murine PCSK9 antibody (m7F8) that specifically binds to the prodomain of PCSK9 was humanized. In brief, this was accomplished using CDR grafting. A human germline framework with maximum homology to the native mouse sequence was selected for the CDR grafting, and the CDRs from the m7F8 were cloned into this framework. The human heavy chain sequence chosen was IGHV1-46*01 | JH1; the light chain was IGKV1-16*01 | JK4. The humanized variable regions were cloned into a vector containing an IgG2 HC constant region and a κ LC constant region to yield a humanized antibody, 7F8.1.

Additionally, a single amino acid in the light chain framework was mutated back to the original mouse sequence (shown in boldface in SEQ ID NO:2). This change improved the binding affinity of 7F8.1 as shown in Figure 1 and Table 3. The resulting antibody is referred to as h7F8.

Table 3

	Human PCSK9 (ELISA)	Rhesus PCSK9 (ELISA)	Human PCSK9 (Yeast / FACS)	Dissociation rate (SPR) (k_{off} , s^{-1})
ch7F8	0.413 nM	1.53 nM	1.2 nM	7.27 e-4
7F8.1	290 nM	--	--	--
h7F8	1.6 nM	--	3.2 nM	14.21 e-4
H7F8m12	0.166 nM	0.186 nM	0.101 nM	0.36 e-4

Humanized h7F8 was expressed both transiently in HEK293 and from HEK293 cells stably transfected with lentiviral vectors. Purification was performed using Protein A chromatography.

h7F8 VK (CDRs underlined)

DIQMTQSPSSLSASVGDRVTITCRTSENVYSYLTWFQQKPGKAPK~~LL~~LIY
NAKTLAEGVPSRFSSGSGTDFLTITSSLPEDFATYYCQHHYGTPYTFGGGTKVEIK
 (SEQ ID NO:2)

H7F8 VH (CDRs underlined)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYPIDWVRQAPGQGLEWMGNFHP
YNDDTNYAQKFQGRVTMTRDTSTSTVYMESSLRSEDTAVYYCARRGELAWFAYWG
 QGTLVTVSS (SEQ ID NO:3)

Example 2: Affinity maturation

To increase the affinity of h7F8 for human PCSK9, the antibody was mutated and evolved, resulting in h7F8m12 containing mutations in both heavy and light chain CDRs. The CDRs are listed in Table 4 (SEQ ID NOs:5, 6, 7, 8, 9, and 10).

Table 4 (mutations in underlined and in boldface)

Designation	Heavy chain CDRs	Light chain CDRs
H7F8m12	GY <u>HFT</u> <u>N</u> YPID (SEQ ID NO:5; CDR1), NFHPYNDD <u>A</u> N (SEQ ID NO:6; CDR2), <u>R</u> <u>M</u> EL <u>S</u> <u>V</u> FAY (SEQ ID NO:7; CDR3)	RTS <u>D</u> NVY <u>R</u> YLT (SEQ ID NO:8; CDR1), <u>Y</u> AKTLAE (SEQ ID NO:9; CDR2), QHHYGTPYT (SEQ ID NO:10; CDR3)

Affinity of antibody containing CDRs with the mutations was measured in multiple experiments. First, a binding titration on the surface of yeast expressing human PCSK9 was performed. Results of this experiment are shown in Figure 2; the binding affinity of h7F8m12 (labeled as “mutant” in Figure 2) was 101 pM whereas the binding affinity of the original h7F8

antibody (labeled as “human” in Figure 2) was 3.2 nM. These data demonstrate that the affinity of the h7F8m12 is about 30-fold improved over the original h7F8 antibody.

Binding to both human and rhesus PCSK9 were also measured by ELISA. Full-length h7F8 IgG2 antibody was expressed in HEK293 cells and purified. ELISAs were carried out by immobilizing purified antibody at 1 ug/mL in PBS at 4°C overnight. Plates were blocked with PBS+2% BSA and then His-tagged PCSK9 (PCSK9-His) was titrated at a range of concentrations between 5 nM and 6.8 pM. After incubation, binding was detected with an anti-His-HRP antibody. The fitted K_d is shown in Table 3 and demonstrates that h7F8m12 binds both human and rhesus PCSK9 with roughly equal affinity. Finally, 7F8m12, h7F8, and the murine 7F8 sequences were each expressed as Fab fragments in HEK293 cells and purified. The Fab fragments were used in studies to determine off-rates using surface plasmon resonance (SPR; Reichert, Inc.). Briefly, in these experiments, human PCSK9 was immobilized on a chip using standard EDC-NHS chemistry and the purified Fabs were flowed over the chip.

Fits to the off-rate data are shown in Table 3, and demonstrate that h7F8m12 has a significantly slower dissociation rate than either the murine or humanized parent versions of 7F8.

Further sequences of h7F8m12 are provided below.

The heavy chain variable domain sequence of h7F8m12 is

QVQLVQSGAEVKKPGASVKVSCKASGYHFTNYPIDWVRQAPGQGLEWMGNFH
 PYNDDANYAQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCARRMELSVF
 AYWGQGTLVTVSS
 (SEQ ID NO:17).

The light chain variable domain sequence of h7F8m12 is

DIQMTQSPSSLSASVGDRVTITCRTSDNVYRYLTWVQKPGKAPKLLIYYAKTLA
 EGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQHHYGTPYTFGGGTKVEIK
 (SEQ ID NO:18).

The heavy chain sequence of of h7F8m12 is

QVQLVQSGAEVKKPGASVKVSCKASGYHFTNYPIDWVRQAPGQGLEWMGNFH
 PYNDDANYAQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCARRMELSVF
 AYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW

NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDPHKPSNTKV
 TVERKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 QFNWYVDGVEVHNAKTKPREEQFNSTFRVVSFLTQVHVDWLNQKEYKCKVSN
 KGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEV
 5 ESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN
 HYTKSLSLSPGK (SEQ ID NO:19).

The light chain sequence of h7F8m12 is

DIQMTQSPSSLSASVGDRVTITCRTSDNVYRYLTWFQQKPGKAPKLLIYYAKTLA
 10 EGVPSRFGSGSGTDFTLTISLQPEDFATYYCQHHYGTPTYTFGGGTKVEIKTVAA
 PSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO:20).

15 **Example 3: *In vivo* inhibition of PCSK9**

To test the ability of PCSK9 antibodies to influence LDL levels, experiments were designed using cynomolgus monkeys and carried out by Covance Laboratories (Dedham, MA). In these experiments, six healthy male cynomolgus macaque monkeys (divided into two groups of three monkeys each) received a single bolus intravenous injection of h7F8m12. Monkeys
 20 received 3 mg/kg or 5 mg/kg. Blood samples were collected pre-injection (at days -8, -4 and 0), and then at the following time points post-injection: 15 minutes, 1 day, 2 days, 3 days, 4 days, 7 days, 10 days, 14 days, 17 days and 21 days. Monkeys were fasted overnight before each blood collection. For each time point, freshly collected serum was aliquoted and analyzed for LDL cholesterol on a Beckman Coulter AU480 Chemistry System and the remainder of the serum was
 25 frozen at -70°C for subsequent analysis.

In both groups, LDL-c levels decreased starting at day 1 following injection (Fig. 3). LDL reached a minimum at 7-10 days post-injection depending on dose, and they returned to approximately baseline (pre-injection) levels by day 21. There was a slight increase in duration of LDL lowering effect at the higher dose of antibody.

Also, in both groups, triglycerides decreased following administration of h7F8m12 (Fig. 4); a maximum decrease of 57% was observed at day 3. Total cholesterol decreased up to 28% after injection (Fig. 5); no changes in HDL were observed over the length of the experiment.

These data demonstrate that a high affinity PCSK9 antibody targeted to the prodomain of PCSK9 can significantly reduce LDL-c.

PK Assay

Serum samples from all three groups of monkeys were analyzed for antibody PK using the Human Therapeutic IgG2 EIA kit (Cayman Chemical, Ann Arbor, MI). In these analyses, microplate wells were pre-coated with an anti-human IgG capture antibody, and captured IgG was detected with a separate anti-human IgG-HRP detection antibody. Standard curves for each group were generated using the respective chimeric antibody diluted in 5% monkey serum (Bioreclamation Inc., Westbury, NY) at concentrations between 6.25 ng/ml and 400 ng/mL. Test samples were also diluted to appropriate concentrations (between 20- and 200-fold) in 5% monkey serum and were run in duplicate.

Fig. 6 shows the data from which the average terminal half-lives are calculated.

Free (unbound) PCSK9 Assay

For both groups of monkeys, freely circulating PCSK9 levels were quantified using a sandwich ELISA. Plates were coated with h7F8m12 as a capture antibody then blocked with PBS + 2% BSA. Standards (rhesus PCSK9 at 0.5 ng/ml to 400 ng/mL) or samples (serum samples at a 5-fold dilution) were prepared in PBS + 2% BSA + 0.1% Tween®-20 and incubated in the plate for 1 hour. A biotinylated human anti-PCSK9 antibody that does not compete with h7F8m12 was then added to the wells and incubated for 1 hour before detecting with streptavidin-HRP. Samples were run in duplicate.

Analysis of data from both groups showed that within 15 minutes following antibody injection, all PCSK9 was antibody-bound, leaving no free circulating PCSK9 in serum (Fig. 7). In both monkeys, this persisted until 7-10 days after injection; after this point a return to baseline levels was observed. The timing of the increase in free PCSK9 corresponds with the increase in LDL cholesterol.

Immunogenicity

For both groups, the immunogenicity of dosed antibody was measured by ELISA. Two assays were developed, one to measure IgG response and one to measure IgM. For the IgM ELISA, plates were coated with the dosed anti-PCSK9 chimera and blocked. Serum from various time points was titrated at 10-fold dilutions and incubated. Plates were washed, then any bound monkey antibodies were detected using an anti-monkey IgM-HRP antibody (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The IgG assay was carried out similarly, except that plates were coated with the purified Fab version of the dosed antibody, and the detection antibody was an anti-monkey IgG (Fc specific)-HRP (AbD Serotec, Raleigh, NC).

No change in either IgG or IgM titers was observed in any monkeys over the course of the experiment, thereby demonstrating relatively low immunogenicity of h7F8m12.

Example 4: Octet[®] measurements of antibody affinity

Affinity measurements for ch7F8 and h7F8m12 binding to human and rhesus PCSK9 were performed on an Octet RED96 System (FortéBIO, Menlo Park, CA). All equilibration, binding, and wash steps were performed in PBS + 1% BSA + 0.05% Tween-20 with 200 μ L of sample in a 96 well, black plate (VWR cat# 82050-784). Anti-human-IgG-Fc capture tips (FortéBIO, cat# 18-5060) were pre-equilibrated in buffer for 10 minutes then incubated with 50 nM ch7F8 or h7F8m12 to capture antibody to an optical thickness value > 1. The tips were dipped in buffer for 2 minutes to measure baseline then incubated with human or rhesus PCSK9 at a range of concentrations to measure association. After 1-2 minutes, tips were switched into buffer and ligand dissociation measured for 5-15 minutes. Optical thickness traces were fit to a 1:1 biomolecular interaction model to determine k_{on} , k_{off} , and K_d values.

The Octet[®] experiments confirm the increased affinity of h7F8m12 compared to ch7F8 (Table 5). Additionally, both antibodies bind with similar affinity to human and rhesus PCSK9.

Table 5 – Binding constant values for PCSK9 antibodies measured by Octet®

Antibody	PCSK9	K_D (M)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
ch7F8	Human	2.7×10^{-9}	3.2×10^5	8.4×10^{-4}
ch7F8	Rhesus	1.9×10^{-9}	2.2×10^5	4.2×10^{-4}
h7F8-m12	Human	1.3×10^{-11}	4.8×10^5	6.3×10^{-6}
h7F8-m12	Rhesus	1.5×10^{-11}	3.8×10^5	5.9×10^{-6}

Other embodiments are within the scope of the following claims.

What is claimed is:

1. An antibody or antigen binding fragment thereof comprising a heavy chain variable domain comprising SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

5

2. The antibody or antigen binding fragment of claim 1 comprising a light chain variable domain comprising SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10.

10 3. The antibody or antigen binding fragment of claim 1 or 2, wherein the antibody or antigen binding fragment can bind, e.g., specifically bind, to a human PCSK9 (e.g., SEQ ID NO:1).

15 4. The antibody or antigen binding fragment of claim 3, wherein the antibody or antigen binding fragment can bind, e.g., specifically bind, to the prodomain of a human PCSK9 (e.g., SEQ ID NO:12).

5. The antibody or antigen binding fragment of claim 3, wherein the antibody or antigen binding fragment binds to the human PCSK9 with a K_d of 10 to 200 pM.

20 6. The antibody, fragment, or derivative thereof of any one of the preceding claims, wherein the antibody, fragment, or derivative thereof can bind to S127, D129, or S127 and D129 of SEQ ID NO:1.

25 7. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment comprises a heavy chain variable domain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:17 and a light chain variable domain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:18.

30 8. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment comprises a heavy chain variable domain sequence as

set forth in SEQ ID NO:17 and a light chain variable domain sequence as set forth in SEQ ID NO:18.

9. The antibody or antigen binding fragment of any one of the preceding claims, wherein
5 the antibody or antigen binding fragment comprises a heavy chain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:19 and a light chain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:20.

10. The antibody or antigen binding fragment of any one of the preceding claims,
10 wherein the antibody or antigen binding fragment comprises a heavy chain sequence as set forth in SEQ ID NO:19 and a light chain sequence as set forth in SEQ ID NO:20.

11. An antibody or antigen binding fragment thereof comprising a heavy chain sequence as set forth in SEQ ID NO:19 and a light chain sequence as set forth in SEQ ID NO:20.

12. An antibody or antigen binding fragment comprising a heavy chain variable domain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:17 and a light chain variable domain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:18.

13. The antibody or antigen binding fragment of claim 12, wherein the antibody or antigen binding fragment comprises a heavy chain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:19 and a light chain that is at least 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:20.

14. A method of inhibiting PCSK9, the method comprising contacting a sample comprising a PCSK9 with the antibody or antigen binding fragment of any one of the preceding claims.

15. A method of detecting a PCSK9 polypeptide in a sample, the method comprising
30 a. providing a sample,

b. contacting the sample with the antibody or antigen binding fragment of any one of the preceding claims under conditions suitable for specific binding of a PCSK9 polypeptide, and

5 c. detecting a complex comprising the PCSK9 antibody or antigen binding fragment.

10 16. A method of treating a subject in need of treatment with a PCSK9 inhibitor, the method comprising administering to the subject a therapeutically effective amount of the antibody or antigen binding fragment of any one of the preceding claims.

17. The method of claim 16, further comprising determining the level of free PCSK9 in a sample (e.g., a blood, plasma, or serum sample) obtained from the subject before and after treatment with the antibody or antigen binding fragment, wherein the level of free PCSK9 in the sample obtained from the subject after treatment is reduced compared with the level of free
15 PCSK9 in the sample obtained from the subject before treatment.

18. The method of claim 16 or 17, wherein the subject is at risk for or has been diagnosed with a cholesterol-related disorder.

20 19. The method of any one of claims 16 to 18, wherein the subject is has been diagnosed with hypercholesterolemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease, atherosclerosis, or a dyslipidemia.

25 20. The method of claim 19, wherein the dyslipidemia is elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated very low density lipoprotein (VLDL), and/or low HDL.

21. The method of any one of claims 16 to 19, further comprising treating the subject with a second agent.

30 22. The method of claim 21, wherein the second agent is another PCSK9 inhibitor.

23. The method of claim 21, wherein the second agent is a statin, a fibrate, ezetimibe, cholestyramine, colestipol, colesevelam, torcetrapib, avasimibe, implitapide, or niacin.

5 24. The method of claim 23, wherein the statin is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, pitavastatin, and mevastatin.

10 25. The method of claim 23, wherein the fibrate is selected from the group consisting of clofibrate, ciprofibrate, bezafibrate, fenofibrate, gemfibrozil, and clinofibrate.

26. A vector comprising a nucleic acid sequence encoding the antibody or antigen binding fragment of any one of claims 1 to 13.

15 27. A host cell comprising the vector of claim 26.

28. A pharmaceutical composition comprising the antibody or antigen binding fragment of any one of claims 1 to 13 and a pharmaceutically acceptable excipient.

20 29. A kit comprising the antibody or antigen binding fragment of any one of claims 1 to 13 and instructions for use of the antibody or antigen binding fragment.

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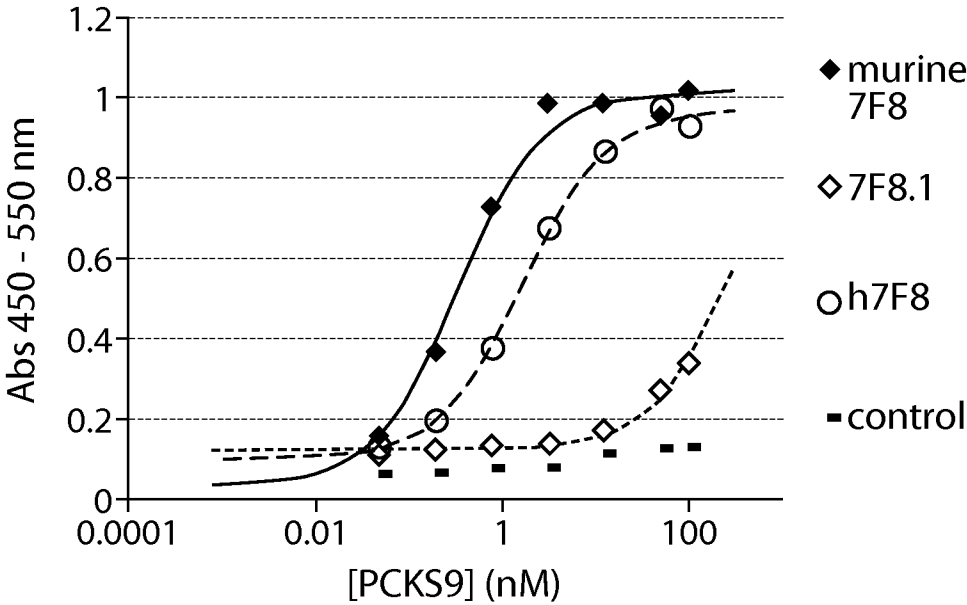


Fig. 1

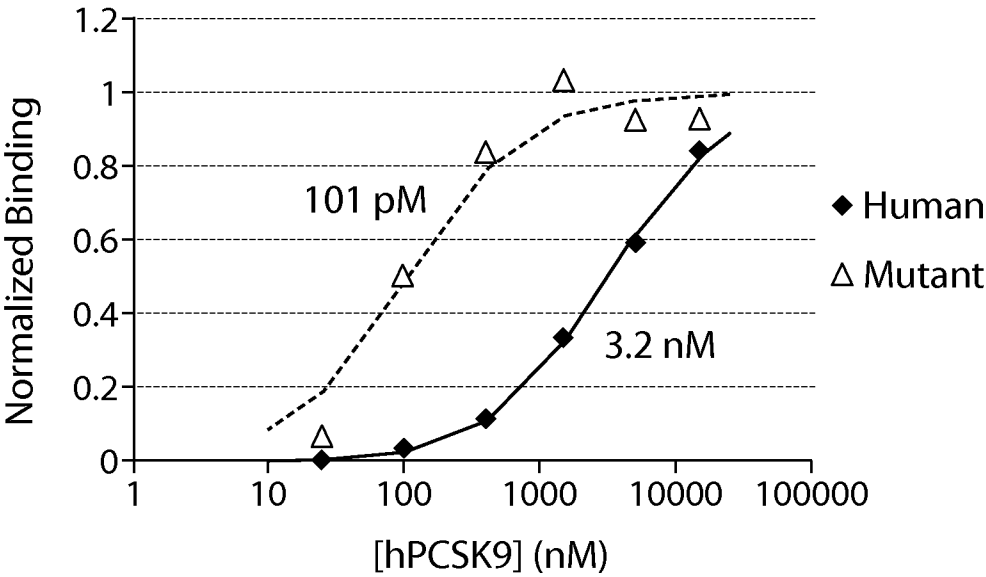


Fig. 2

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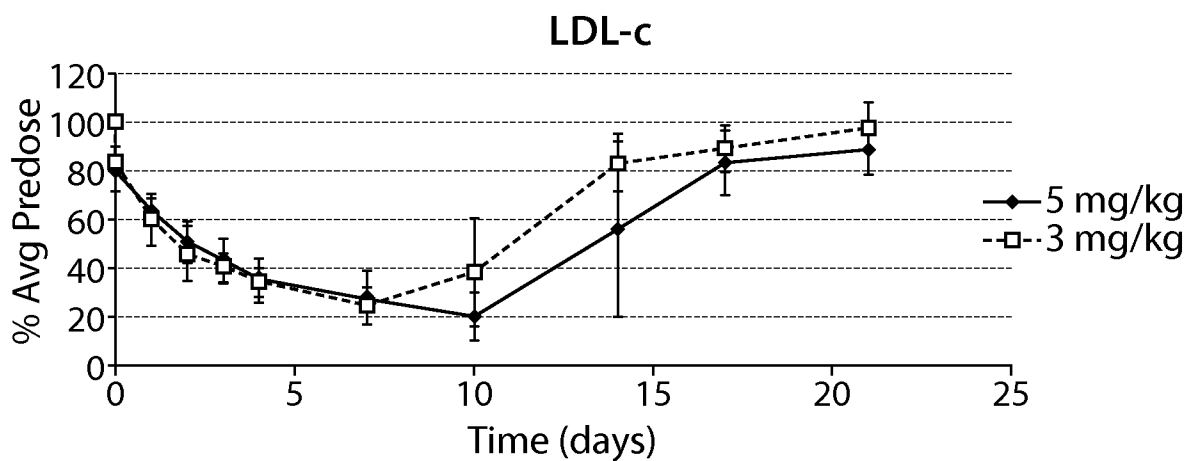


Fig. 3

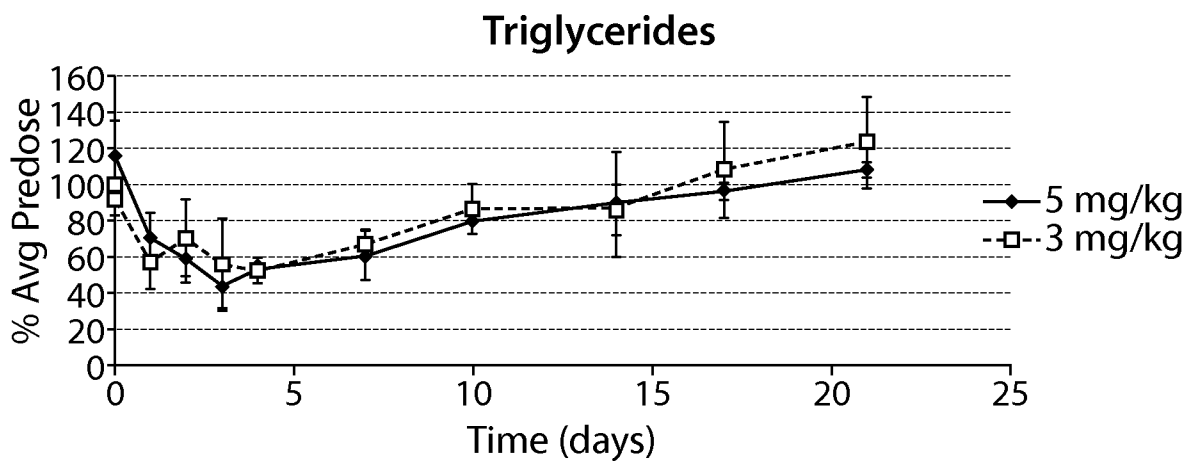


Fig. 4

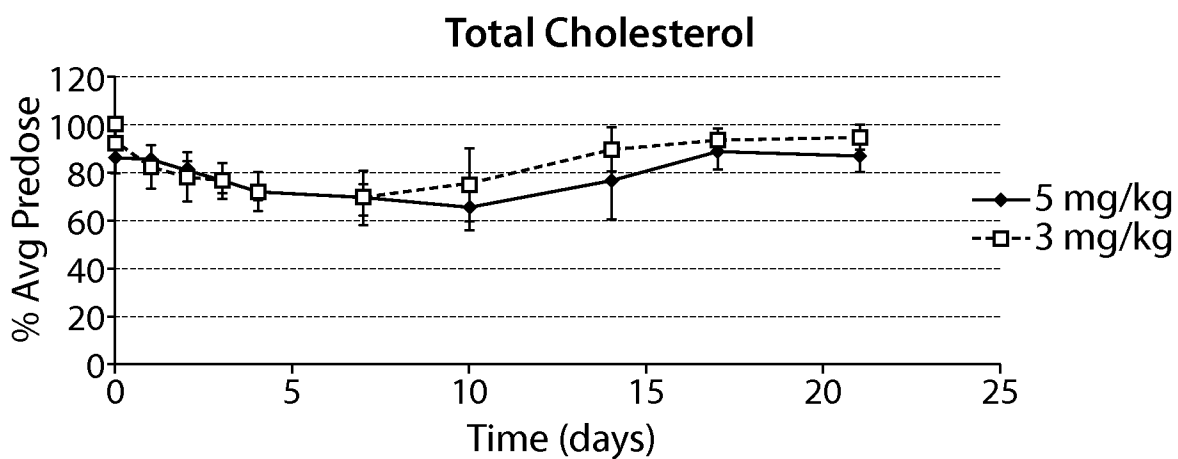


Fig. 5

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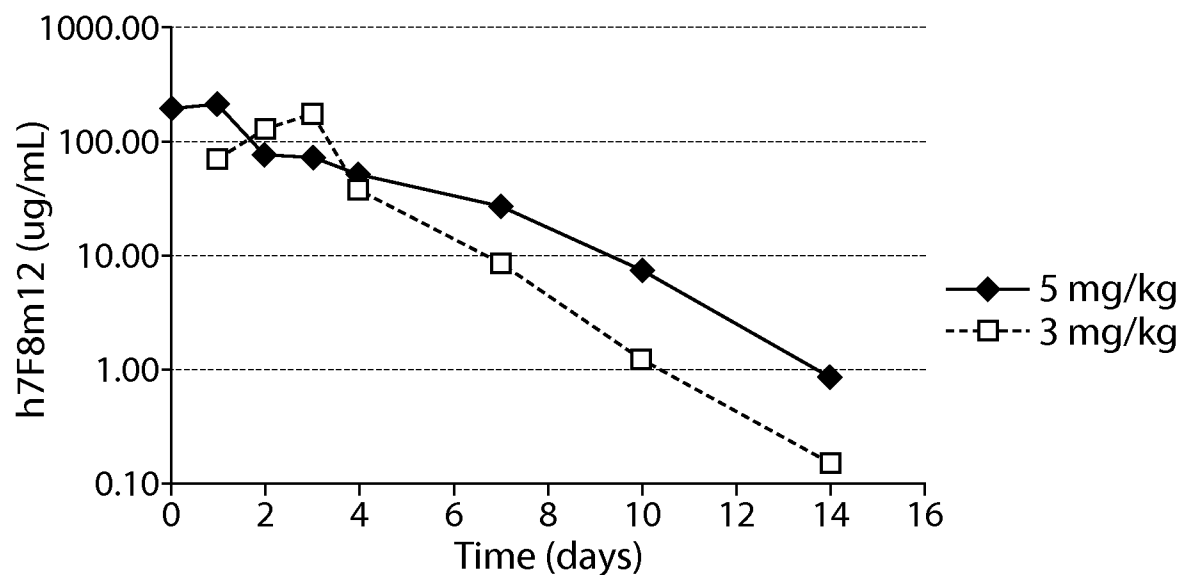


Fig. 6

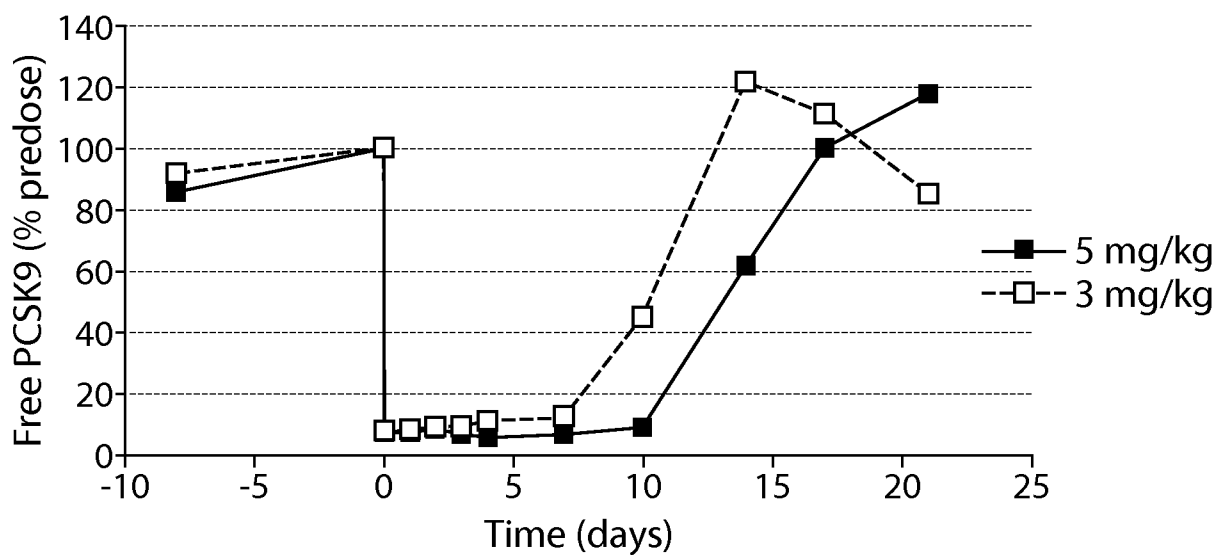


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2015/037345

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K16/40 A61K39/00
 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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/166768 A1 (SLEEMAN MARK W [US] ET AL) 1 July 2010 (2010-07-01) page 114 - page 127 examples 3-9 page 179	1-29
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"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

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"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

25 August 2015

Date of mailing of the international search report

02/09/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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 Fax: (+31-70) 340-3016

Authorized officer

Fellows, Edward

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/037345

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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