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(54) Title: METHODS AND COMPOSITIONS FOR TREATING A PROPROTEIN CONVERTASE SUBTILISIN KEXIN (PCSK9) GENE-ASSOCIATED DISORDER

(57) Abstract: The invention relates to methods of inhibiting the expression of a PCSK9 gene in a subject, as well as therapeutic and prophylactic methods for treating subjects having a lipid disorder, such as a hyperlipidemia using RNAi agents, e.g., double- stranded RNAi agents, targeting the PCSK9 gene.

METHODS AND COMPOSITIONS FOR TREATING A PROPROTEIN CONVERTASE SUBTILISIN KEXIN (PCSK9) GENE-ASSOCIATED DISORDER

Related Applications

5   This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/209,526, filed on August 25, 2015, the entire contents of which are incorporated herein by reference.

This application is related to U.S. Provisional Application No. 61/733,518, filed on December 5, 2012; U.S. Provisional Application No. 61/793,530, filed on March 15, 2013;

10   U.S. Provisional Application No. 61/886,916, filed on October 4, 2013; U.S. Provisional Application No. 61/892,188, filed on October 17, 2013; PCT Application No. PCT/US2013/073349, filed on December 5, 2013; and U.S. Patent Application No. 14/650,128, filed on June 5, 2015. The entire contents of each of the foregoing patent applications are hereby incorporated herein by reference.

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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 ASCII copy, created on August 24, 2016, is named 121301-04420_SL.txt and is 188,218

20   bytes in size.

Background of the Invention

Proprotein convertase subtilisin kexin 9 (PCSK9) is a member of the subtilisin serine protease family. The other eight mammalian subtilisin proteases, PCSK1-PCSK8 (also called

25   PC1/3, PC2, furin, PC4, PC5/6, PACE4, PC7, and S1P/SKI-1) are proprotein convertases that process a wide variety of proteins in the secretory pathway and play roles in diverse biological processes (Bergeron, F. (2000) *J. Mol. Endocrinol.* 24, 1-22, Gensberg, K., (1998) *Semin. Cell Dev. Biol.* 9, 11-17, Seidah, N. G. (1999) *Brain Res.* 848, 45-62, Taylor, N. A., (2003) *FASEB J.* 17, 1215-1227, and Zhou, A., (1999) *J. Biol. Chem.* 274, 20745-20748).

30   PCSK9 has been proposed to play a role in cholesterol metabolism. PCSK9 mRNA expression is down-regulated by dietary cholesterol feeding in mice (Maxwell, K. N., (2003) *J. Lipid Res.* 44, 2109-2119), up-regulated by statins in HepG2 cells (Dubuc, G., (2004) *Arterioscler. Thromb. Vasc. Biol.* 24, 1454-1459), and up-regulated in sterol regulatory element binding protein (SREBP) transgenic mice (Horton, J. D., (2003) *Proc. Natl. Acad.*

35   *Sci. USA* 100, 12027-12032), similar to the cholesterol biosynthetic enzymes and the low-density lipoprotein receptor (LDLR). Furthermore, PCSK9 missense mutations have been found to be associated with a form of autosomal dominant hypercholesterolemia (Hchola3) (Abifadel, M., et al. (2003) *Nat. Genet.* 34, 154-156, Timms, K. M., (2004) *Hum. Genet.* 114,

349-353, Leren, T. P. (2004) *Clin. Genet.* 65, 419-422). PCSK9 may also play a role in determining LDL cholesterol levels in the general population, because single-nucleotide polymorphisms (SNPs) have been associated with cholesterol levels in a Japanese population (Shioji, K., (2004) *J. Hum. Genet.* 49, 109-114).

5   Autosomal dominant hypercholesterolemias (ADHs) are monogenic diseases in which patients exhibit elevated total and LDL cholesterol levels, tendon xanthomas, and premature atherosclerosis (Rader, D. J., (2003) *J. Clin. Invest.* 111, 1795-1803). The pathogenesis of ADHs and a recessive form, autosomal recessive hypercholesterolemia (ARH) (Cohen, J. C., (2003) *Curr. Opin. Lipidol.* 14, 121-127), is due to defects in LDL uptake by the liver. ADH

10   may be caused by LDLR mutations, which prevent LDL uptake, or by mutations in the protein on LDL, apolipoprotein B, which binds to the LDLR. ARH is caused by mutations in the ARH protein that are necessary for endocytosis of the LDLR-LDL complex via its interaction with clathrin. Therefore, if PCSK9 mutations are causative in Hchola3 families, it seems likely that PCSK9 plays a role in receptor-mediated LDL uptake.

15   Overexpression studies point to a role for PCSK9 in controlling LDLR levels and, hence, LDL uptake by the liver (Maxwell, K. N. (2004) *Proc. Natl. Acad. Sci. USA* 101, 7100-7105, Benjannet, S., *et al.* (2004) *J. Biol. Chem.* 279, 48865-48875, Park, S. W., (2004) *J. Biol. Chem.* 279, 50630-50638). Adenoviral-mediated overexpression of mouse or human PCSK9 for 3 or 4 days in mice results in elevated total and LDL cholesterol levels; this effect

20   is not seen in LDLR knockout animals (Maxwell, K. N. (2004) *Proc. Natl. Acad. Sci. USA* 101, 7100-7105, Benjannet, S., *et al.* (2004) *J. Biol. Chem.* 279, 48865-48875, Park, S. W., (2004) *J. Biol. Chem.* 279, 50630-50638). In addition, PCSK9 overexpression results in a severe reduction in hepatic LDLR protein, without affecting LDLR mRNA levels, SREBP protein levels, or SREBP protein nuclear to cytoplasmic ratio.

25   While hypercholesterolemia itself is asymptomatic, longstanding elevation of serum cholesterol can lead to atherosclerosis. Over a period of decades, chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries which can lead to progressive stenosis or even complete occlusion of the involved arteries. In addition, smaller plaques may rupture and cause a clot to form and obstruct blood flow resulting in, for

30   example, myocardial infarction and/or stroke. If the formation of the stenosis or occlusion is gradual, blood supply to the tissues and organs slowly diminishes until organ function becomes impaired.

Accordingly, there is a need in the art for effective treatments for PCSK9-associated diseases, such as a hyperlipidemia, *e.g.,* hypercholesterolemia.

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Summary of the Invention

The present invention is based, at least in part, on the surprising discovery that a single dose of a double-stranded RNAi agent comprising chemical modifications shows an exceptional potency and durability to inhibit expression of PCSK9. Specifically, a single fixed dose, *e.g.*, a fixed dose of about 300 mg to about 500 mg, of RNAi agents targeting a human PCSK9 gene, *e.g.*, nucleotides 3544-3623 of a human PCSK9 gene (nucleotides 3544-3623 of SEQ ID NO:1), *e.g.*, nucleotides 3601-3623 of SEQ ID NO:1, including a GalNAc ligand are shown herein to be exceptionally effective and durable in silencing the activity of a PCSK9 gene.

Accordingly, the present invention provides methods for inhibiting expression of a PCSK9 gene in a subject and methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a PCSK9 gene, *e.g.*, a disorder mediated by PCSK9 expression, such as a hyperlipidemia, *e.g.*, hypercholesterolemia, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a PCSK9 gene.

In one aspect, the methods of the present invention for inhibiting expression of a PCSK9 gene in a subject and methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a PCSK9 gene, *e.g.*, a disorder mediated by PCSK9 expression, such as a hyperlipidemia, *e.g.*, hypercholesterolemia, include administering to a subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby inhibiting the expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a fixed

dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1 thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having hpercholesterolemia.

The fixed dose may administered to the subject at an interval of once a week, once every two weeks, once a month,once a quarter, or bianually.

In one embodiment, the subject is administered a fixed dose of about 25 mg to about 50 mg once a week. In another embodiment, the subject is administered a fixed dose of about 50 mg to about 100 mg once every two weeks. In another embodiment, the subject is administered a fixed dose of about 100 mg to about 200 mg once a month. In yet another embodiment, the subject is administered a fixed dose of about 300 mg to about 800 mg once a quarter. In another embodiment, the subject is administered a fixed dose of about 300 mg to about 800 mg biannually.

The present invention also provides methods in which the RNAi agent is administered in a dosing regimen that includes a loading phase and a maintenance phase.

Accordingly, in one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering

afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject , and wherein the maintenance phase comprises administering a fixed dose of about 25 mg to about 100 mg of the RNAi agent to the subject about once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby inhibiting the expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mg of the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mgof the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mg of the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of

the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having hperlipidemia.

The double stranded RNAi agent may be administered to the subject subcutaneously, *e.g.*, by subcutaneous injection, or intramuscularly.

In one embodiment, the antisense strand comprises a nucleotide sequence selected from the group consisting of any one of the unmodified nucleotide sequences provided in Table 1. In one embodiment, the double-stranded RNAi agent targets nucleotides 3601-3623 of SEQ ID NO:1. In one embodiment, the agent targeting nucleotides 3601-3623 of SEQ ID NO:1 is AD-60212.

In one embodiment, the antisense strand comprises the nucleotide sequence 5'-ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685).

In one embodiment, the sense strand comprises the nucleotide sequence 5'-CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686).

In one embodiment, the double-stranded ribonucleic acid RNAi agent comprises at least one modified nucleotide.

In one embodiment, substantially of the nucleotides of the sense strand are modified nucleotides. In another embodiment, substantially all of the nucleotides of the antisense strand are modified nucleotides. In yet another embodment, substantially of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, all of the nucleotides of the sense strand are modified nucleotides. In another embodiment, all of the nucleotides of the antisense strand are modified nucleotides. In yet another embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense

5   strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby

10   decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an

15   antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby

20   treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a

25   double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the

30   subject having hyperlipidemia.

In one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a

35   double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all

of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3'(SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having hyperlipidemia.

The fixed dose may administered to the subject at an interval of once a week, once every two weeks, once a month,once a quarter, or bianually.

In one embodiment, the subject is administered a fixed dose of about 25 mg to about 50 mg once a week. In another embodiment, the subject is administered a fixed dose of about 50 mg to about 100 mg once every two weeks. In another embodiment, the subject is administered a fixed dose of about 100 mg to about 200 mg once a month. In yet another embodiment, the subject is administered a fixed dose of about 300 mg to about 800 mg once a quarter. In another embodiment, the subject is administered a fixed dose of about 300 mg to about 800 mg biannually.

In one aspect, the present inventionprovide methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides method sof treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having hyperlipidemia.

In one aspect, the present inventionprovide methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand

comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding

5   fragment thereof, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering

10   afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-

15   ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding

20   fragment thereof, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides method sof treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading

25   phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide

30   sequence 5'- ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding

35   fragment thereof, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a double-stranded

ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-ACAAAGCAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having hyperlipidemia.

In one embodiment, the subject is a human.

In one embodiment, the disorder that would benefit from reduction in PCSK9 expression is hyperlipidemia, such as hypercholesterolemia.

In one embodiment, the hyperlipidemia is hypercholesterolemia.

The double stranded RNAi agent may be administered to the subject subcutaneously, *e.g.,* by subcutaneous injection, or intramuscularly.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage.

In one embodiment, the double-stranded ribonucleic acid RNAi agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the double-stranded ribonucleic acid RNAi agent.

In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is

In one embodiment, the double-stranded ribonucleic acid RNAi agent is conjugated to the ligand as shown in the following schematic

and, wherein X is O or S. In one embodiment, the X is O.

In one embodiment, PCSK9 expression is inhibited by at least about 30%.

In one embodiment, the methods of the invention further comprise determining an LDLR genotype or phenotype of the subject.

In one embodiment, administering the double-stranded RNAi agent results in a decrease in serum cholesterol in the subject and/or a decrease in PCSK9 protein accumulation.

In one embodiment, the methods of the invention further comprise determining the serum cholesterol level in the subject.

In one embodiment, the methods of the invention further comprise comprising administering an additional therapeutic agent to the subject, *e.g.*, a statin and/or an anti-PCSK9 antibody. In one embodiment, the anti-PCSK9 antibody is selected from the group consisting of alirocumab (Praluent), evolocumab (Repatha), and bococizumab.

In one embodiment, the RNAi agent is administered as a pharmaceutical compositon.

The RNAi agent may be administered in an unbuffered solution, such as saline or water., or administered with a buffer solution. In one embodiment, the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In another embodiment, the buffer solution is phosphate buffered saline (PBS).

In one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a single fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having hyperlipidemia.

In one embodiment, the subject is administered a fixed dose of about 200 mg to about 800 mg once a quarter. In another embodiment, the subject is administered a fixed dose of about 200 mg to about 800 mg biannually.

In one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 800 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby inhibiting expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein

the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby decreasing the level of LDLc in the subject.

In yet another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject , and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage, and administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof, thereby treating the subject having hyperlipidemia.

In one embodiment, the subject is administered the maintenance does as a fixed dose of about 200 mg to about 800 mg once a quarter. In another embodiment, the subject is administered the maintenance does as a fixed dose of about 200 mg to about 800 mg biannually.

In one embodiment, the double-stranded ribonucleic acid RNAi agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the double-stranded ribonucleic acid RNAi agent.

In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is

In one embodiment, the double-stranded ribonucleic acid RNAi agent is conjugated to the ligand as shown in the following schematic

and, wherein X is O or S. In one embodiment, the X is O.

In one embodiment, the anti-PCSK9 antibody, or antigen-binding fragment thereof, is selected from the group consisting of alirocumab (Praluent), evolocumab (Repatha), and bococizumab.

In one embodiment, the methods further include administering an additional therapeutic agent, *e.g.*, a statin, to the subject.

In one aspect, the present invention provides kits for performing the method of the invention. The kits include the RNAi agent, and instructions for use, and optionally, means for administering the RNAi agent to the subject.

5   The present invention is further illustrated by the following detailed description and drawings.

Brief Description of the Drawings

Figure 1 is a graph showing the knockdown of PCSK9 protein levels, shown as a

10   percent mean PCSK9 knockdown relative to baseline, in subjects receiving a single fixed dose of AD-60212.

Figure 2 is a graph showing the lowering of LDL-c levels, shown as a percent mean LCL-C lowering relative to baseline, in subjects receiving a single fixed dose of AD-60212.

Figure 3 is a graph showing the knockdown of PCSK9 protein levels, shown as a

15   percent mean PCSK9 knockdown relative to baseline, in subjects receiving multiple fixed doses of AD-60212.

Figure 4 is a graph showing the lowering of LDL-c levels, shown as a percent mean LCL-C lowering relative to baseline, in subjects receiving multiple fixed doses of AD-60212.

20   **Detailed Description of the Invention**

The present invention is based, at least in part, on the surprising discovery that a single dose of a double-stranded RNAi agent comprising chemical modifications shows an exceptional potency and durability to inhibit expression of PCSK9. Specifically, a single fixed dose, *e.g.*, a fixed dose of about 300 mg to about 500 mg, of RNAi agents targeting a

25   human PCSK9 gene, *e.g.*, nucleotides 3544-3623 of a human PCSK9 gene (nucleotides 3544-3623 of SEQ ID NO:1), *e.g.*, nucleotides 3601-3623 of SEQ ID NO:1, including a GalNAc ligand are shown herein to be exceptionally effective and durable in silencing the activity of a PCSK9 gene.

Accordingly, the present invention provides methods for inhibiting expression of a

30   PCSK9 gene and methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a PCSK9 gene, *e.g.*, a disorder mediated by PCSK9 expression, such as a hyperlipidemia, *e.g.*, hypercholesterolemia, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a PCSK9 gene.

35

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a PCSK9 gene, as well as compositions, uses,

and methods for treating subjects having diseases and disorders that would benefit from
inhibition and/or reduction of the expression of this gene.

I.   Definitions

In order that the present invention may be more readily understood, certain terms are
first defined. In addition, it should be noted that whenever a value or range of values of a
parameter are recited, it is intended that values and ranges intermediate to the recited values
are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at
least one) of the grammatical object of the article. By way of example, "an element" means
one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the
phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term
"and/or," unless context clearly indicates otherwise. For example, "sense strand or antisense
strand" is understood as "sense strand or antisense strand or sense strand and antisense
strand."

The term "about" is used herein to mean within the typical ranges of tolerances in the
art. For example, "about" can be understood as about 2 standard deviations from the mean.
In certain embodiments, about means +10%. In certain embodiments, about means +5%.
When about is present before a series of numbers or a range, it is understood that "about" can
modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers is understood to include the
number adjacent to the term "at least", and all subsequent numbers or integers that could
logically be included, as clear from context. For example, the number of nucleotides in a
nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21
nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated
property. When at least is present before a series of numbers or a range, it is understood that
"at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to
the phrase and logical lower values or intergers, as logical from context, to zero. For
example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0
nucleotide overhang. When "no more than" is present before a series of numbers or a range,
it is understood that "no more than" can modify each of the numbers in the series or range.

As used herein, "PCSK9" refers to the proprotein convertase subtilisin kexin 9 gene or
protein. PCSK9 is also known as FH3, HCHOLA3, NARC-1, or NARC1. The term PCSK9
includes human PCSK9, the amino acid and nucleotide sequence of which may be found in,
for example, GenBank Accession No. GI:299523249 (SEQ ID NO:1); mouse PCSK9, the

amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:163644257; rat PCSK9, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:77020249.

Additional examples of PCSK9 mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

In one embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in PCSK9 expression; a human at risk for a disease, disorder or condition that would benefit from reduction in PCSK9 expression; a human having a disease, disorder or condition that would benefit from reduction in PCSK9 expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in PCSK9 expression as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with a disorder that would benefit from reduction in PCSK9 expression, or slowing or reversing the progression of such a disorder, whether detectable or undetectable. For example, in the context of hyperlipidemia, treatment may include a decrease in serum lipid levels, *e.g.*, a decrease in low density lipoprotein cholesterol (LDLc). "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of a PCSK9 gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with a disease, disorder, or condition mediated by PCSK9 expression, *e.g.*, a symptom such as cardiovascular disease, *e.g.*, coronary artery disease (CAD) (also known as coronary heart disease (CHD)), or transient ischemic attack (TIA) or stroke. The likelihood of developing a such a symptom is reduced, for example, when an individual having one or more risk factors (*e.g.*, diabetes, previous personal history of CHD or noncoronary atherosclerosis (*e.g.*, abdominal aortic aneurysm, peripheral artery disease, and carotid artery stenosis), family history of cardiovascular disease, *e.g.*, in male relatives younger than 50 years or in female relatives younger than age 60 years, tobacco use, hypertension, and/or obesity (BMI ≥30)) for a disease, disorder, or condition mediated by PCSK9 expression, *e.g.*, hypercholesterolemia, either fails to develop, for example, coronary artery disease, or develops, *e.g.*, coronary artery disease, with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention. Prevention can require administration of more than one dose.

The interchangeably used terms "PCSK9-associated disease" and "disorder that would benefit from a reduction in PCSK9 expression," as used herein, are intended to include any disease, disorder, or condition associated with the PCSK9 gene or protein. Such a disease may be caused, for example, by excess production of the PCSK9 protein, by PCSK9

5   gene mutations, by abnormal cleavage of the PCSK9 protein, by abnormal interactions between PCSK9 and other proteins or other endogenous or exogenous substances. Exemplary PCSK9-associated diseases include lipidemias, *e.g.*, a hyperlipidemia, and other forms of lipid imbalance such as hypercholesterolemia, hypertriglyceridemia and the pathological conditions associated with these disorders, *e.g.*, CHD and atherosclerosis.

10   As used herein the term "hypercholesterolemia" refers to a form of hyperlipidemia (elevated levels of lipids in the blood) in which there are high levels of cholesterol in the serum of a subject, *e.g.*, at least about 240 mg/dL of total cholesterol.

As used herein, the term "cardiovascular disease " refers to a disease affecting the heart or blood vessels, which includes, for example, arteriosclerosis, coronary artery disease

15   (or narrowing of the arteries), heart valve disease, arrhythmia, heart failure, hypertension, orthostatic hypotension, shock, endocarditis, diseases of the aorta and its branches, disorders of the peripheral vascular system, heart attack,cardiomyopathy, and congenital heart disease.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a patient for treating a PCSK9 associated

20   disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by PCSK9 expression, the types of preceding or concomitant

25   treatments, if any, and other individual characteristics of the patient to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject who does not yet experience or display symptoms of a PCSK9-associated disease, but who may be predisposed to the disease, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease.

30   Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

35   A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. RNAi gents employed in the

methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a PCSK9 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a PCSK9 gene.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about 25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.*, Table B). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms "iRNA", "RNAi agent," "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.*, inhibits, the expression of PCSK9 in a cell, *e.g.*, a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, a PCSK9 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, a PCSK9 gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded siRNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150: 883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In another embodiment, an "iRNA" for use in the compositions, uses, and methods of the invention is a double-stranded RNA and is referred to herein as a "double stranded RNAi agent," "double-stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA" refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.*, a PCSK9 gene. In some embodiments of the invention, a double-stranded RNA (dsRNA) triggers the

degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any

overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12,

5   13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

10   In one embodiment, an RNAi agent of the invention is a dsRNA agent, each strand of which comprises 19-23 nucleotides that interacts with a target RNA sequence, *i.e.*, a PCSK9 target mRNA sequence. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the

15   dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more

20   endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188).

In another embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a PCSK9 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double

25   stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases

30   unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188).

As used herein, the term "nucleotide overhang" refers to at least one unpaired

35   nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three

nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on
5   the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1
10   nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3,
15   2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

20   In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the sense strand of the duplex. In certain embodiments,
25   an extended overhang is present on the 5'end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the
30   overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNAi agent, *i.e.*, no nucleotide overhang. A "blunt ended" RNAi agent is a
35   dsRNA that is double-stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with nucleotide overhangs at one end (*i.e.*, agents with one overhang and one blunt end) or with nucleotide overhangs at both ends.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.*, a PCSK9 mRNA. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.*, a PCSK9 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, 2, or 1 nucleotides of the 5'- and/or 3'-terminus of the iRNA. In one embodiment, a double stranded RNAi agent of the invention includea a nucleotide mismatch in the antisense strand. In another embodiment, a double stranded RNAi agent of the invention includea a nucleotide mismatch in the sense strand. In one embodiment, the nucleotide mismatch is, for example, within 5, 4, 3, 2, or 1 nucleotides from the 3'-terminus of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA. .

The term "sense strand," or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of an iRNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding PCSK9). For example, a polynucleotide is complementary to at least a part of a PCSK9 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding PCSK9.

In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, an "iRNA" may include ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such

modifications, as used in an iRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In one aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense RNA molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense RNA molecule may be about 15 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

II. Methods of the Invention

The present invention provides methods of inhibiting the expression of a Proprotein Convertase Subtilisin Kexin 9 (PCSK9) gene in a subject. The present invention also provides therapeutic and prophylactic methods for treating or preventing diseases and conditions that can be modulated by down regulating PCSK9 gene expression. For example, the compositions described herein can be used to treat lipidemia, *e.g.*, a hyperlipidemia and other forms of lipid imbalance such as hypercholesterolemia, hypertriglyceridemia and the pathological conditions associated with these disorders such as heart and circulatory diseases. Other diseases and conditions that can be modulated by down regulating PCSK9 gene expression include lysosomal storage diseases including, but not limited to, Niemann-Pick disease, Tay-Sachs disease, Lysosomal acid lipase deficiency, and Gaucher Disease. The methods include administering to the subject a therapeutically effective amount or prophylactically effective amount of an RNAi agent of the invention. In some embodiments, the method includes administering an effective amount of a PCSK9 iRNA agent to a patient having a heterozygous LDLR genotype.

As PCSK9 regulates the levels of the LDL receptor, which in turn removes cholesterol-rich LDL particles from the plasma, the effect of the decreased expression of a PCSK9 gene preferably results in a decrease in LDLc (low density lipoprotein cholesterol) levels in the blood, and more particularly in the serum, of the mammal. In some embodiments, LDLc levels are decreased by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, as compared to pretreatment levels. Accordingly, the present invention also provides methods for lowering the level of low density cholesterol (LDLc) in the serum of a subject.

In certain embodiments of the invention, the double-stranded RNAi agent is administered to a subject as a fixed dose. A "fixed dose" (*e.g.*, a dose in mg) means that one dose of an iRNA agent is used for all subjects regardless of any specific subject-related factors, such as weight. In other embodiments, an iRNA agent of the invention is administered to a subject as a weight-based dose. A "weight-based dose" (*e.g.*, a dose in mg/kg) is a dose of the iRNA agent that will change depending on the subject's weight.

In certain embodiments of an RNAi agent is administered to the subject as a fixed dose of about 100 mg to about 700 mg, about 150 mg to about 700 mg, about 200 mg to about 700 mg, about 250 mg to about 700 mg, about 300 mg to about 700 mg, about 350 mg to about 700 mg, about 400 mg to about 700 mg, about 450 mg to about 700 mg, about 500 mg to about 700 mg, about 550 mg to about 700 mg, about 600 to about 700 mg, about 650 to about 700 mg, about 100 mg to about 650 mg, about 150 mg to about 650 mg, about 200 mg to about 650 mg, about 250 mg to about 650 mg, about 300 mg to about 650 mg, about 350 mg to about 650 mg, about 400 mg to about 650 mg, about 450 mg to about 650 mg, about 500 mg to about 650 mg, about 550 mg to about 650 mg, about 600 to about 650 mg, about 100 mg to about 600 mg, about 150 mg to about 600 mg, about 200 mg to about 600 mg, about 250 mg to about 600 mg, about 300 mg to about 600 mg, about 350 mg to about 600 mg, about 400 mg to about 600 mg, about 450 mg to about 600 mg, about 500 mg to about 600 mg, about 550 mg to about 600 mg, about 100 mg to about 550 mg, about 150 mg to about 550 mg, about 200 mg to about 550 mg, about 250 mg to about 550 mg, about 300 mg to about 550 mg, about 350 mg to about 550 mg, about 400 mg to about 550 mg, about 450 mg to about 550 mg, about 500 mg to about 550 mg, about 100 mg to about 500 mg, about 150 mg to about 500 mg, about 200 mg to about 500 mg, about 250 mg to about 500 mg, about 300 mg to about 500 mg, about 350 mg to about 500 mg, about 400 mg to about 500 mg, or about 450 mg to about 500 mg, *e.g.*, a fixed dose of about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, or about 700 mg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention.

The administration may be repeated, for example, on a regular basis. For example, the fixed dose may administered to the subject at an interval of once a week, once every two weeks, once a month, once a quarter, or bianuallyfor six months or a year or longer, *i.e.*, chronic administration.

In one embodiment, the subject is administered a fixed dose of about 25 mg to about 50 mg once a week. In another embodiment, the subject is administered a fixed dose of about 50 mg to about 100 mg once every two weeks. In another embodiment, the subject is administered a fixed dose of about 100 mg to about 200 mg once a month. In yet another

embodiment, the subject is administered a fixed dose of about 300 mg to about 600 mg once a quarter. In another embodiment, the subject is administered a fixed dose of about 300 mg to about 600 mg biannually (*i.e.*, twice a year).

Accordingly, in one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent, *e.g.*, a dsRNA, of the invention (*e.g.*, a pharmaceutical composition comprising a dsRNA of the invention), wherein a total of about 200 mg to about 600 mg of the double-stranded RNAi agent is administered to the subject every quarter or biannually, and wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1.

In another aspect, the present invention provides methods of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent, wherein a total of about 200 mg to about 600 mg of the double-stranded RNAi agent is administered to the subject every quarter or biannually, and wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, such as a hyperlipidemia, *e.g.*, hypercholesterolemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent, *e.g.*, a dsRNA, of the invention (*e.g.*, a pharmaceutical composition comprising a dsRNA of the invention), wherein a total of about 200 mg to about 600 mg of the double-stranded RNAi agent is administered to the subject every quarter or biannually, and wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia, such as hypercholestrolemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent, *e.g.*, a dsRNA, of the invention (*e.g.*, a pharmaceutical composition comprising a dsRNA of the invention), wherein a total of about 200 mg to about 600 mg of the double-stranded RNAi agent is administered to the

subject every quarter or biannually, and wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1.

As indicated above, the administration of the RNAi agents to a subject may be repeated on a regular basis, for example, at an interval of once a week, once every two weeks, once a month, once a quarter, or bianually.

Accordingly, in some embodiments, the RNAi agent is administered in a dosing regimen that includes a "loading phase" of closely spaced administrations that may be followed by a "maintenance phase", in which the RNAi agent is administred at longer spaced intervals. For example, after administration weekly or biweekly for one month, administration can be repeated once per month, for six months or a year or longer, *i.e.,* chronic administration.

In one embodiment, the loading phase comprises a single administration of the RNAi agent during the first week. In another embodiment, the loading phase comprises a single administration of the RNAi agent during the first two weeks. In yet another embodiment, the loading phase comprises a single administration of the RNAi agent during the first month.

In certain embodiments of an RNAi agent is administered to the subject during a loading phase as a fixed dose of about 100 mg to about 700 mg, about 150 mg to about 700 mg, about 200 mg to about 700 mg, about 250 mg to about 700 mg, about 300 mg to about 700 mg, about 350 mg to about 700 mg, about 400 mg to about 700 mg, about 450 mg to about 700 mg, about 500 mg to about 700 mg, about 550 mg to about 700 mg, about 600 to about 700 mg, about 650 to about 700 mg, about 100 mg to about 650 mg, about 150 mg to about 650 mg, about 200 mg to about 650 mg, about 250 mg to about 650 mg, about 300 mg to about 650 mg, about 350 mg to about 650 mg, about 400 mg to about 650 mg, about 450 mg to about 650 mg, about 500 mg to about 650 mg, about 550 mg to about 650 mg, about 600 to about 650 mg, about 100 mg to about 600 mg, about 150 mg to about 600 mg, about 200 mg to about 600 mg, about 250 mg to about 600 mg, about 300 mg to about 600 mg, about 350 mg to about 600 mg, about 400 mg to about 600 mg, about 450 mg to about 600 mg, about 500 mg to about 600 mg, about 550 mg to about 600 mg, about 100 mg to about 550 mg, about 150 mg to about 550 mg, about 200 mg to about 550 mg, about 250 mg to about 550 mg, about 300 mg to about 550 mg, about 350 mg to about 550 mg, about 400 mg to about 550 mg, about 450 mg to about 550 mg, about 500 mg to about 550 mg, about 100 mg to about 500 mg, about 150 mg to about 500 mg, about 200 mg to about 500 mg, about 250 mg to about 500 mg, about 300 mg to about 500 mg, about 350 mg to about 500 mg, about 400 mg to about 500 mg, or about 450 mg to about 500 mg, *e.g.,* a fixed dose of about100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250

mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, or about 700 mg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention.

In one embodiment , the maintenance phase comprises administration of a dose of the RNAi agent to the subject once a month, once every two months, once every three months, once every four months, once every five months, or once every six months. In one particular embodiment, the maintenance dose is administered to the subject once a month.

The maintenance dose or doses can be the same or lower than the initial dose, *e.g.*, one-half of the initial dose. For example, a maintenance dose may be about 25 mg to about 100 mg administered to the subject monthly, for example about 25 mg to about 75 mg, about 25 mg to about 50 mg, or about 50 mg to about 75 mg, *e.g.*, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention.

Any of these schedules may optionally be repeated for one or more iterations. The number of iterations may depend on the achievement of a desired effect, *e.g.*, the suppression of a PCSK9 gene, and/or the achievement of a therapeutic or prophylactic effect, *e.g.*, reducing serum cholesterol levels or reducing a symptom of hypercholesterolemia. Following treatment, the patient can be monitored for changes in his/her condition. The dosage of the RNAi agent may either be increased in the event the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disease state is observed, if the disease state has been ablated, or if undesired side-effects are observed.

Accordingly, in one aspect, the present invention provides methods of inhibiting the expression of a PCSK9 gene in a subject. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering afixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject , and wherein the maintenance phase comprises administering a fixed dose of about 25 mg to about 100 mg of the RNAi agent to the subject about once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby inhibiting the expression of the PCSK9 gene in the subject.

In another aspect, the present invention provides method s of decreasing the level of low densiy lipoprotein (LDLc) in a subject. The emthods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mg of the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby decreasing the level of LDLc in the subject.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PCSK9 expression. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mgof the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

In yet another aspect, the present invention provides methods of treating a subject having hyperlipidemia. The methods include administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase, wherein the loading phase comprises administering to the subject a fixed dose of about 200 mg to about 600 mg of the RNAi agent, and wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a month, wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having hperlipidemia.

In one embodiment, the double-stranded ribonucleic acid (RNAi) agent for use in the methods of the present invention comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'-

ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.

As used herein, a "subject" includes a human or non-human animal, preferably a vertebrate, and more preferably a mammal. A subject may include a transgenic organism. Most preferably, the subject is a human, such as a human suffering from or predisposed to developing a PCSK9-associated disease.

The methods and uses of the invention include administering a composition described herein such that expression of the target PCSK9 gene is decreased, for an extended period of time, such as, for about 80 days, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, or about 180 days, or longer.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of PCSK9 may be determined by determining the mRNA expression level of PCSK9 using methods routine to one of ordinary skill in the art, *e.g.,* Northern blotting, qRT-PCR, by determining the protein level of PCSK9 using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques, and/or by determining a biological activity of PCSK9, such as the effect on one or more serum lipid parameters, such as, for example, total cholesterol levels, high density lipoprotein cholesterol (HDL) levels, non-HDL levels, very low density lipoprotein cholesterol (VLDL) levels, triglyceride levels, Lp(a) levels, and lipoprotein particle size.

Administration of the dsRNA according to the methods and uses of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a disorder that would benefit from reduction in PCSK9 expression. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, serum lipid levels (*e.g.,* LDLc levels), quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a hyperlipidemia may be

assessed, for example, by periodic monitoring of LDLc levels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters.

5   A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given iRNA

10   drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

          Alternatively, the efficacy can be measured by a reduction in the severity of disease as

15   determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

          In general, the iRNA agent does not activate the immune system, *e.g.*, it does not

20   increase cytokine levels, such as TNF-alpha or IFN-alpha levels. For example, when measured by an assay, such as an in vitro PBMC assay, such as described herein, the increase in levels of TNF-alpha or IFN-alpha, is less than 30%, 20%, or 10% of control cells treated with a control dsRNA, such as a dsRNA that does not target PCSK9.

          In another embodiment, administration can be provided when Low Density

25   Lipoprotein cholesterol (LDLc) levels reach or surpass a predetermined minimal level, such as greater than 70mg/dL, 130 mg/dL, 150 mg/dL, 200 mg/dL, 300 mg/dL, or 400 mg/dL.

          The effect of the decreased PCSK9 gene preferably results in a decrease in LDLc (low density lipoprotein cholesterol) levels in the blood, and more particularly in the serum, of the mammal. In some embodiments, LDLc levels are decreased by at least 10%, 15%, 20%, 25%,

30   30%, 40%, 50%, 60%, 70%, 80%, 90% or more, as compared to pretreatment levels.

          In some embodiments of the methods of the invention, PCSK9 expression is decreased for an extended duration, *e.g.*, at least one week, two weeks, three weeks, or four weeks or longer. For example, in certain instances, expression of the PCSK9 gene is suppressed by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by

35   administration of an iRNA agent described herein. In some embodiments, the PCSK9 gene is suppressed by at least about 60%, 70%, or 80% by administration of the iRNA agent. In some embodiments, the PCSK9 gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide.

The RNAi agents of the invention may be administered to a subject using any mode of administration known in the art, including, but not limited to subcutaneous, intravenous, intramuscular, intraocular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In preferred embodiments, the
5   agents are administered subcutaneously.

In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of PCSK9, or a therapeutic or prophylactic effect. A depot injection may also
10   provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a
15   subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi agent to the liver.
20   Other modes of administration include epidural, intracerebral, intracerebroventricular, nasal administration, intraarterial, intracardiac, intraosseous infusion, intrathecal, and intravitreal, and pulmonary. The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.
25   The iRNA can be administered by intravenous infusion over a period of time, such as over a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or about a 25 minute period. The administration may be repeated, for example, on a regular basis, such as weekly, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a
30   less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

Administration of the iRNA can reduce PCSK9 levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%,
35   28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic

5   reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

Owing to the inhibitory effects on PCSK9 expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

10   An iRNA of the invention may be administered in "naked" form, or as a "free iRNA." A naked iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer

15   solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

The invention further provides methods and uses for the use of an iRNA or a

20   pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction and/or inhibition of PCSK9 expression, *e.g.*, a subject having hyperlipidemia, *e.g.*, hypercholesterolemia, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. The siRNA and an

25   additional therapeutic agent can be administered in combination in the same composition, e.g., parenterally, or the additional therapeutic agent can be administered as part of a separate composition or by another method described herein.

Examples of additional therapeutic agents include those known to treat an agent known to treat a lipid disorders, such as hypercholesterolemia, atherosclerosis or

30   dyslipidemia. For example, a siRNA featured in the invention can be administered with, *e.g.*, an HMG-CoA reductase inhibitor (*e.g.*, a statin), a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist (*e.g.*, losartan potassium, such as Merck & Co. 's Cozaar®), an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a

35   cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular protectant (*e.g.*, AGI-1067, from Atherogenics), a glycoprotein IIb/IIIa inhibitor, aspirin or an aspirin-

like compound, an IBAT inhibitor (*e.g.*, S-8921 , from Shionogi), a squalene synthase inhibitor, or a monocyte chemoattractant protein (MCP)-I inhibitor. Exemplary HMG-CoA reductase inhibitors include atorvastatin (Pfizer's Lipitor®/Tahor/Sortis/Torvast/Cardyl), pravastatin (Bristol-Myers Squibb 's Pravachol, Sankyo's Mevalotin/Sanaprav), simvastatin

5   (Merck's Zocor®/Sinvacor, Boehringer Ingelheim's Denan, Banyu's Lipovas), lovastatin (Merck's Mevacor/Mevinacor, Bexal's Lovastatina, Cepa; Schwarz Pharma's Liposcler), fluvastatin (Novartis' Lescol®/Locol/Lochol, Fujisawa's Cranoc, Solvay's Digaril), cerivastatin (Bayer's Lipobay/GlaxoSmithKline's Baycol), rosuvastatin (AstraZeneca' s Crestor®), and pitivastatin (itavastatin/risivastatin) (Nissan Chemical, Kowa Kogyo, Sankyo,

10   and Novartis). Exemplary fibrates include, *e.g.*, bezafibrate (*e.g.*, Roche's Befizal®/Cedur®/Bezalip®, Kissei's Bezatol), clofibrate (*e.g.*, Wyeth's Atromid-S®), fenofibrate (*e.g.*, Fournier's Lipidil/Lipantil, Abbott's Tricor®, Takeda's Lipantil, generics), gemfibrozil (*e.g.*, Pfizer' s Lopid/Lipur) and ciprofibrate (Sanofi-Synthelabo's Modalim®). Exemplary bile acid sequestrants include, *e.g.*, cholestyramine (Bristol-Myers Squibb's

15   Questran® and Questran Light™), colestipol (*e.g.*, Pharmacia's Colestid), and colesevelam (Genzyme/Sankyo's WelChol™). Exemplary niacin therapies include, *e.g.*, immediate release formulations, such as Aventis' Nicobid, Upsher-Smith's Niacor, Aventis' Nicolar, and Sanwakagaku's Perycit. Niacin extended release formulations include, *e.g.*, Kos Pharmaceuticals' Niaspan and Upsher-Smith's SIo- Niacin. Exemplary antiplatelet agents

20   include, *e.g.*, aspirin (*e.g.*, Bayer's aspirin), clopidogrel (Sanofi-Synthelabo/Bristol-Myers Squibb's Plavix), and ticlopidine (*e.g.*, Sanofi-Synthelabo's Ticlid and Daiichi's Panaldine). Other aspirin-like compounds useful in combination with a dsRNA targeting PCSK9 include, *e.g.*, Asacard (slow-release aspirin, by Pharmacia) and Pamicogrel (Kanebo/Angelini Ricerche/CEPA). Exemplary angiotensin-converting enzyme inhibitors include, *e.g.*, ramipril

25   (*e.g.*, Aventis' Altace) and enalapril (*e.g.*, Merck & Co.'s Vasotec). Exemplary acyl CoA cholesterol acetyltransferase (AC AT) inhibitors include, e.g., avasimibe (Pfizer), eflucimibe (BioMsrieux Pierre Fabre/Eli Lilly), CS-505 (Sankyo and Kyoto), and SMP-797 (Sumito). Exemplary cholesterol absorption inhibitors include, *e.g.*, ezetimibe (Merck/Schering-Plough Pharmaceuticals Zetia®) and Pamaqueside (Pfizer). Exemplary CETP inhibitors include, *e.g.*,

30   Torcetrapib (also called CP-529414, Pfizer), JTT-705 (Japan Tobacco), and CETi-I (Avant Immunotherapeutics). Exemplary microsomal triglyceride transfer protein (MTTP) inhibitors include, *e.g.*, implitapide (Bayer), R-103757 (Janssen), and CP-346086 (Pfizer). Other exemplary cholesterol modulators include, e.g., NO- 1886 (Otsuka/TAP Pharmaceutical), CI-1027 (Pfizer), and WAY- 135433 (Wyeth-Ayerst).

35   Exemplary bile acid modulators include, *e.g.*, HBS-107 (Hisamitsu/Banyu), Btg-511 (British Technology Group), BARI-1453 (Aventis), S-8921 (Shionogi), SD-5613 (Pfizer), and AZD- 7806 (AstraZeneca). Exemplary peroxisome proliferation activated receptor (PPAR) agonists include, *e.g.*, tesaglitazar (AZ-242) (AstraZeneca), Netoglitazone (MCC-

555) (Mitsubishi/ Johnson & Johnson), GW-409544 (Ligand
Pharniaceuticals/GlaxoSmithKline), GW-501516 (Ligand
Pharmaceuticals/GlaxoSmithKline), LY-929 (Ligand Pharmaceuticals and Eli Lilly), LY-
465608 (Ligand Pharmaceuticals and Eli Lilly), LY-518674 (Ligand Pharmaceuticals and Eli
Lilly), and MK-767 (Merck and Kyorin). Exemplary gene-based therapies include, *e.g.* ,
AdGWEGF 121.10 (GenVec), ApoA1 (UCB Pharma/Groupe Fournier), EG-004 (Trinam)
(Ark Therapeutics), and ATP -binding cassette transporter- A1 (ABCA1) (CV
Therapeutics/Incyte, Aventis, Xenon). Exemplary Glycoprotein IIb/IIIa inhibitors include,
e.g., roxifiban (also called DMP754, Bristol-Myers Squibb), Gantofiban (Merck
KGaA/Yamanouchi), and Cromafiban (Millennium Pharmaceuticals). Exemplary squalene
synthase inhibitors include, *e.g.*, BMS- 1884941 (Bristol-Myers Squibb), CP-210172 (Pfizer),
CP-295697 (Pfizer), CP-294838 (Pfizer), and TAK-475 (Takeda). An exemplary MCP-I
inhibitor is, *e.g.*, RS-504393 (Roche Bioscience). The anti-atherosclerotic agent BO- 653
(Chugai Pharmaceuticals), and the nicotinic acid derivative Nyclin (Yamanouchi
Pharmacuticals) are also appropriate for administering in combination with a dsRNA featured
in the invention. Exemplary combination therapies suitable for administration with a dsRNA
targeting PCSK9 include, *e.g.*, advicor (Niacin/lovastatin from Kos Pharmaceuticals),
amlodipine/atorvastatin (Pfizer), and ezetimibe/simvastatin (*e.g.*, Vytorin® 10/10, 10/20,
10/40, and 10/80 tablets by Merck/Schering-Plough Pharmaceuticals). Agents for treating
hypercholesterolemia, and suitable for administration in combination with a dsRNA targeting
PCSK9 include, *e.g.*, lovastatin, niacin Altoprev® Extended-Release Tablets (Andrx Labs),
lovastatin Caduet® Tablets (Pfizer), amlodipine besylate, atorvastatin calcium Crestor®
Tablets (AstraZeneca), rosuvastatin calcium Lescol® Capsules (Novartis), fluvastatin sodium
Lescol® (Reliant, Novartis), fluvastatin sodium Lipitor® Tablets (Parke-Davis), atorvastatin
calcium Lofibra® Capsules (Gate), Niaspan Extended-Release Tablets (Kos), niacin
Pravachol Tablets (Bristol-Myers Squibb), pravastatin sodium TriCor® Tablets (Abbott),
fenofibrate Vytorin® 10/10 Tablets (Merck/Schering-Plough Pharmaceuticals), ezetimibe,
simvastatin WelChol™ Tablets (Sankyo), colesevelam hydrochloride Zetia® Tablets
(Schering), ezetimibe Zetia® Tablets (Merck/Schering-Plough Pharmaceuticals), and
ezetimibe Zocor® Tablets (Merck).

In one embodiment, an iRNA agent is administered in combination with an
ezetimibe/simvastatin combination (*e.g.*, Vytorin® (Merck/Schering-Plough
Pharmaceuticals)).

In another embodiment, an iRNA agent is administered in combination with an anti-
PCSK9 antibody. Exemplary anti-PCSK9 antibodies for use in the combination therapies of
the invention inclue, for example, alirocumab (Praluent), evolocumab (Repatha),
bococizumab (PF-04950615, RN316, RN-316, L1L3; Pfizer, Rinat) , lodelcizumab
(LFU720, pJG04; Novartis), ralpancizumab (RN317, PF-05335810; Pfizer, Rinat), RG7652 (

MPSK3169A, YW508.20.33b; Genentech), LY3015014 (Lilly), LPD1462 (h1F11; Schering-Plough), AX1(AX189, 1B20, 1D05; Merck & Co), ALD306 (Alder); mAb1 (Boehringer), andIg1-PA4 (Nanjing Normal U.).

In one embodiment, the iRNA agent is administered to the patient, and then the additional therapeutic agent is administered to the patient (or *vice versa*). In another embodiment, the iRNA agent and the additional therapeutic agent are administered at the same time.

In another aspect, the invention features, a method of instructing an end user, *e.g.*, a caregiver or a subject, on how to administer an iRNA agent described herein. The method includes, optionally, providing the end user with one or more doses of the iRNA agent, and instructing the end user to administer the iRNA agent on a regimen described herein, thereby instructing the end user.

In one aspect, the invention provides a method of treating a patient by selecting a patient on the basis that the patient is in need of LDL lowering, LDL lowering without lowering of HDL, ApoB lowering, or total cholesterol lowering. The method includes administering to the patient a siRNA in an amount sufficient to lower the patient's LDL levels or ApoB levels, *e.g.*, without substantially lowering HDL levels.

Genetic predisposition plays a role in the development of target gene associated diseases, *e.g.*, hyperlipidemia. Therefore, a patient in need of a siRNA can be identified by taking a family history, or, for example, screening for one or more genetic markers or variants. Examples of genes involved in hyperlipidemia include but are not limited to, *e.g.*, LDL receptor (LDLR), the apoliproteins (ApoAl, ApoB, ApoE, and the like), Cholesteryl ester transfer protein (CETP), Lipoprotein lipase (LPL), hepatic lipase (LIPC), Endothelial lipase (EL), Lecithinxholesteryl acyltransferase (LCAT).

A healthcare provider, such as a doctor, nurse, or family member, can take a family history before prescribing or administering an iRNA agent of the invention. In addition, a test may be performed to determine a geneotype or phenotype. For example, a DNA test may be performed on a sample from the patient, *e.g.*, a blood sample, to identify the PCSK9 genotype and/or phenotype before a PCSK9 dsRNA is administered to the patient. In another embodiment, a test is performed to identify a related genotype and/or phenotype, *e.g.*, a LDLR genotype. Example of genetic variants with the LDLR gene can be found in the art, *e.g.*, in the following publications which are incorporated by reference: Costanza *et al* (2005) *Am J Epidemiol.* 15;161(8):714-24; Yamada *et al.* (2008) *J Med Genet.* Jan;45(l):22-8, Epub 2007 Aug 31; and Boes *et al* (2009) *Exp. Gerontol* 44: 136-160, Epub 2008 Nov 17.

The present invention further provides methods of inhibiting expression of a Proprotein Convertase Subtilisin Kexin 9 (PCSK9) in a cell, such as a cell within a subject, *e.g.*, a human subject.

Accordingly, the present invention provides methods of inhibiting expression of a PCSK9 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, in an amount effective to inhibit expression of the PCSK9 gene in the cell, thereby inhibiting expression of the PCSK9 in the cell.

5   Contacting of a cell with a double stranded RNAi agent may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. Contacting may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand,

10   including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc3 ligand, or any other ligand that directs the RNAi agent to a site of interest, *e.g.*, the liver of a subject.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating" and other similar terms, and includes any level of inhibition.

15   The phrase "inhibiting expression of a PCSK9" is intended to refer to inhibition of expression of any PCSK9 gene (such as, *e.g.*, a mouse PCSK9 gene, a rat PCSK9 gene, a monkey PCSK9 gene, or a human PCSK9 gene) as well as variants or mutants of a PCSK9 gene. Thus, the PCSK9 gene may be a wild-type PCSK9 gene, a mutant PCSK9 gene, or a transgenic PCSK9 gene in the context of a genetically manipulated cell, group of cells, or

20   organism.

"Inhibiting expression of a PCSK9 gene" includes any level of inhibition of a PCSK9 gene, *e.g.*, at least partial suppression of the expression of a PCSK9 gene. The expression of the PCSK9 gene may be assessed based on the level, or the change in the level, of any variable associated with PCSK9 gene expression, *e.g.*, PCSK9 mRNA level, PCSK9 protein

25   level, or lipid levels. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with PCSK9 expression compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose

30   baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of a PCSK9 gene is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about

35   45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

Inhibition of the expression of a PCSK9 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a PCSK9 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an RNAi agent of the invention, or by administering an RNAi agent of the invention to a subject in which the cells are or were present) such that the expression of a PCSK9 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

Alternatively, inhibition of the expression of a PCSK9 gene may be assessed in terms of a reduction of a parameter that is functionally linked to PCSK9 gene expression, *e.g.*, PCSK9 protein expression, such as lipid levels, cholesterol levels, *e.g.*, LDLc levels. PCSK9 gene silencing may be determined in any cell expressing PCSK9, either constitutively or by genomic engineering, and by any assay known in the art. The liver is the major site of PCSK9 expression. Other significant sites of expression include the pancreas, kidney, and intestines.

Inhibition of the expression of a PCSK9 protein may be manifested by a reduction in the level of the PCSK9 protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of a PCSK9 gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of PCSK9 mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of PCSK9 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the PCSK9 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase

protection assays (Melton *et al.*, *Nuc. Acids Res.* 12:7035), Northern blotting, *in situ* hybridization, and microarray analysis.

In one embodiment, the level of expression of PCSK9 is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of
5   selectively binding to a specific PCSK9. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but
10   are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to PCSK9 mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA
15   from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of PCSK9 mRNA.

An alternative method for determining the level of expression of PCSK9 in a sample
20   involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.*
25   (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present
30   in very low numbers. In particular aspects of the invention, the level of expression of PCSK9 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

The expression levels of PCSK9 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound
35   nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of PCSK9 expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

The level of PCSK9 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

The term "sample" as used herein refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, lymph, urine, cerebrospinal fluid, saliva, ocular fluids, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In preferred embodiments, a "sample derived from a subject" refers to blood or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue derived from the subject.

In some embodiments of the methods of the invention, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of PCSK9 may be assessed using measurements of the level or change in the level of PCSK9 mRNA or PCSK9 protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is sthe liver. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

III. iRNAs for Use in the Methods of the Invention

Described herein are methods for the use of double-stranded RNAi agents which inhibit the expression of a PCSK9 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having a PCSK9-associated disorder, *e.g.*, a hyperlipidemia, *e.g.*, hypercholesterolemia.

Accordingly, the invention provides double-stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, a PCSK9 gene) *in vivo* for use in the claimed methods.

In one embodiment, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-
5   modified, and does not comprise, *e.g.*, chemical modifications and/or conjugations known in the art and described herein. In another embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain aspects of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. For example substantially all of the nucleotides of the sense strand
10   are modified nucleotides, and/or substantially all of the nucleotides of the antisense strand are modified nucleotides and/or substantially all of the nucleotides of both the sense strand and the antisense strand are modified nucleotides. In other embodiments of the invention, all of the nucleotides of an iRNA of the invention are modified. For example all of the nucleotides of the sense strand are modified nucleotides, and/or all of the nucleotides of the antisense
15   strand are modified nucleotides and/or all of the nucleotides of both the sense strand and the antisense strand are modified nucleotides. iIRNAs of the invention in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

The dsRNA includes an antisense strand having a region of complementarity which is
20   complementary to at least a part of an mRNA formed in the expression of a PCSK9 gene. The region of complementarity is about 30 nucleotides or less in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the PCSK9 gene, the iRNA inhibits the expression of the PCSK9 gene (*e.g.*, a humanPCSK9 gene) by at least about 10% as assayed by, for example, a PCR or branched
25   DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially
30   complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a PCSK9 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art,
35   the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is between 15 and 30 base pairs in length, *e.g.*, between, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18,

15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is between 15 and 30 nucleotides in length, *e.g.,* between 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the dsRNA is about 15 to about 20 nucleotides in length, or between about 25 and about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

In certain embodiments, a dsRNA agent of the invention may include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a PCSK9 gene. These dsRNA agents with the longer length antisense strands preferably include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-

28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs.

Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target PCSK9 expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double-stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in Table 1, and the corresponding antisense strand of the sense strand is selected from the group of sequences of Table 1. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a PCSK9 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in Table 1, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in Table 1. In one embodiment, the substantially complementary sequences of the dsRNA are contained on

separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although some of the sequences in Table 1 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in Table 1 that is un-modified, un-conjugated, and/or modified and/or conjugated differently than described therein.

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 1, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of any one of Table 1 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of any one of Tables 3, 4, 5, 6, 18, 19, 20, 21, and 23, and differing in their ability to inhibit the expression of a PCSK9 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Table 1 identify a site(s) in a PCSK9 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided in Table 1 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a PCSK9 gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be

identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with
5   an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in aTable 1 represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.
10   Further, it is contemplated that for any sequence identified, *e.g.*, in Table 1, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of
15   iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the
20   molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.
            An iRNA as described herein can contain one or more mismatches to the target
25   sequence. In one embodiment, an iRNA as described herein contains no more than
3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides
30   from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of a PCSK9 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a
35   PCSK9 gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a PCSK9 gene is important, especially if the particular region of complementarity in a PCSK9 gene is known to have polymorphic sequence variation within the population.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example,

5   end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement

10   of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this

15   specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral

20   phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked

25   analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863;

30   4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805;

35   7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed

heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl

5   backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

         Representative U.S. patents that teach the preparation of the above oligonucleosides

10   include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

15   In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide

20   nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the

25   entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

         Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--

30   CH_2-, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], -- CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have

35   morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

         Modified RNAs can also contain one or more substituted sugar moieties.   The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein

the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_{.n}OCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$.

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

The RNA of an iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils

and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH,

5   2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for

10   increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and

15   Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

       Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to,

20   the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein

25   by reference.

       The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A"bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In

30   certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include the RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a

35   bicyclic sugar moiety comprising a 4'-CH2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen,

J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH2)—O-2' (LNA); 4'-(CH2)—S-2'; 4'-(CH2)2—O-2' (ENA); 4'-CH(CH3)—O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH2OCH3)—O-2' (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4'-C(CH3)(CH3)—O-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4'-CH2—N(OCH3)-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4'-CH2—O—N(CH3)-2' (see, e.g.,U.S. Patent Publication No. 2004/0171570); 4'-CH2—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4'-CH2—C(H)(CH3)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH2—C(=CH2)-2' (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133;7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH3)-0-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5' carbons of ribose . CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT

publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

One or more of the nucleotides of an iRNA of the invention may also include a hydroxymethyl substituted nucleotide. A "hydroxymethyl substituted nucleotide" is an acyclic 2'-3'-seco-nucleotide, also referred to as an "unlocked nucleic acid" ("UNA") modification

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

A. Modified iRNAs Comprising Motifs

In certain aspects of the invention, the double-stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in U.S. Patent Publication No. 2014/0315835 and PCT Publication No. WO 2013/075035, the entire contents of each of which are incorporated herein by reference, the entire contents of each of which are incorporated herein by reference. As shown herein and inU.S. Patent Publication No. 2014/0315835 and PCT Publication No. WO 2013/075035, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand and/or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense and/or antisense strand. The RNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand. The resulting RNAi agents present superior gene silencing activity.

More specifically, it has been surprisingly discovered that when the sense strand and antisense strand of the double-stranded RNAi agent are completely modified to have one or

more motifs of three identical modifications on three consecutive nucleotides at or near the
cleavage site of at least one strand of an RNAi agent, the gene silencing acitivity of the RNAi
agent was superiorly enhanced.

Accordingly, the invention provides double-stranded RNAi agents capable of
inhibiting the expression of a target gene (*i.e.*, a PCSK9 gene) *in vivo*. The RNAi agent
comprises a sense strand and an antisense strand. Each strand of the RNAi agent may range
from 12-30 nucleotides in length. For example, each strand may be between 14-30
nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30
nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19
nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21
nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA
("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent
may be 12-30 nucleotide pairs in length. For example, the duplex region can be between 14-
30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in
length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide
pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21
nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in
length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22,
23, 24, 25, 26, and 27 nucleotides in length.

In one embodiment, the RNAi agent may contain one or more overhang regions
and/or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The
overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5
nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in
length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. The
overhangs can be the result of one strand being longer than the other, or the result of two
strands of the same length being staggered. The overhang can form a mismatch with the
target mRNA or it can be complementary to the gene sequences being targeted or can be
another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to
form a hairpin, or by other non-base linkers.

In one embodiment, the nucleotides in the overhang region of the RNAi agent can
each independently be a modified or unmodified nucleotide including, but no limited to 2'-
sugar modified, such as, 2-F, 2'-Omethyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine
(Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo),
and any combinations thereof. For example, TT can be an overhang sequence for either end
on either strand. The overhang can form a mismatch with the target mRNA or it can be
complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-terminal end of the sense strand or, alternatively, at the 3'-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the RNAi has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In one embodiment, the RNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In another embodiment, the RNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In yet another embodiment, the RNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand. When the 2 nucleotide overhang

is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.*, in an alternating motif. Optionally, the RNAi agent further comprises a ligand (preferably GalNAc$_3$).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19

nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand

For an RNAi agent having a duplex region of 17-23 nucleotide in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, i.e., at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistry of the motifs are distinct from each other and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing

modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the RNAi agent may contain more than one motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In one embodiment, every nucleotide in the sense strand and antisense strand of the RNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′ hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5' terminal position, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10

nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of a RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

It may be possible, e.g., to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, e.g., in a 5' or 3' overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, e.g., with a modification described herein. Modifications can include, e.g., the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, e.g., the use of deoxyribonucleotides, , 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase , and modifications in the phosphate group, e.g., phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In one embodiment, each residue of the sense strand and antisense strand is independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'- O-methyl or 2'-fluoro modifications, or others.

In one embodiment, the N_a and/or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB…," "AABBAABBAABB…," "AABAABAABAAB…," "AAABAAABAAAB…," "AAABBBAAABBB…," or "ABCABCABCABC…," etc.

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, i.e., modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several

possibilities of modifications within the alternating motif such as "ABABAB…", "ACACAC…" "BDBDBD…" or "CDCDCD…," etc.

In one embodiment, the RNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5'-3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisenese strand may start with "BBAABBAA" from 5'-3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one embodiment, the RNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'- O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand and/or antisense strand interrupts the initial modification pattern present in the sense strand and/or antisense strand. This interruption of the modification pattern of the sense and/or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense and/or antisense strand surprisingly enhances the gene silencing acitivty to the target gene.

In one embodiment, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "…N_aYYYN_b…," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Altnernatively, N_a and/or N_b may be present or absent when there is a wing modification present.

The RNAi agent may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate

internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand and/or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand and/or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-standed RNAi agent comprises 6-8phosphorothioate internucleotide linkages. In one embodiment, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-terminus or the 3'-terminus.

In one embodiment, the RNAi comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, and/or the 5'end of the antisense strand.

In one embodiment, the 2 nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the RNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the

duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). In one embodiment, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense and/or antisense strand.

In one embodiment, the sense strand sequence may be represented by formula (I):

$$5' \; n_p\text{-}N_a\text{-}(X\,X\,X\;)_i\text{-}N_b\text{-}Y\,Y\,Y\text{-}N_b\text{-}(Z\,Z\,Z\;)_j\text{-}N_a\text{-}n_q \; 3' \quad (I)$$

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In one embodiment, the N_a and/or N_b comprise modifications of alternating pattern.

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p-N_a-YYY- N_a-n_q 3' (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

5' $n_{q'}$-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_l$-N'_a-$n_{p'}$ 3'   (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification;

and X'X'X', Y'Y'Y' and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, the N_a' and/or N_b' comprise modifications of alternating pattern.

The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23nucleotidein length, the Y'Y'Y' motif can occur at positions 9, 10, 11;10, 11, 12; 11, 12, 13; 12, 13, 14 ; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In one embodiment, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

5' $n_{q'}$-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-$n_{p'}$ 3'   (IIb);

5' $n_{q'}$-N_a'-Y'Y'Y'-N_b'-X'X'X'-$n_{p'}$ 3' (IIc); or

5' $n_{q'}$-N_a'- Z'Z'Z'-N_b'-Y'Y'Y'-N_b'- X'X'X'-N_a'-$n_{p'}$ 3' (IId).

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6. In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (Ia).

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of X', Y' and Z' may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X', Y' and Z', in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In one embodiment the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain X'X'X' motif or Z'Z'Z' motifs as wing modifications at the opposite end of the duplex region; and X'X'X' and Z'Z'Z' each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the RNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

sense: 5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

antisense: 3' $n_p{'}$ -$N_a{'}$-(X'X'X')$_k$-$N_b{'}$ -Y'Y'Y'-$N_b{'}$ -(Z'Z'Z')$_l$-$N_a{'}$ -$n_q{'}$ 5'

(III)
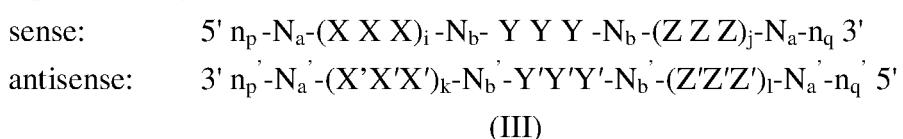
wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and $N_a{'}$ independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and $N_b{'}$ independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein

each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

5   In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

          Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

10   5' n_p- N_a -Y Y Y -N_a-n_q 3'
          3' n_p' -N_a' -Y'Y'Y' -N_a'n_q' 5'
                    (IIIa)
          5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'
          3' n_p' -N_a' -Y'Y'Y'-N_b' -Z'Z'Z'-N_a'n_q' 5'
15   (IIIb)
          5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'
          3' n_p' -N_a' -X'X'X'-N_b' -Y'Y'Y'-N_a' -n_q' 5'
                    (IIIc)
          5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'
20   3' n_p' -N_a' -X'X'X'-N_b' -Y'Y'Y'-N_b' -Z'Z'Z'-N_a-n_q' 5'
                    (IIId)
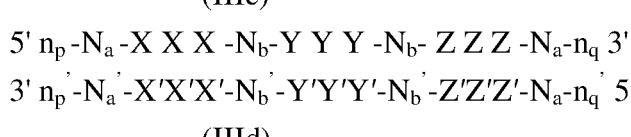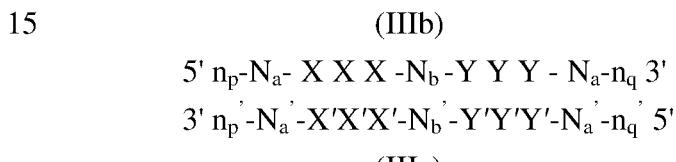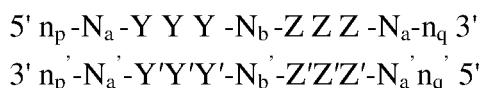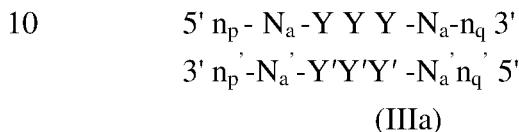
          When the RNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

          When the RNAi agent is represented by formula (IIIb), each N_b independently
25 represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

          When the RNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or
30 0modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising
35 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b and N_b' independently comprises modifications of alternating pattern.

Each of X, Y and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the RNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the RNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

When the RNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

In one embodiment, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In one embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are

connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two RNAi agents represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

Various publications describe multimeric RNAi agents that can be used in the methods of the invention. Such publications include WO2007/091269, US Patent No. 7858769, WO2010/141511, WO2007/117686, WO2009/014887 and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to a RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, e.g., a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, i.e., all ring atoms are carbon atoms, or a heterocyclic ring system, i.e., one or more ring atoms may be a heteroatom, e.g., nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, e.g. fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, e.g. a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, e.g., the phosphate, or modified phosphate, e.g., sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, e.g., a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, e.g., a carbohydrate,

e.g. monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another

5   chemical entity, *e.g.*, a ligand to the constituent ring.

The RNAi agents may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl,

10   quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In certain specific embodiments, the RNAi agent for use in the methods of the invention is an agent selected from the group of agents listed in any one of Tables 3, 4, 5, 6, 18, 19, 20, 21, and 23. These agents may further comprise a ligand.

15

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA suitable for use in the methods of the invention involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA. Such

20   moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an

25   aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine

30   or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

35   In one embodiment, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent

such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, monovalent or multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucoseamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, ligands include monovalent or multivalent galactose. In certain embodiments, ligands include cholesterol.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]$_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc*. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and

oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

5   When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites

10   derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A.   Lipid Conjugates

15   In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be

20   used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

          A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate

25   to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

          In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds

30   HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

          In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that

35   target to kidney cells can also be used in place of or in addition to the lipid based ligand.

          In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type,

e.g., cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 3). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 4) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 5) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 6) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct

conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, a α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:
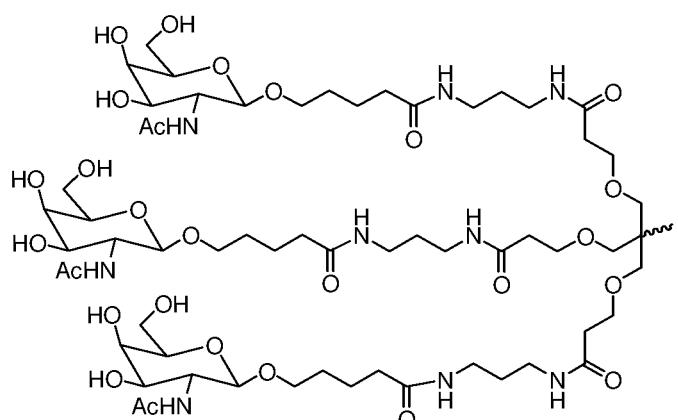
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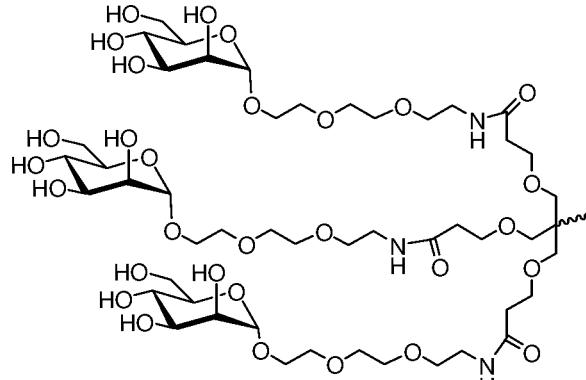
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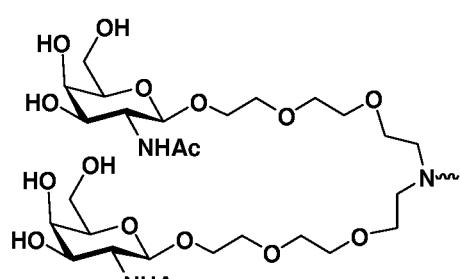
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Formula V,

Formula VI,

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Formula VII,

Formula VIII,

Formula IX,

Formula X,

Formula XI,

5
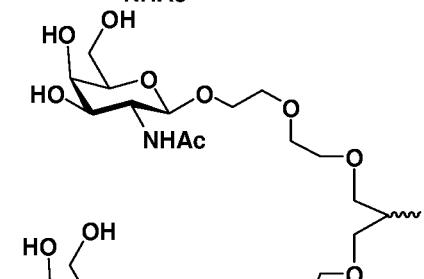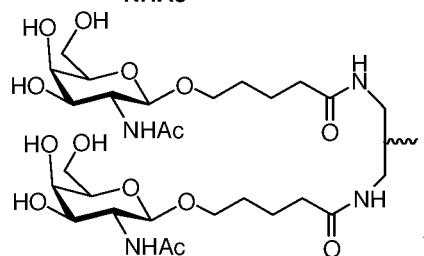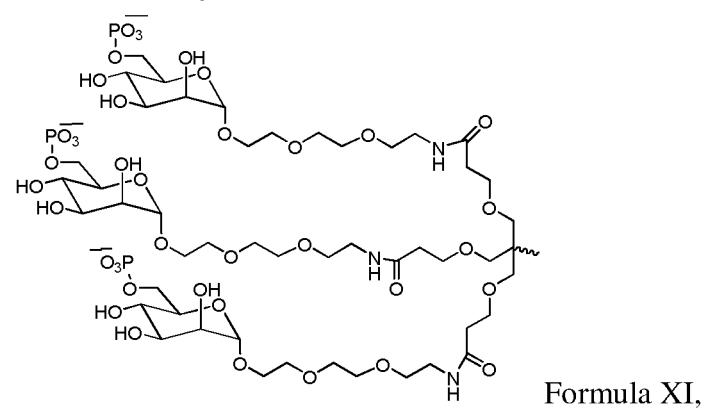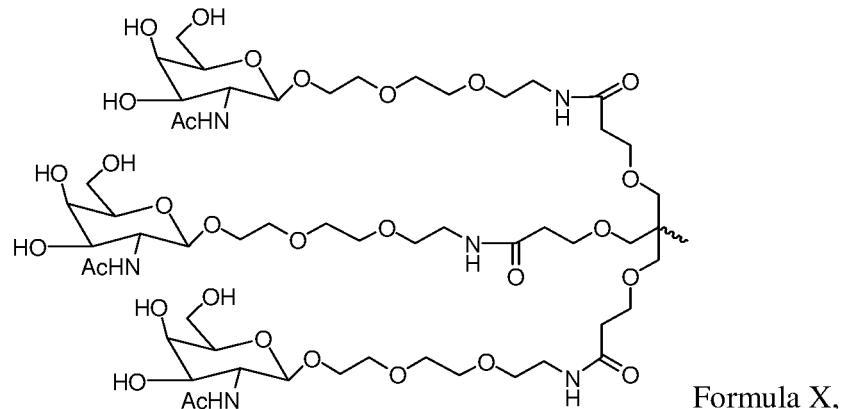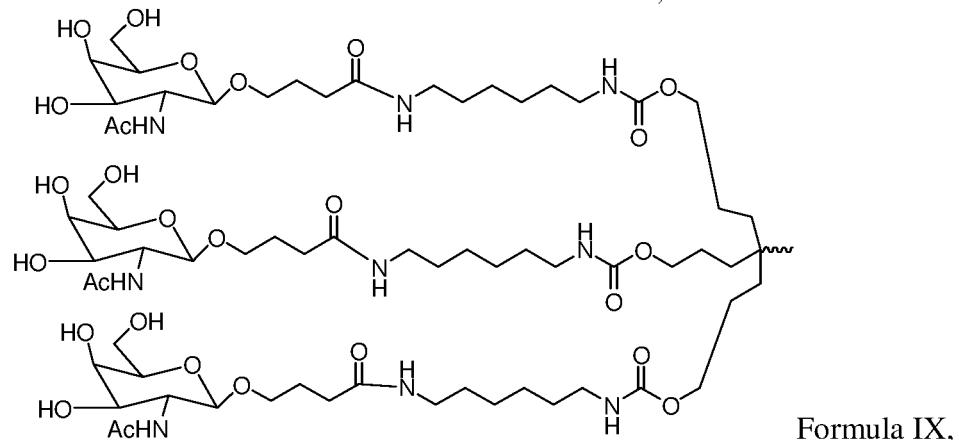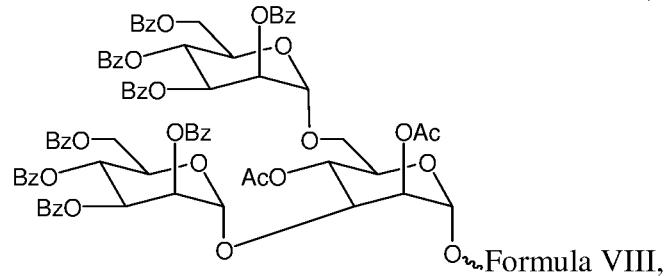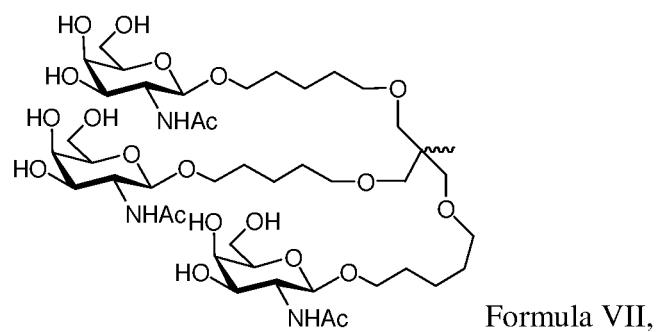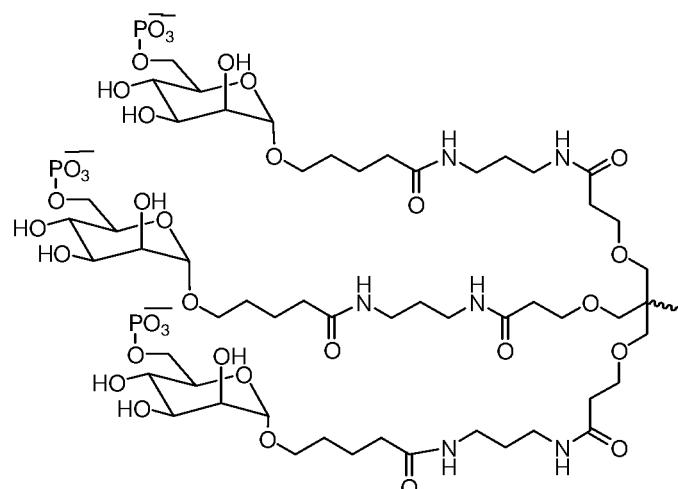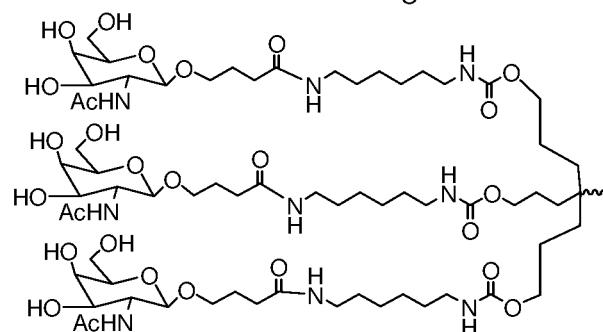
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Formula XIII,
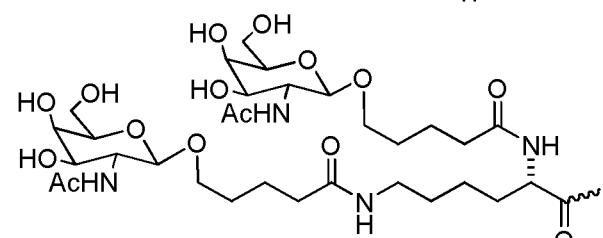
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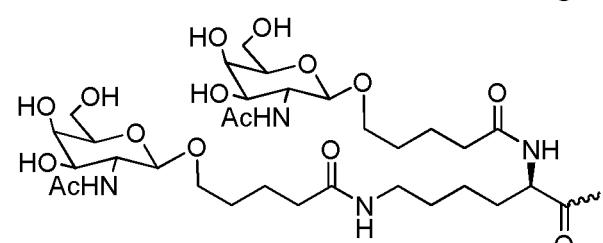
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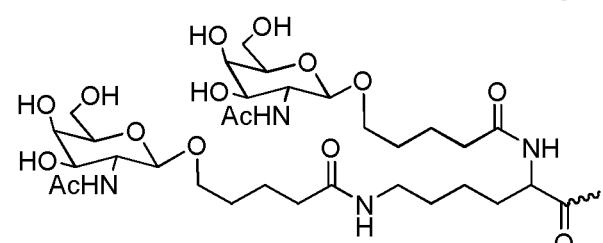
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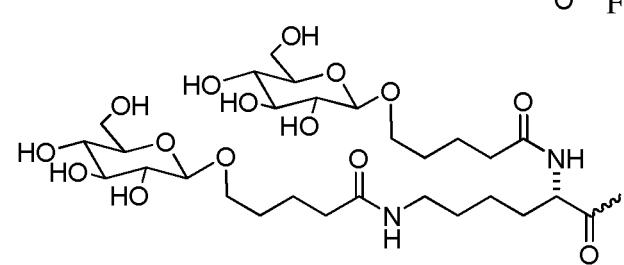
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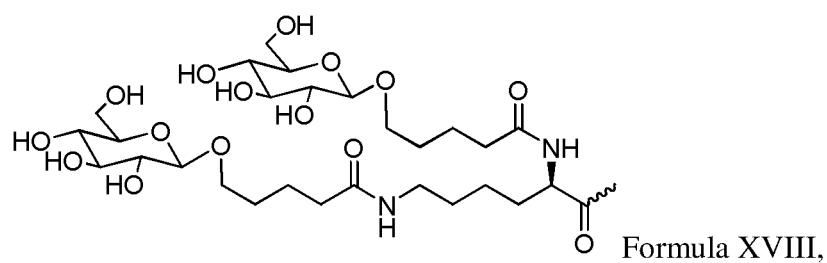
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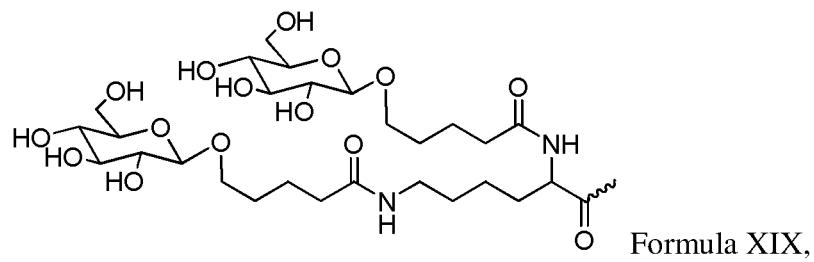
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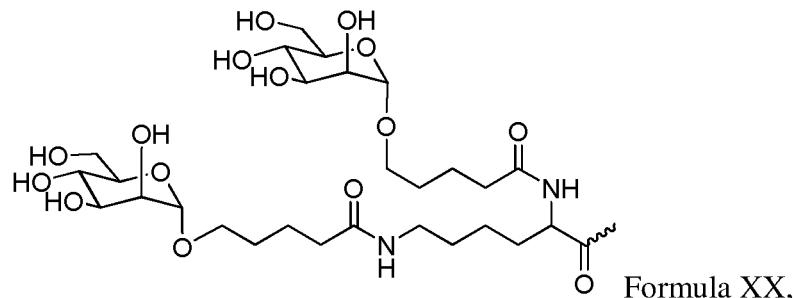
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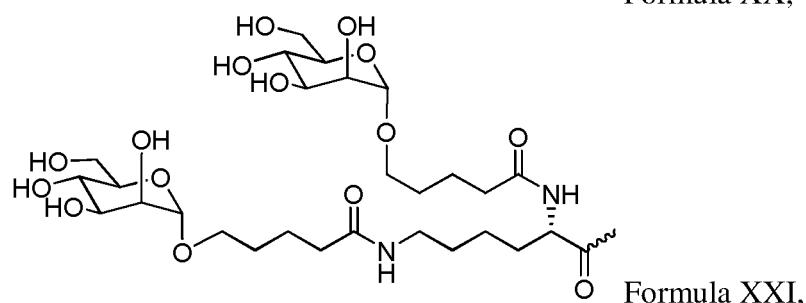
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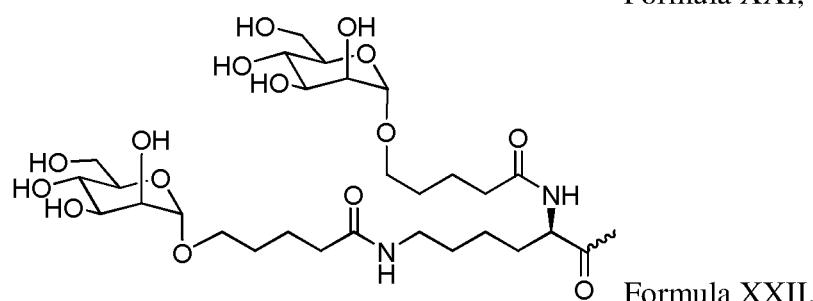
5   Formula XXII.

In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as
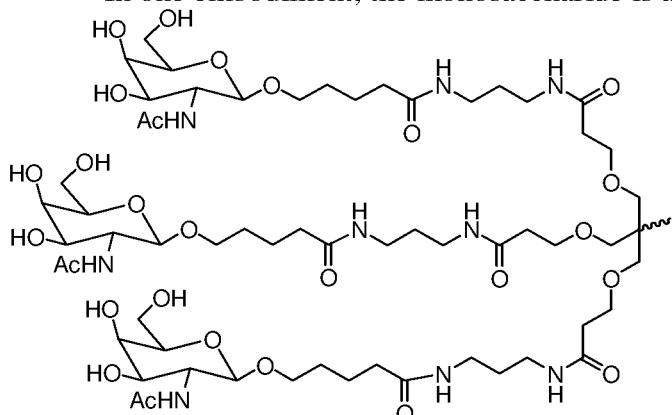
Formula II.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,
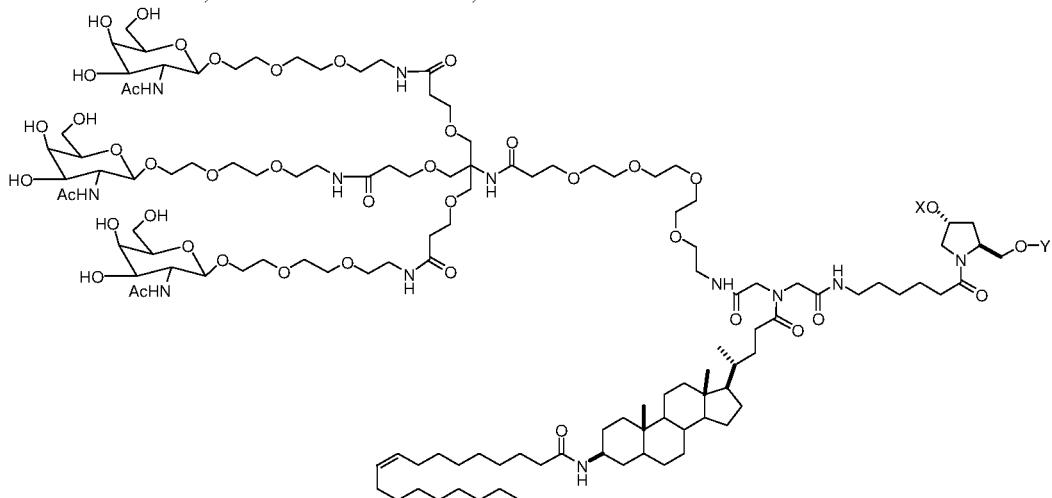
5

(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a

10   bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6)

15   GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a

20   hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a

monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a

5   cell permeation peptide.

Additional carbohydrate conjugates suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

10   *D. Linkers*

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise

15   a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl,

20   cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl,

25   alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O),

30   substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but

35   which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which

can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50,

60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

   i. Redox cleavable linking groups

   In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

   ii. Phosphate-based cleavable linking groups

   In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

   iii. Acid cleavable linking groups

   In another embodiment, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents

such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -

5   C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

        iv. Ester-based linking groups

10   In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated

15   using methods analogous to those described above.

        v. Peptide-based cleaving groups

        In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds

20   formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the

25   amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

30   In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,
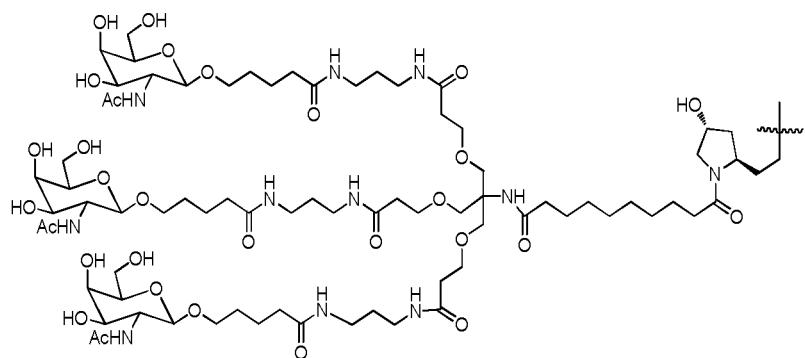
(Formula XXIV),

(Formula XXV),

x = 1-30
y = 1-15

(Formula XXVI),

x = 1-30
y = 1-15

(Formula XXVII),

5

x = 0-30
y = 1-15
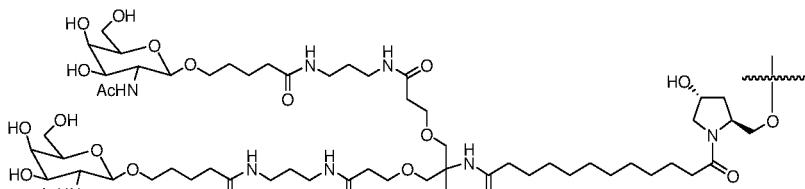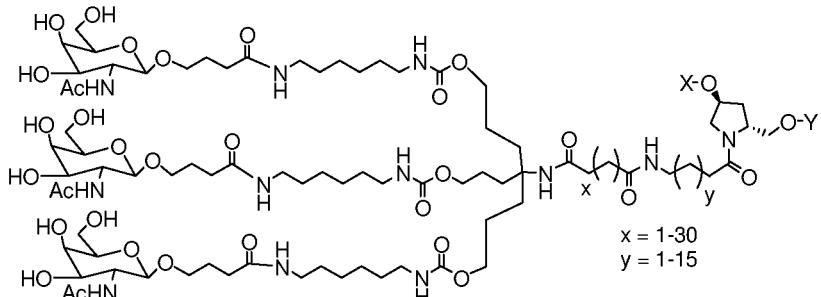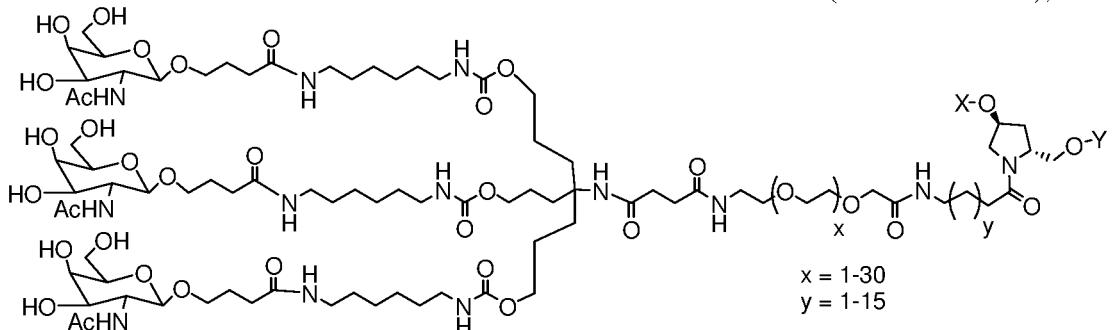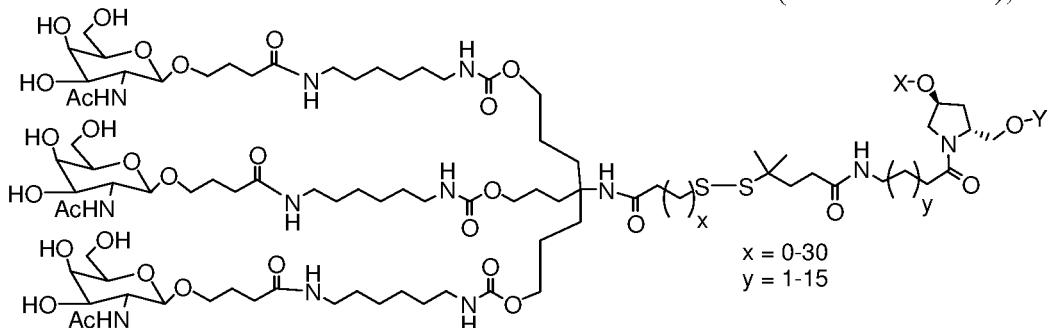
(Formula XXVIII),

(Formula XXIX),
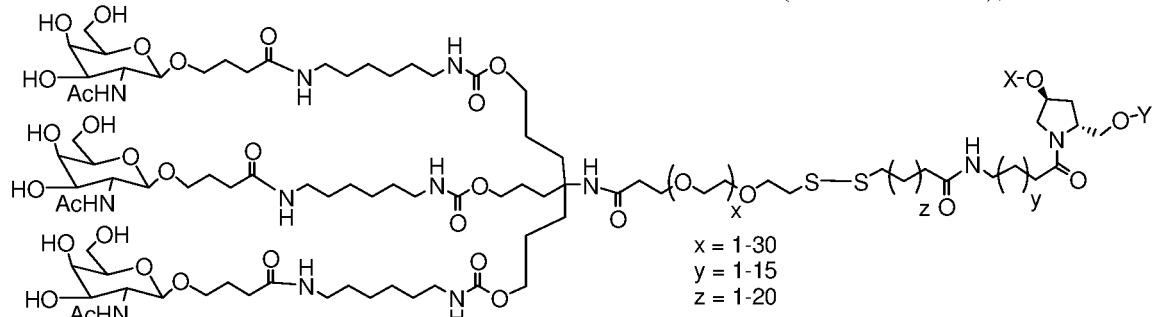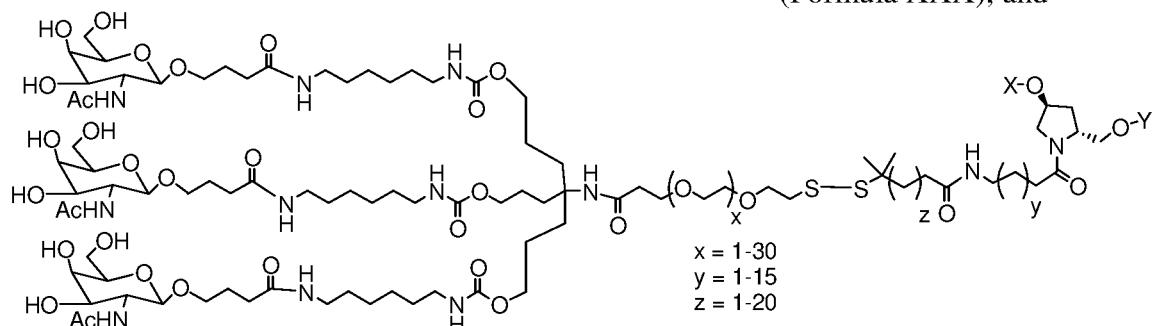
x = 0-30
y = 1-15
z = 1-20

(Formula XXX), and

x = 1-30
y = 1-15
z = 1-20

(Formula XXXI),

x = 1-30
y = 1-15
z = 1-20

when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

Formula XXXII
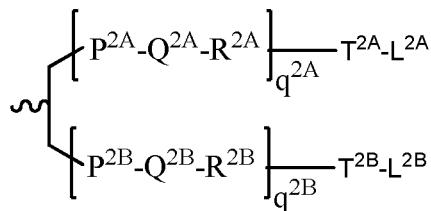

Formula XXXIII

,
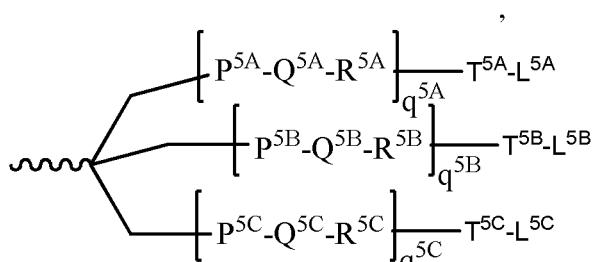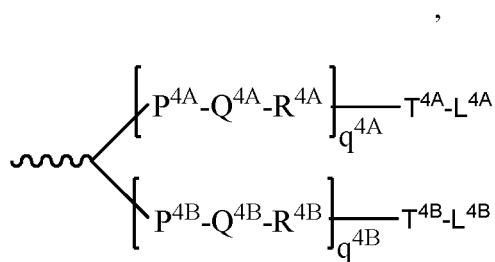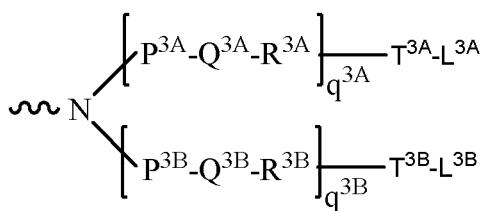
Formula XXXIV

Formula XXXV

;

5   wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or

10   CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence

15   absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, $-C(O)-CH(R^a)-NH-$, CO, CH=N-

or heterocyclyl;
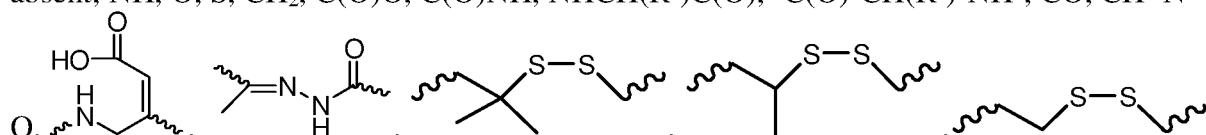
L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide,

20   trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; andR^a is H or amino acid side chain.Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXVI):

Formula XXXVI

$$\left[P^{5A}-Q^{5A}-R^{5A}\right]_{q^{5A}}-T^{5A}-L^{5A}$$
$$\left[P^{5B}-Q^{5B}-R^{5B}\right]_{q^{5B}}-T^{5B}-L^{5B}$$
$$\left[P^{5C}-Q^{5C}-R^{5C}\right]_{q^{5C}}-T^{5C}-L^{5C}$$

,
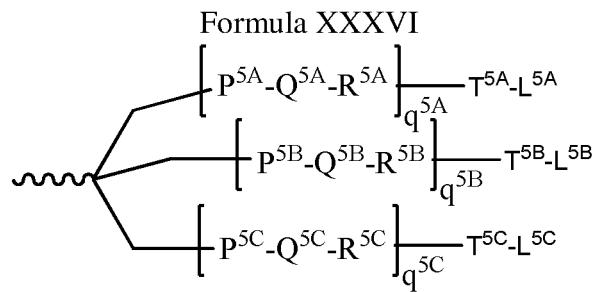
wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, preferably dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric

dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.*, a cell within a subject, such as a human subject (*e.g.*, a subject in need thereof, such as a subject having a disorder that would benefit from reduction in PCSK9 expression) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.*, a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that

encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., *et al* (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ., *et al* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J., *et al* (2005) *Mol. Ther.*11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ., *et al* (2006) *Mol. Ther.* 14:343-350; Li, S., *et al* (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA., *et al* (2006) *Mol. Ther.* 14:476-484; Zhang, X., *et al* (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., *et al* (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate

tumor regression in a mouse model of prostate cancer (McNamara, JO., *et al* (2006) *Nat.*
Biotechnol. 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using
drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a
cationic delivery system. Positively charged cationic delivery systems facilitate binding of an
5   iRNA molecule (negatively charged) and also enhance interactions at the negatively charged
cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers,
or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*,
Kim SH., *et al* (2008) J*ournal of Controlled Release* 129(2):107-116) that encases an iRNA.
The formation of vesicles or micelles further prevents degradation of the iRNA when
10   administered systemically. Methods for making and administering cationic- iRNA complexes
are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J.*
Mol. Biol 327:761-766; Verma, UN., *et al* (2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS
et al (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their
entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery
15   of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003),
supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006)
Nature 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328;
Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al*
(2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.*
20   71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and
polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al*
(1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with
cyclodextrin for systemic administration. Methods for administration and pharmaceutical
compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which
25   is herein incorporated by reference in its entirety.

   A.   *Vector encoded iRNAs of the Invention*

      iRNA targeting the PCSK9 gene can be expressed from transcription units inserted into
DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*,
International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No.
30   WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the
order of hours to weeks) or sustained (weeks to months or longer), depending upon the
specific construct used and the target tissue or cell type. These transgenes can be introduced
as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-
integrating vector. The transgene can also be constructed to permit it to be inherited as an
35   extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

      The individual strand or strands of an iRNA can be transcribed from a promoter on an
expression vector. Where two separate strands are to be expressed to generate, for example, a
dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or

infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

5   iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient
10   restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

        iRNA expression plasmids can be transfected into target cells as a complex with
15   cationic lipid carriers (*e.g.*, Oligofectamine) or non-cationic lipid-based carriers (*e.g.*, Transit-TKO™). Multiple lipid transfections for iRNA-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a
20   fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

        Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors,
25   including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also
30   be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in
35   target cells. Other aspects to consider for vectors and constructs are further described below.

        Vectors useful for the delivery of an iRNA will include regulatory elements (promoter, enhancer, *etc.*) sufficient for expression of the iRNA in the desired target cell or

tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*,

5   circulating glucose levels, or hormones (Docherty *et al.*, 1994, *FASEB J.* 8:20-24). Such inducible expression systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the

10   appropriate regulatory/promoter sequence based on the intended use of the iRNA transgene.

Viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences

15   encoding an iRNA are cloned into one or more vectors, which facilitate delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen *et al.*, *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene

20   therapy are: Clowes *et al.*, *J. Clin. Invest.* 93:644-651 (1994); Kiem *et al.*, *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

25   Adenoviruses are also contemplated for use in delivery of iRNAs of the invention. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting

30   non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science* 252:431-434 (1991); Rosenfeld *et al.*,

35   *Cell* 68:143-155 (1992); Mastrangeli *et al.*, *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, *et al.*, *Gene Therapy* 2:775-783 (1995). A suitable AV vector for expressing an iRNA featured in the invention, a method for constructing the

recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

Adeno-associated virus (AAV) vectors may also be used to delivery an iRNA of the invention (Walsh *et al.*, Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Pat. No. 5,436,146). In one embodiment, the iRNA can be expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter. Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), *J. Virol.* 61: 3096-3101; Fisher K J *et al.* (1996), *J. Virol,* 70: 520-532; Samulski R *et al.* (1989), *J. Virol.* 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

Another viral vector sui for delivery of an iRNA of the inevtion is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate. For example, lentiviral vectors can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors can be made to target different cells by engineering the vectors to express different capsid protein serotypes; see, *e.g.*, Rabinowitz J E *et al.* (2002), *J Virol* 76:791-801, the entire disclosure of which is herein incorporated by reference.

The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

V.   Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and

animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of a PCSK9, *e.g.* a disease or disorder that would benefit from reduction in PCSK9 expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. Another example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a PCSK9 gene.

Preferably, in the methods of the invention an iRNA agent is administered to a subject as a fixed dose. In one particular embodiment, a fixed dose of an iRNA agent of the invention is based on a predetermined weight or age.

In some embodiments, the RNAi agent is administered as a fixed dose of between about 200 mg to about 850 mg, between about 200 mg to about 500 mg, between about 200 mg to about 400 mg, between about 200 mg to about 300 mg, between about 100 mg to about

800 mg, between about 100 mg to about 750 mg, between about 100 mg to about 700 mg, between about 100 mg to about 650 mg, between about 100 mg to about 600 mg, between about 100 mg to about 550 mg, between about 100 mg to about 500 mg, between about 200 mg to about 850 mg, between about 200 mg to about 800 mg, between about 200 mg to about 750 mg, between about 200 mg to about 700 mg, between about 200 mg to about 650 mg, between about 200 mg to about 600 mg, between about 200 mg to about 550 mg, between about 200 mg to about 500 mg, between about 300 mg to about 850 mg, between about 300 mg to about 800 mg, between about 300 mg to about 750 mg, between about 300 mg to about 700 mg, between about 300 mg to about 650 mg, between about 300 mg to about 600 mg, between about 300 mg to about 550 mg, between about 300 mg to about 500 mg, between about 400 mg to about 850 mg, between about 400 mg to about 800 mg, between about 400 mg to about 750 mg, between about 400 mg to about 700 mg, between about 400 mg to about 650 mg, between about 400 mg to about 600 mg, between about 400 mg to about 550 mg, or between about 400 mg to about 500 mg.

In some embodiments, the RNAi agent is administered as a fixed dose of about about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, or about 850 mg.

In some embodiments, subjects are administered, *e.g.*, subcutaneously or intramuscularly, multiple doses of a therapeutic amount of iRNA.

The iRNA may be formualted in a pharmaceutical composition at a suitable concentration such that a suitable volume of the compsosition is administered to the subject, such as about 1.0 mls, 1.1 mls, 1.2 mls, 1.3 mls, 1.4 mls, 1.5 mls, 1.6 mls, 1.7 mls, 1.8 mls, 1.9 mls, or about 2.0 mls of a pharmaceutical composition. For example, in one embodiment, an iRNA agent of the invention is formulated in a suitable pharmaceutical formulation at about 200 mg/ml such that administration of about 1.5 mls of the formautlion to a subject provides a 300 mg fixed dose of the agent.

As described herein, a single dose of the iRNA agents or pharmaceutical compositions comprising such agents can be long lasting, such that subsequent doses are administered at not more than 1 week, 2 weeks, 1 month, 2 month, 3 month, 4 month , 5 month, or 6 month intervals.

In some embodiments, subjects are administered, *e.g.*, subcutaneously or intramuscularly, a repeat dose of a therapeutic amount of iRNA. A repeat-dose regimine may include administration of a therapeutic amount of iRNA on a regular basis, such as once a month, once every two months, once a quarter, once every four months, once every five months, or biannually. In some embodiments of the invention, a single dose of the

pharmaceutical compositions of the invention is administered once per quarter (qQ). In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bi-annually (*i.e.*, every six months). Administration can be repeated, *e.g.*, once every quarter for 6 months, one year, two years or longer, *e.g.*,

5   administered chronically.

In some embodiments, the RNAi agent is administered in a dosing regimen that includes a loading phase followed by a maintenance phase.

The loading phase may include a single administration of the RNAi agent during the first week, a single administration of the RNAi agent during the first two weeks, or a single

10   administration of the RNAi agent during the first month at a fixed dose of, for example, about 100 mg to about 700 mg, about 150 mg to about 700 mg, about 200 mg to about 700 mg, about 250 mg to about 700 mg, about 300 mg to about 700 mg, about 350 mg to about 700 mg, about 400 mg to about 700 mg, about 450 mg to about 700 mg, about 500 mg to about 700 mg, about 550 mg to about 700 mg, about 600 to about 700 mg, about 650 to about 700

15   mg, about 100 mg to about 650 mg, about 150 mg to about 650 mg, about 200 mg to about 650 mg, about 250 mg to about 650 mg, about 300 mg to about 650 mg, about 350 mg to about 650 mg, about 400 mg to about 650 mg, about 450 mg to about 650 mg, about 500 mg to about 650 mg, about 550 mg to about 650 mg, about 600 to about 650 mg, about 100 mg to about 600 mg, about 150 mg to about 600 mg, about 200 mg to about 600 mg, about 250 mg

20   to about 600 mg, about 300 mg to about 600 mg, about 350 mg to about 600 mg, about 400 mg to about 600 mg, about 450 mg to about 600 mg, about 500 mg to about 600 mg, about 550 mg to about 600 mg, about 100 mg to about 550 mg, about 150 mg to about 550 mg, about 200 mg to about 550 mg, about 250 mg to about 550 mg, about 300 mg to about 550 mg, about 350 mg to about 550 mg, about 400 mg to about 550 mg, about 450 mg to about

25   550 mg, about 500 mg to about 550 mg, about 100 mg to about 500 mg, about 150 mg to about 500 mg, about 200 mg to about 500 mg, about 250 mg to about 500 mg, about 300 mg to about 500 mg, about 350 mg to about 500 mg, about 400 mg to about 500 mg, or about 450 mg to about 500 mg, *e.g.*, a fixed dose of about100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325

30   mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, or about 700 mg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention.

The maintenance phase may include administration of a dose of the RNAi agent to the

35   subject once a month, once every two months, once every three months, once every four months, once every five months, or once every six months. In one particular embodiment, the maintenance dose is administered to the subject once a month.

The maintenance dose or doses can be the same or lower than the initial dose, *e.g.*, one-half of the initial dose. For example, a maintenance dose may be about 25 mg to about 100 mg administered to the subject monthly, for example about 25 mg to about 75 mg, about 25 mg to about 50 mg, or about 50 mg to about 75 mg, *e.g.*, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, or about 100 mg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention.

The pharmaceutical composition can be administered by intravenous infusion over a period of time, such as over a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21, 22, 23, 24, or about a 25 minute period. The administration may be repeated, for example, on a regular basis, such as weekly, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (*e.g.*, by a transdermal patch), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular tissue, such as the liver (*e.g.*, the hepatocytes of the liver).

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be

useful. Suitable topical formulations include those in which the iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines;

polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.*, p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

A. *Additional Formulations*

i. *Emulsions*

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion.

Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and

Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example,

carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

5   Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are
10   also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

         The application of emulsion formulations via dermatological, oral and parenteral
15   routes and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery
20   have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume
25   1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

         ii. Microemulsions
30   In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.),
35   New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an

intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M.,

5   Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant

10   molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug

15   Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions,

20   microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310),

25   hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and

30   consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The

35   oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, *Pharmaceutical Research*, 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, *Pharmaceutical Research*, 1994, 11, 1385; Ho *et al.*, *J. Pharm. Sci.,* 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or iRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the iRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five broad categories-- surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

an RNAi agent of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated

with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of iRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, *J. Pharm. Pharmacol.*, 1988, 40, 252).

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-20} alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.*, Touitou, E., *et al.* Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate),

glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*,

5   Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, *J. Pharm. Exp. Ther.*,

10   1992, 263, 25; Yamashita *et al.*, *J. Pharm. Sci.*, 1990, 79, 579-583).

Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of iRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage

15   of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino

20   acyl derivatives of beta-diketones (enamines)(see *e.g.*, Katdare, A. *et al.*, Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, *J. Control Rel.*, 1990, 14, 43-51).

25   As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of iRNAs through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl-

30   and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, *J. Pharm. Pharmacol.*, 1987, 39, 621-626).

Agents that enhance uptake of iRNAs at the cellular level can also be added to the

35   pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available

transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen;

5   Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene

10   (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass^a D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™

15   (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San

20   Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

25   Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

        v. Carriers

        Certain compositions of the present invention also incorporate carrier compounds in

30   the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a

35   carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially

phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, DsRNA Res. Dev., 1995, 5, 115-121; Takakura *et al.*, DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

5   *vi.   Excipients*

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for 10   the desired bulk, consistency, *etc.*, when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or 15   calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc*).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-20   parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

25   Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously 30   react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

vii.   Other Components

35   The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local

anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the

5   biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

10   Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non-RNAi

15   mechanism and which are useful in treating a hemolytic disorder. Examples of such agents include, but are not lmited to an anti-inflammatory agent, anti-steatosis agent, anti-viral, and/or anti-fibrosis agent. In addition, other substances commonly used to protect the liver, such as silymarin, can also be used in conjunction with the *iRNAs described herein*. Other agents useful for treating liver diseases include telbivudine, entecavir, and protease inhibitors

20   such as telaprevir and other disclosed, for example, in Tung *et al.*, U.S. Application Publication Nos. 2005/0148548, 2004/0167116, and 2003/0144217; and in Hale *et al.*, U.S. Application Publication No. 2004/0127488.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the

25   LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in

30   formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated

35   initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which

achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by PCSK9 expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Kits

The present invention also provides kits for using any of the iRNA agents and/or performing any of the methods of the invention. Such kits include one or more RNAi agent(s) and instructions for use, *e.g.*, instructions for inhibiting expression of a PCSK9 in a cell by contacting the cell with the RNAi agent(s) in an amount effective to inhibit expression of the PCSK9. The kits may optionally further comprise means for contacting the cell with the RNAi agent (*e.g.*, an injection device), or means for measuring the inhibition of PCSK9 (*e.g.*, means for measuring the inhibition of PCSK9 mRNA protein). Such means for measuring the inhibition of PCSK9 may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for administering the RNAi agent(s) to a subject or means for determining the therapeutically effective or prophylactically effective amount.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the iRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein, as well as the Sequence Listing and Figures, are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1.   *Synthesis of GalNAc-Conjugated Oligonucleotides*

A series of siRNA duplexes targeting nucleotides 3544-3623 of the human PCSK9 gene (SEQ ID NO:1) were designed, synthesized. These same sequences were also

synthesized with various nucleotide modifications and conjugated with a trivalent GalNAc. The sense and antisense strand sequences of the modified duplexes are shown in Table 1.

Table B: Abbreviations of nucleotide monomers used in nucleic acid sequence
representation.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate

Abbreviation	Nucleotide(s)
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
dT	2`-deoxythymidine-3`-phosphate
dTs	2`-deoxythymidine-3`-phosphorothioate
dU	2`-deoxyuridine-3`-phosphate
dUs	2`-deoxyuridine-3`-phosphorothioate
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(Aeo)	2'-O-methoxyethyladenosine-3'-phosphate
(Aeos)	2'-O-methoxyethyladenosine-3'-phosphorothioate
(Geo)	2'-O-methoxyethylguanosine-3'-phosphate
(Geos)	2'-O-methoxyethylguanosine-3'- phosphorothioate
(Teo)	2'-O-methoxyethyl-5-methyluridine-3'-phosphate
(Teos)	2'-O-methoxyethyl-5-methyluridine-3'- phosphorothioate
(m5Ceo)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphate
(m5Ceos)	2'-O-methoxyethyl-5-methylcytidine-3'- phosphorothioate
(A3m)	3`-O-methyladenosine-2`-phosphate
(A3mx)	3'-O-methyl-xylofuranosyladenosine-2'-phosphate
(G3m)	3`-O-methylguanosine-2`-phosphate
(G3mx)	3'-O-methyl-xylofuranosylguanosine-2'-phosphate
(C3m)	3`-O-methylcytidine-2`-phosphate
(C3mx)	3'-O-methyl-xylofuranosylcytidine-2'-phosphate
(U3m)	3`-O-methyluridine-2`-phosphate
(U3mx)	3'-O-methylxylouridine-2'-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(pshe)	Hydroxyethylphosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ggn)	2'-O-hexadecyl-cytidine-3'-phosphate
(Agn)	Adenosine-glycol nucleic acid (GNA)
P	5'-phosphate

Abbreviation	Nucleotide(s)
(m5Cam)	2`-O-(N-methylacetamide)-5-methylcytidine-3`-phosphate
(m5Cams)	2`-O-(N-methylacetamide)-5-methylcytidine-3`-phosphorothioate
(Tam)	2`-O-(N-methylacetamide)thymidine-3`-phosphate
(Tams)	2`-O-(N-methylacetamide)thymidine-3`-phosphorothioate
(Aam)	2`-O-(N-methylacetamide)adenosine-3`-phosphate
(Aams)	2`-O-(N-methylacetamide)adenosine-3`-phosphorothioate
(Gam)	2`-O-(N-methylacetamide)guanosine-3`-phosphate
(Gams)	2`-O-(N-methylacetamide)guanosine-3`-phosphorothioate
(Uyh)	2'-O-(1-hexyl-4-methylene-1,2,3-triazolyl)-uridine-3'-phosphate
(Ayh)	2'-O-(1-hexyl-4-methylene-1,2,3-triazolyl)-adenosine-3'-phosphate
(Gyh)	2'-O-(1-hexyl-4-methylene-1,2,3-triazolyl)-guanosine-3'-phosphate
(Cyh)	2'-O-(1-hexyl-4-methylene-1,2,3-triazolyl)-cytidine-3'-phosphate
(iA)	inverted adenosine-5'-phosphate
(iC)	inverted cytidine-5'-phosphate

Table 1. Double-Stranded Ribonucleic Acid (RNAi) Agents Targeting Nucleotides 3544-3623 of Human PCSK9 (SEQ ID NO:1).

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-53806	A-110717	7	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	8	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717	9	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	10	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717	11	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	12	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717	13	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	14	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717	15	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	16	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717	17	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	18	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717.6	19	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	20	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717.7	21	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	22	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717.8	23	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	24	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-53806	A-110717.9	25	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	26	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56979	A-116393	27	caAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	28	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56979	A-116393	29	caAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	30	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56975	A-116394	31	(iC)aAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	32	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56975	A-116394	33	(iC)aAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	34	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56975	A-116394	35	(iC)aAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	36	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56975	A-116394	37	(iC)aAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	38	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56975	A-116394	39	(iC)aAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	40	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56983	A-116400	41	CbaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	42	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56983	A-116400	43	CbaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	44	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu
AD-56983	A-116400	45	CbaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	46	aAfaAfaGfaUfaAfaugUfaAfaugUfcUfgcCfuUfgsCfsu

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56983	A-116400	47	CbaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	48	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56983	A-116400	49	CbaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	50	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56977	A-116406	51	CfaagCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	52	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56977	A-116406	53	CfaagCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	54	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56977	A-116406	55	CfaagCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	56	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56976	A-116407	57	CfaagCfaGfaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	58	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56976	A-116407	59	CfaagCfaGfaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	60	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56980	A-116408	61	CfaagCfagaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	62	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56980	A-116408	63	CfaagCfagaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	64	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56984	A-116409	65	CfaagCfagaCfAfUfuUfaucUfuuuUfL96	3544	A-109589	66	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56984	A-116409	67	CfaagCfagaCfAfUfuUfaucUfuuuUfL96	3544	A-109589	68	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56987	A-116410	69	CfaagCfagaCfAfUfuUfaucUfuuuuUfL96	3544	A-109589	70	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56987	A-116410	71	CfaagCfagaCfAfUfuUfaucUfuuuuUfL96	3544	A-109589	72	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56991	A-116415	73	CfaagCfagaCfAfUfuUfaucuuuuuL96	3544	A-109589	74	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56993	A-116416	75	CfaagcagaCfAfUfuUfaucuuuuuL96	3544	A-109589	76	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56995	A-116417	77	CfaagcagaCfAfUfuuaucuuuuuL96	3544	A-109589	78	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56978	A-116418	79	CfaAfgfCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	80	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56978	A-116418	81	CfaAfgfCfaGfaCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	82	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56981	A-116419	83	CfaAfgfCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	84	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56985	A-116420	85	CfaAfgfCfCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	86	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56988	A-116421	87	CfaAfgfCfAfGfaCfAfUfuUfaUfcUfUfuUfuUfL96	3544	A-109589	88	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56988	A-116421	89	CfaAfgfCfAfGfaGfaCfAfUfuUfAfUfcUfuUfuUfL9	3544	A-109589	90	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56988	A-116421	91	CfaAfGfCfAfGfaCfaCfAfUfuUfAfUfAfUfcCfUfuUfuUfL96	3544	A-109589	92	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56982	A-116426	93	CfaAfgcaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	94	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56982	A-116426	95	CfaAfgcaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	96	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56986	A-116428	97	CfaAfgCfagaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	98	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56986	A-116428	99	CfaAfgCfagaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	100	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56989	A-116430	101	CfaAfgCfagacAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	102	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56990	A-116432	103	CfaAfgCfaGfaCfAfuuUfaUfcUfuUfuUfL96	3544	A-109589	104	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56992	A-116434	105	CfaAfgCfaGfaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	106	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56992	A-116434	107	CfaAfgCfaGfaCfAfUfuUfaucUfuUfuUfL96	3544	A-109589	108	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56994	A-116436	109	CfaAfgCfaGfaCfAfUfuUfaUfcUfuuuUfL96	3544	A-109589	110	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56994	A-116436	111	CfaAfgCfaGfaCfAfUfuUfaUfcUfuuuUfL96	3544	A-109589	112	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56996	A-116438	113	caagCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	114	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57001	A-116440	115	CfaAfgcagaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	116	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57007	A-116442	117	CfaAfgCfaGfaCfAfuuuaUfcUfuUfuUfL96	3544	A-109589	118	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57013	A-116444	119	CfaAfgCfaGfaCfAfUffaucuuUfuUfuUfL96	3544	A-109589	120	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57019	A-116446	121	CfaAfgCfaGfaCfAfUfuUfaUfcUfuuuuL96	3544	A-109589	122	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57022	A-116448	123	CfaAfgCfaGfaCfAfUfuUfaUfcUfCfUfuUfuUfL96	3544	A-109589	124	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57025	A-116449	125	CfaAfgCfaGfaCfAfUfuUfaUfcUfCfUfuUfuUfL96	3544	A-109589	126	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56997	A-116450	127	CfaAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	128	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57002	A-116452	129	CfaAfgCfaGfaCfAfUfUfUfaUfcUfuUfuUfL96	3544	A-109589	130	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57008	A-116453	131	CfaAfgCfaGfAfCfAfcfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	132	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-57014	A-116454	133	CfaAfgCfAfGfaCfAfcfAfUfuUfaUfcUfuUfuUfL96	3544	A-109589	134	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57020	A-116455	135	CfAfAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-1095893	136	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57020	A-116455	137	CfAfAfgCfaGfaCfAfUfuUfaUfcUfuUfuUfL96	3544	A-1095893	138	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57026	A-116457	139	CfaAfgCfaGfaCfAfUfuUfaUfcuuUfuUfL96	3544	A-1095893	140	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57003	A-116460	141	CfaAfgCfaGfaCfAfUfuuaUfcUfuUfuUfuUfL96	3544	A-109589	142	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57009	A-116462	143	CfaAfgCfaGfaCfauuUfaUfcUfuUfuUfuUfL96	3544	A-109589	144	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57015	A-116464	145	CfaAfgCfaGfacaUfuUfaUfcUfuUfuUfuUfL96	3544	A-109589	146	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57023	A-116467	147	CfaAfgCfaGfaCfAfUfuUfaucUfUfUfuUfuUfL96	3544	A-109589	148	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57027	A-116469	149	CfaAfgCfaGfaCfAfUfuuaUfcUfuUfUfUfuUfL96	3544	A-109589	150	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56998	A-116471	151	CfaAfgCfagaCfAfUfuUfaUfcUfuUfUfUfuUfL96	3544	A-109589	152	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57004	A-116473	153	CfaAfgCfagaCfaAfUfuUfaUfcUfuUfUfUfuUfL96	3544	A-109589	154	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57010	A-116475	155	CfaagCfaGfaCfAfUfuUfaUfcUfuUfUfUfuUfL96	3544	A-109589	156	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57016	A-116477	157	caAfgCfaGfaCfAfUfuUfaUfcUfuUfUfUfuUfL96	3544	A-109589	158	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56999	A-116479	159	CfaAfgCfaGfaGfaCfAfUfuUfaUfcUfuUfUfUfuUfL96	3544	A-109589	160	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-56999	A-116479	161	CfaAfgCfaGfaCfAfUfuUfaAfUfcUfUfUfuUfuUfL96	3544	A-109589	162	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57021	A-116481	163	CfaAfgCfAfGfaCfAfUfuUfaUfcUfUfUfuUfuUfL96	3544	A-109589	164	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57024	A-116483	165	CfaAfgfCfaGfaCfAfUfuUfaUfcUfUfUfuuuUfuUfL96	3544	A-109589	166	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57005	A-116486	167	CfaAfgCfaGfaCfAfUfuUfaUfcCfUfuuuUfuUfL96	3544	A-109589	168	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57011	A-116488	169	CfaAfgCfaGfaCfAfUfuuaUfUfuUfuUfuUfL96	3544	A-109589	170	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57017	A-116490	171	CfaAfgfCfagaCfAfUfuUfaUfcUfuUfuUfuUfL96	3544	A-109589	172	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-57000	A-116492	173	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfaUfaUfcUf(Teo)JUf(Teo)UfL96	3544	A-109589	174	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57000	A-116492	175	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfaUfaUfcUf(Teo)JUf(Teo)UfL96	3544	A-109589	176	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57000	A-116492	177	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfaUfaUfcUf(Teo)JUf(Teo)UfL96	3544	A-109589	178	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57006	A-116494	179	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfuUf(Aeo)Uf(m5Ceo)Uf(Teo)UfL96	3544	A-109589	180	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57006	A-116494	181	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfuUf(Aeo)Uf(m5Ceo)Uf(Teo)UfL96	3544	A-109589	182	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57006	A-116494	183	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfuUf(Aeo)Uf(m5Ceo)Uf(Teo)UfL96	3544	A-109589	184	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57012	A-116498	185	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfaUfaUfcUf(Teo)JUf(Teo)UbL96	3544	A-109589	186	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-57018	A-116500	187	Cf(Aeo)Af(Geo)CfaGfaCfAfUfuUfaUfaUfcUf(Aeo)Uf(m5Ceo)Uf(Teo)UbL96	3544	A-109589	188	aAfaAfaGfaUfaAfaugUfcUfgCfuUfgsCfsu
AD-53812	A-110718	189	AfaGfcAfgAfcAfAfUfuUfuAfucCfuUfuUfgAfL96	3545	A-109591	190	uCfaAfaAfgAfuAfaauGfuCfuUfgGfcUfusGfsc
AD-53818	A-110719	191	AfgCfaGfaCfaUfUfuUfaUfcUfuUfuUfgGfgAfL96	3546	A-109593	192	uCfcAfaAfaGfaUfaaaUfgUfcUfgCfusUfsg
AD-53766	A-110679	193	GfcAfgAfcAfuUfUfAfucfUfuUfuUfgGfgUfL96	3547	A-109513	194	aCfcCfaAfaAfgAfuaaAfuGfuCfuGfcsUfsu
AD-53772	A-110680	195	AfgAfcAfuUfuAfUfcfUfuUfuUfgGfgUfgUfL96	3549	A-109515	196	aGfaCfcCfaAfaAfgauAfaAfuGfuCfusGfsc
AD-53824	A-110720	197	GfaCfaUfuUfaUfcUffUfuUfuGfgGfcfUfuL96	3550	A-109595	198	aAfgAfcCfcAfaAfagaUfaAfaUfgUfcsUfsg
AD-53778	A-110681	199	AfcAfUfuUfaUfcUfUfuUfUfgGfgUfgUfL96	3551	A-109517	200	aCfaGfaCfcCfaAfaagAfuAfaAfuGfusCfsu
AD-53784	A-110682	201	UfuUfaUfcUfuUfUfgGfgfuCfuUfUfgGfUfL96	3554	A-109519	202	aGfgAfcAfgAfcfCfcAfaAfgAfuAfaAfsUfsg
AD-53829	A-110721	203	UfuAfUfcUfuUfUfgGfgfgUfcUfgUfcfCfuUfL96	3555	A-109597	204	aAfgGfcCfaGfaCfcfCfaAfaAfgAfuAfasAfsu
AD-53790	A-110683	205	UfaUfcUfuUfUfgGfgfuCfuGfuCfcfUfcUfL96	3556	A-109521	206	aGfaGfgAfcAfgAfcfCfcAfaAfgAfuaGfaUfasAfsa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-53835	A-110722	207	AfuCfuUfuUfgGfGfUfcUfgUfcCfuCfuUfL96	3557	A-109599	208	aAfgAfgGfaCfaGfaccCfaAfaAfgAfusAfsa
AD-53796	A-110684	209	UfcUfuUfuGfgGfUfCfuGfuCfuUfcUfL96	3558	A-109523	210	aGfaGfaGfgAfgacCfcAfaAfaGfaAfasUfsa
AD-53802	A-110685	211	UfuUfuGfgGfuCfUfGfuCfcUfcUfcUfgUfL96	3560	A-109525	212	aCfaGfaGfgAfgAfcagAfcCfcAfaAfasGfsa
AD-53808	A-110686	213	UfuUfgGfgUfcUfGfUfcUfcUfcUfgUfuUfL96	3561	A-109527	214	aAfcAfgAfgGfacaGfaCfcCfaAfasAfsg
AD-53795	A-110723	215	UfuGfgGfuCfuGfUfcUfcUfcUfgUfuUfL96	3562	A-109601	216	aAfaCfaGfaGfgacAfgAfcCfcAfaAfsa
AD-53801	A-110724	217	UfgGfgUfcUfgUfcCfcUfcUfcUfgAfcUfgAfL96	3563	A-109603	218	uCfaAfcAfgAfgGfaCfaGfaCfcCfasAfsa
AD-53807	A-110725	219	GfgGfuCfuGfuCfcCfuUfcUfgUfgGfcAfL96	3564	A-109605	220	uGfcAfcAfgAfgGfaggAfcAfgAfcCfcsAfsa
AD-53814	A-110687	221	GfgUfcUfgUfcCfUfcUfcUfgUfgGfcUfcUfL96	3565	A-109529	222	aGfgCfaAfcAfgAfgagAfgGfaCfaGfaCfcsCfsa
AD-53820	A-110688	223	GfuCfuGfuCfcCfUfcUfcUfgUfgGfcCfuUfL96	3566	A-109531	224	aAfgGfcAfaCfaGfagaGfgAfcAfgAfcAfcsCfsc
AD-53825	A-110689	225	UfcUfgUfcCfcUfcUfUfcUfgGfuUfuUfgCfcAfL96	3567	A-109533	226	aAfaGfgCfaAfcAfgagAfgGfaCfaGfaGfasCfsc
AD-53831	A-110690	227	CfuGfuCfcUfcUfcUfgUfUfgUfgfcCfuUfuUfL96	3568	A-109535	228	aAfaAfgGfcAfaCfagaGfaGfgAfcAfgAfgsAfsc
AD-53791	A-110691	229	UfgUfcCfcUfcUfcUfgUfUfuUfgGfcfcCfuUfuUfL96	3569	A-109537	230	aAfaAfaGfgCfaAfcagAfgAfgGfaCfaCfasGfsa
AD-53797	A-110692	231	GfuCfcUfcUfcUfgUfgGfccCfuUfuUfAfL96	3570	A-109539	232	uAfaAfaAfaGfgCfAfacaGfaGfgAfgAfcsAfsg
AD-58902		233	UfsusUfcuUfaAfgcAffcCfuGfuUfuUfgCfuUfL96	3597		234	asAfsgCfaAfaAfcAfcAfgguCfuAfgAfaAfasgsu
AD-53803	A-110693	235	UfuUfcUfaGfaCffCfUfgUfuUfuUfgCfcUfuUfL96	3600	A-109541	236	aAfaGfcAfaAfaCfaggUfcUfaGfaAfaAfasAfsg
AD-59232		237	CfsusAfgAfcCfuGfUfuUfuUfgCfuUfuUfgfcUfL96	3600		238	PasCfsaAfaAfgCfaAfaacAfgCfuAfgsasa
AD-59212		239	CfsusAfgAfcCfuGfUfuUfuUfgCfuUfuUfgfcUfL96	3600		240	PasCfsaAfaAfsgCfaAfaacAfgCfuCfsuAfgsasa
AD-53809	A-110694	241	UfuCfuAfgAfcCfUfGfuUfuUfgCfuUfuUfgfcUfL96	3601	A-109543	242	aAfaAfgCfaAfaAfaAfcagGfuCfuAfgAfaAfasAfsa
AD-53815		243	CfuAfgAfcCfuGfUfuUfuUfgCfuUfuUfgfcUfL96	3601		244	aCfaAfaAfgCfaAfaAfacAfgGfuCfuAfgsAfsa
AD-579280		245	CfsusAfgAfcCfuGfUfUfuUfuUfgCfuUfuUfgfcUfL96	3601		246	asCfsaAfaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59182		247	CfsusAfgAfcCfuGfUfUfuUfuugCfuuuuuguL96	3601		248	asCfsaAfaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59184		249	CfsusAfgAfcCfuGfUfUfuUfuugCfuuuuuguL96	3601		250	asCfsaAfaAfaAfgCfaAfaacAfgGfuCfuAfgsasa

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-59186		251	CfsusAfgAfcCfuGfUfuuugCfuuuuguL96	3601		252	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59171		253	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		254	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59176		255	CfsusAfgAfcCfuGfuuuugcuuuuguL96	3601		256	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59170		257	CfsusagacCfuGfuuuugCfuuuuguL96	3601		258	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59175		259	CfsusagacCfuGfuuuugcuuuuguL96	3601		260	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59179		261	csusagacCfuGfuuuugcuuuuguL96	3601		262	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59218		263	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		264	asCfsaAfaAfgCfaAfAfgCfaAfAfcAfgGfuCfuAfgsasa
AD-59222		265	CfsusAfgAfcCfuGfuuuugcuuuuguL96	3601		266	asCfsaAfaAfgCfaAfAfgCfaAfAfAfcAfgGfuCfuAfgsasa
AD-59226		267	CfsusagacCfuGfuuuugCfuuuuguL96	3601		268	asCfsaAfaAfgCfaAfAfgCfaAfAfcAfgGfuCfuAfgsasa
AD-59230		269	CfsusagacCfuGfuuuugcuuuuguL96	3601		270	asCfsaAfaAfgCfaAfAfgCfaAfAfcAfgGfuCfuAfgsasa
AD-59235		271	csusagacCfuGfuuuugcuuuuguL96	3601		272	asCfsaAfaAfgCfaAfAfgCfaAfAfcAfgGfuCfuAfgsasa
AD-59207		273	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		274	asCfsaAfAfAfgCfaAfAfcAfgGfuCfuAfgCfuagsasa
AD-59211		275	CfsusAfgAfcCfuGfuuuugcuuuuguL96	3601		276	asCfsaAfAfAfgCfaAfAfcAfgCfaAfAfcAfgGfuCfuagsasa
AD-59215		277	CfsusagacCfuGfuuuugCfuuuuguL96	3601		278	asCfsaAfAfAfgCfaAfAfcAfgCfaAfAfcAfgGfuCfuagsasa
AD-59219		279	CfsusagacCfuGfuuuugcuuuuguL96	3601		280	asCfsaAfAfAfgCfaAfAfcAfgCfaAfAfcAfgGfuCfuagsasa
AD-59223		281	csusagacCfuGfuuuugcuuuuguL96	3601		282	asCfsaAfAfAfgCfaAfAfcAfgCfaAfAfcAfgGfuCfuagsasa
AD-59181		283	CfsusAfgAfcCfuGfUfuUfuUfgCfuUfuUfgsUfL96	3601		284	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59172		285	CfsusAfgAfcCfuGfUfuUfuUfgCfuUfuUfsgsUfL96	3601		286	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59177		287	CfsusAfgAfcCfuGfUfuUfuUfgCfsuUfsuUfsgsUfL96	3601		288	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59180		289	CfsusAfgAfcCfuGfUfuUfuUfgCfsuUfsuUfsgsUfsL96	3601		290	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-59183		291	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfsgsUfsL96	3601		292	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59185		293	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfsL96	3601		294	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59173		295	CfsusAfgAfcCfuGfUfUfuugCfuuuugsuL96	3601		296	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59236		297	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3601		298	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59216		299	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgsUfL96	3601		300	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59220		301	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfsgsUfL96	3601		302	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59224		303	CfsusAfgAfcCfuGfUfUfuUfgCfsuUfsuUfsgsUfL96	3601		304	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59228		305	CfsusAfgAfcCfuGfUfUfuUfgCfsuUfsuUfsgsUfsL96	3601		306	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59233		307	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfsgsUfsL96	3601		308	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59237		309	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfsL96	3601		310	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59209		311	CfsusAfgAfcCfuGfUfUfuugCfuuuugsuL96	3601		312	asCfsaAfaAfsgCfaAfaacAfgGfuCfsuAfgsasa
AD-59208		313	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3601		314	asCfsaAfaAfsgCfaAfaacAfgGfuCfsusAfgsasa
AD-59210		315	csusAGaccuGuuuuGcuuuuGuL96	3601		316	AscsAAAAGcAAAAcAGGucuAGsasa
AD-59227		317	CfsusAfgAfAfccuGfuuuuGfcuuuuGfuL96	3601		318	asCfsAfAfAfAfGfcAfAfAfAfAfcAfGfGfucuUfAfGfsasa
AD-59231		319	CfsusAfgAfAfccuGfuuuuGfcuuuuGfuL96	3601		320	asCfsAfAfAfAfGfcAfAfaacAfgGfucuUfAfgsasa
AD-59198		321	(C3m)usAfgAfcCfuGfUfUfuUfuUfgCfuUfuUfgUfL96	3601		322	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-59200		323	(C3m)(U3m)AfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		324	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59203		325	(m5Cam)usAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		326	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59204		327	(m5Cam)(Tam)AfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		328	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59188		329	(m5Cams)(Tams)AfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		330	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59191		331	(m5Cams)usAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		332	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59213		333	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		334	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59217		335	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		336	asCfsaAfaAfgCfaAfaacAfgGfuCfuAf(G3m)(A3m)a
AD-59221		337	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		338	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(Aam)a
AD-59225		339	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		340	asCfsaAfaAfgCfaAfaacAfgGfuCfuAf(Gam)(Aam)a
AD-59229		341	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		342	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(Aams)a
AD-59234		343	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		344	asCfsaAfaAfgCfaAfaacAfgGfuCfuAf(Gams)(Aams)a
AD-59238		345	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		346	(A3m)CfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59241		347	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		348	as(C3m)aAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59245		349	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		350	(Aam)CfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59250		351	CfsusAfgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		352	as(m5Cam)aAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59196		353	usAfsgAfcCfuGfuUfuUfgCfuUfuUfgUfL96	3601		354	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-59189		355	AfsgsAfcCfuGfUfUfUfgCfuUfuUfgUfL96	3601		356	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59190		357	usCfsuAfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3601		358	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59192		359	UfsusCfuAfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUf L96	3601		360	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59240		361	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		362	asCfsaAfaAfsgCfaAfaacAfgGfuCfuAfgs(A3m) a
AD-59244		363	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		364	asCfsaAfaAfsgCfaAfaacAfgGfuCfuAfgsasa
AD-59202.7		365	(C3m)usagaccuguuuugcuuuuguL96	3601		366	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59195		367	(C3m)usAfgAfcCfuGfuuuugCfuuuuguL96	3601		368	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59249		369	CfsusAfgAfcCfuGfUfUfUfuugCfuuuuguL96	3601		370	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59254		371	CfsusAfgAfcCfuGfuuuugCfuuuuguL96	3601		372	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59259		373	(C3m)usAfgAfcCfuGfuuuugCfuuuuguL96	3601		374	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59264		375	(C3m)usagaccuguuuugcuuuuguL96	3601		376	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59264		377	(C3m)usagaccuguuuugcuuuuguL96	3601		378	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-59255		379	CsusagaccuGfUfUfuugcuuuuguL96	3601		380	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgs(A3m)a
AD-57928		381	CfsusAfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3601		382	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-58893		383	CfsuAfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3601		384	asCfaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-58894		385	CfusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3601		386	aCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsaa
AD-58895		387	CfuAfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3601		388	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-58896		389	CfsusAfgAfcCfuGfUfUfuUfuUfgCfuUfuUfgUfL96	3601		390	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgaa
AD-58897		391	CfsusAfsgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL9 6	3601		392	asCfsasAfaAfgCfaAfaacAfgGfuCfuAfsgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-58898		393	CfsusAfsgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3601		394	asCfsaAfaAfgCfsaAfaacAfsgGfuCfuAfsgsasa
AD-58899		395	CfsusAfsgAfcCfuGfUfuUfgCfuUfuUfsgUfL96	3601		396	asCfsaAfaAfgCfsaAfaacAfsgGfuCfuAfsgsasa
AD-53813	A-110726	397	UfcUfaGfaCfcUfGfUfuUfuGfcUfuUfuUfL96	3602	A-109607	398	aAfaAfaGfcAfaAfacaGfgUfCfuaGfasAfsa
AD-59246		399	CfsusAfsgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602		400	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfsgsa
AD-59253		401	usAfsgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3602		402	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfsgsa
AD-59242		403	AfsgsAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3602		404	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfsgsa
AD-59253		405	usAfsgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3602		406	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfsgsa
AD-59258		407	usasgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3602		408	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfsgsa
AD-53815	A-110695	409	CfuAfgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	410	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-53815	A-110695	411	CfuAfgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	412	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-53815	A-110695	413	CfuAfgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	414	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56633	A-115520	415	cuAfgAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	416	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56617	A-115535	417	CfuagAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	418	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56623	A-115536	419	CfuagAfcCfuGfUfuUfuUfgcuUfuUfguL96	3603	A-109545	420	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56629	A-115537	421	CfuagAfccuGfUfuUfuUfgcuUfuguL96	3603	A-109545	422	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56635	A-115538	423	CfuagAfccuGfUfuUfuugcuUfuugcuL96	3603	A-109545	424	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56641	A-115539	425	CfuagaccuGfUfuugcuuuuguL96	3603	A-109545	426	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56625	A-115542	427	CfuAfGfAfcCfuGfUfuUfuGfcUfuUfuUfgUfL96	3603	A-109545	428	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56631	A-115543	429	CfuAfGfAfcCfuGfUfuUfuGfCfuUfuUfgUfL96	3603	A-109545	430	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56637	A-115544	431	CfuAfGfAfcCfuGfUfuUfuGfCfuUfUfUfUfgUfL96	3603	A-109545	432	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56643	A-115545	433	CfuAfGfAfcCfuCfuGfUfuUfuGfCfuUfUfUfUfGfUfL	3603	A-109545	434	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56649	A-115546	435	CfuFAfGfAfCfCfuGfUfUfUfUfGfCfUfUfUfUfGfUfL96	3603	A-109545	436	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56655	A-115547	437	CfuFAfGfAfCfCfUfGfUfUfUfUfUfGfCfUfUfUfUfGfUfL96	3603	A-109545	438	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56615	A-110695	439	CfuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115519	440	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfsa
AD-56621	A-115520	441	cuAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3603	A-115519	442	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfsa
AD-56627	A-115521	443	cuAfgAfcCfuGfUfUfuugCfuUfuugUfL96	3603	A-115519	444	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfsa
AD-56639	A-115520	445	cuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115522	446	ACfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56645	A-110695.6	447	CfuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115522	448	ACfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56651	A-115523	449	(iC)uAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115524	450	(iA)CfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfs(iA)
AD-56610	A-115523	451	(iC)uAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115525	452	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfs(iA)
AD-56616	A-115523	453	(iC)uAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115526	454	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfs(iA)
AD-56622	A-115527	455	(iC)uAfgAfcCfuGfUfUfuUfgCfuUfuugUfL96	3603	A-115526	456	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfs(iA)
AD-56628	A-115527	457	(iC)uAfgAfcCfuGfUfUfUfUfgCfuUfuugUfL96	3603	A-115528	458	(iA)caAfaAfgcaAfaacAfgGfuCfuAfgsAfs(iA)
AD-56634	A-115529	459	CbuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115530	460	AbCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsAb
AD-56640	A-115529	461	CbuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115531	462	aCfaAfaAfgCfaAfaacAfgGfuCfuAfgsAfsAb
AD-56646	A-115529	463	CbuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115532	464	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfsAb
AD-56652	A-115533	465	CbuAfgAfcCfuGfUfUfuUfgCfuuugUfL96	3603	A-115532	466	acaAfaAfgcaAfaacAfgGfuCfuAfgsAfsAb
AD-56611	A-115533	467	CbuAfgAfcCfuGfUfUfuUfgCfuuugUfL96	3603	A-115534	468	(iA)caAfaAfgcaAfaacAfgGfuCfuAfgsAfsAb
AD-56647	A-110695.7	469	CfuAfgAfcCfuGfUfUfUfUfgCfuUfuUfgUfL96	3603	A-115540	470	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-56653	A-115535	471	CfuagAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115540	472	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-56612	A-115536	473	CfuagAfcCfuGfUfUfuUfgCfuUfuUfgcuUfUfguL96	3603	A-115540	474	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56618	A-115537	475	CfuagAfccuGfUfUfgfcuUfuUfguL96	3603	A-115540	476	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-56624	A-115538	477	CfuagAfccuGfUfUfuugcuUfuuuguL96	3603	A-115540	478	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-56630	A-115539	479	CfuagaccuGfUfUfuugcuuuuuguL96	3603	A-115540	480	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-56636	A-110695.8	481	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115541	482	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56642	A-115535	483	CfuagAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115541	484	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56648	A-115536	485	CfuagAfcChuGfUfUfuUfgcuUfuUfgguL96	3603	A-115541	486	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56654	A-115537	487	CfuagAfccuGfUfUfuUfgcuUfuUfgguL96	3603	A-115541	488	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56613	A-115538	489	CfuagAfccuGfUfUfuugcuUfuuuguL96	3603	A-115541	490	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56619	A-115539	491	CfuagaccuGfUfUfuugcuuuuuguL96	3603	A-115541	492	aCfaaaAfgCfaAfaacAfgguCfuAfgsasa
AD-56614	A-110695.9	493	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115548	494	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56620	A-115542	495	CfuAfgfAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115548	496	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56626	A-115543	497	CfuAfgfAfcCfuGfUfUfuUfgfCfuUfuUfgUfL96	3603	A-115548	498	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56632	A-115544	499	CfuAfgfAfcCfuGfUfUfuUfgfCfuUfuUfgUfgUfL96	3603	A-115548	500	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56638	A-115545	501	CfuAfgfAfcCfuCfuGfUfUfuUfgfCfuUfuUfgUfgUfL96	3603	A-115548	502	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56644	A-115546	503	CfUfUfAfgfAfcCfuCfuUfuUfgfCfUfUfUfUfgUfgUfgUff L96	3603	A-115548	504	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56650	A-115547	505	CfUfUfAfgfAfcCfuCfuUfuUfgfCfUfUfUfUfgUfUfUfgUf UfL96	3603	A-115548	506	aCfaAfAfAfgCfaAfaacAfgGfUfCfuAfgsAfsa
AD-56656	A-110695	507	CfuAfgAfcCfuGfUfUfuUfgcuUfuUfgUfL96	3603	A-115549	508	aCfaAfAfAfgfCfaAfaacAfgGfrfuCfuAfgsAfsa
AD-56662	A-115542	509	CfuAfgfAfcCfuGfUfUfuUfgcuUfuUfgUfL96	3603	A-115549	510	aCfaAfAfAfgfCfaAfaacAfgGfrfuCfuAfgsAfsa
AD-56668	A-115543	511	CfuAfgfAfcCfuGfUfUfuUfgfCfuUfuUfgUfL96	3603	A-115549	512	aCfaAfAfAfgfCfaAfaacAfgGfrfuCfuAfgsAfsa
AD-56673	A-115544	513	CfuAfgfAfcCfuGfUfUfuUfgfCfuUfuUfUfgUfL96	3603	A-115549	514	aCfaAfAfAfgfCfaAfaacAfgGfrfuCfuAfgsAfsa

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56678	A-115545	515	CfuAfgfAfcfCfuGfUfuUfgfCfuUfUfUfgfUfL96	3603	A-115549	516	aCfaAfAfAfGfcfAfaacAfgGfUfCfuAfgsAfsa
AD-56683	A-115546	517	CfUfAfgfAfcfCfuGfUfuUfgfCfCfuUfUfUfgfUfL96	3603	A-115549	518	aCfaAfAfAfGfcfAfaacAfgGfUfCfuAfgsAfsa
AD-56688	A-115547	519	CfUfAfgfAfcfCfCfUfGfUfUfUfUfgfCfCfuUfUfUfgfUfL96	3603	A-115549	520	aCfaAfAfAfGfcfAfaacAfgGfUfCfuAfgsAfsa
AD-56657	A-115550	521	CfuAfgAfcCfuGfUfUfuUfgCfuUfuugUfL96	3603	A-115551	522	aCfAfaFaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56663	A-115552	523	CfuAfgAfcCfuGfUfUfuUfgCfuuuUfgUfL96	3603	A-115553	524	aCfaAfAfAfGfcfAfaacAfgGfuCfuAfgsAfsa
AD-56669	A-115554	525	CfuAfgAfcCfuGfUfUfuUfgcuUfugcuUfgUfL96	3603	A-115555	526	aCfaAfAfAfGfcfAfaacAfgGfuCfuAfgsAfsa
AD-56674	A-115556	527	CfuAfgAfcCfuGfUfUfuuugCfuUfuUfgUfL96	3603	A-115557	528	aCfaAfAfAfgCfAfAfaacAfgGfuCfuAfgsAfsa
AD-56679	A-115558	529	CfuAfgAfccuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115559	530	aCfaAfAfAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56684	A-115560	531	CfuAfgacCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115561	532	aCfaAfAfAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56689	A-115535	533	CfuagAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115562	534	aCfaAfAfAfgCfAfAfaacAfgGfuCfuAfgsAfsa
AD-56693	A-115520	535	cuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115563	536	aCfaAfAfAfgCfaAfaacAfgGfuCfuAfGfsAfsa
AD-56658	A-115564	537	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115565	538	aCfaaaAfgCfaAfaacAfgGfuCfuAfgsAfsa
AD-56664	A-115566	539	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115567	540	aCfaAfaagCfaAfaacAfgGfuCfuAfgsAfsa
AD-56670	A-115568	541	CfuAfgAfcCfuGfUfUfuUfgfCfCfuUfUfUfgfUfL96	3603	A-115569	542	aCfaAfAfAfgcaAfaacAfgGfuCfuAfgsAfsa
AD-56680	A-115572	543	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115573	544	aCfaAfaAfgCfaAfaacagGfuCfuAfgsAfsa
AD-56685	A-115574	545	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115575	546	aCfaAfAfAfgCfaAfaacAfgguCfuAfgsAfsa
AD-56690	A-115542	547	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603	A-115576	548	aCfaAfAfAfgCfaAfaacAfgGfucuAfgsAfsa
AD-56694	A-115577	549	CfUfAfgAfcCfuGfUfUfuUfgCfuUfuUfgfUfL96	3603	A-115578	550	aCfaAfAfAfgCfaAfaacAfgGfuCfuagsAfsa
AD-56659	A-110695	551	CfuAfgAfcCfuGfUfUfuUfgCfuUfuUfgfUfL96	3603	A-115579	552	aCfaAfAfAfgCfaAfaacAfgGfuCfuAfgsasa
AD-59214		553	AsGsAccuGuuuuuGcuuuuGuL96	3603		554	AscsAAAAGcAAAAcAGGucusAsG

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Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-59251		555	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603		556	asCfsaAfaAfgCfaAfaacAfgGfuCfusAfsg
AD-59261		557	AfsgsAfcCfuGfUfUfuUfgCfuUfuuUfgUfL96	3603		558	asCfsaAfaAfgCfaAfaacAfgGfuCfusasg
AD-59262		559	usAfsgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603		560	asCfsaAfaAfgCfaAfaacAfgGfuCfusasg
AD-59265		561	csusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3603		562	asCfsaAfaAfgCfaAfaacAfgGfuCfusasg
AD-53821	A-110696	563	UfaGfaCfcUfgUfUfUfuUfgGfcUfuUfuGfuAfL96	3604	A-109547	564	uAfcAfaAfaGfcAfaaaCfaGfgUfcUfasGfsa
AD-59256		565	usAfsgsAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3604		566	asCfsaAfaAfgCfaAfaacAfgGfuCfsusAf
AD-59247		567	gsAfscCfuGfUfUfuUfgCfuUfuUfgUfL96	3604		568	asCfsaAfaAfgCfaAfaacAfgGfuCfsusa
AD-59252		569	AfsgsAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3604		570	asCfsaAfaAfgCfaAfaacAfgGfuCfsusa
AD-59257		571	usAfsgsAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3604		572	asCfsaAfaAfgCfaAfaacAfgGfuCfsusa
AD-56665	A-115580	573	AfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115581	574	aCfaAfaAfgCfaAfaacAfgGfuCfusAfsg
AD-56671	A-115582	575	AfgAfcCfuGfUfUfuUfgCfuUfuugUfL96	3605	A-115583	576	aCfAfaAfaAfgCfaAfaacAfgGfuCfusAfsg
AD-56676	A-115584	577	AfgAfcCfuGfUfUfuUfgCfuuuUfgUfL96	3605	A-115585	578	aCfaAfAfAfgCfaAfaacAfgGfuCfusAfsg
AD-56681	A-115586	579	AfgAfcCfuGfUfUfuUfgcuUfuUfgUfL96	3605	A-115587	580	aCfaAfaAfAfgCfaAfaacAfgGfuCfusAfsg
AD-56686	A-115588	581	AfgAfcCfuGfUfUfuugCfuUfuUfgUfL96	3605	A-115589	582	aCfaAfaAfgCfAfAfaacAfgGfuCfusAfsg
AD-56691	A-115590	583	AfgAfccuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115591	584	aCfaAfaAfgCfaAfaacAfgGfuCfuCfusAfsg
AD-56695	A-115592	585	AfgacCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115593	586	aCfaAfaAfgCfaAfaacAfgGfuCfusAfsg
AD-56660	A-115594	587	agAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115595	588	aCfaAfaAfgCfaAfaacAfgGfuCfUfsAfsg
AD-56666	A-115596	589	AfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115597	590	aCfaaaAfgCfaAfaacAfgGfuCfusAfsg
AD-56672	A-115598	591	AfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115599	592	aCfaAfaagCfaAfaacAfgGfuCfusAfsg
AD-56677	A-115600	593	AfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3605	A-115601	594	aCfaAfaAfgcaAfaacAfgGfuCfusAfsg
AD-56682	A-115602	595	AfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3605	A-115603	596	aCfaAfaAfgCfaaaacAfgGfuCfusAfsg
AD-56687	A-115604	597	AfgAfcCfuGfUfUfUfuUfgCfuUfuUfgUfL96	3605	A-115605	598	aCfaAfaAfgCfaAfaacagGfuCfusAfsg

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-56692	A-115606	599	AfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3605	A-115607	600	aCfaAfaAfgCfaAfaacAfgguCfusAfsg
AD-56696	A-115608	601	AfGfAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3605	A-115609	602	aCfaAfaAfgCfaAfaacAfgGfucusAfsg
AD-56661	A-115580	603	AfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3605	A-115610	604	aCfaAfaAfgCfaAfaacAfgGfuCfusasg
AD-56667	A-115611	605	gAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3605	A-115612	606	aCfaAfaAfgCfaAfaacAfgGfuCfausa
AD-59260		607	AfsgsAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3605		608	asCfsaAfaAfgCfaAfaacAfgGfusCfsu
AD-59248		609	gsAfscCfuGfUfUfuUfgUfgCfuUfUfuUfgUfL96	3605		610	asCfsaAfaAfgCfaAfaacAfgGfusCfsu
AD-53826	A-110697	611	UfuUfuGfuAfaCfuUfgAfaGfaUfaUfuUfL96	3618	A-109549	612	aAfaUfaUfcUfuCfaagUfuAfcAfaAfasGfsc
AD-53832	A-110698	613	UfuUfgUfaAfcUfuUfGfaAfgAfuAfuuAfL96	3619	A-109551	614	uAfaAfaUfcUfuCfaaGfuUfcaaGfuaCfaAfasAfsg
AD-53792	A-110699	615	UfuGfuAfaCfuuGfAfaGfaUfaUfuUfaUfL96	3620	A-109553	616	aUfaAfaUfaUfcUfucaAfgUfuAfcAfasAfsa
AD-53798	A-110700	617	UfgUfaAfcUfuGfAfAfgAfaUfaUfuAfuUfL96	3621	A-109555	618	aAfuAfaAfuAfuCfuucAfaGfuUfaAfcAfaAfsa
AD-53819	A-110727	619	GfuAfaCfuUfgAfAfgAfaUfaUfuAfUfuUfL96	3622	A-109609	620	aAfaUfaAfaUfaUfcuuCfaAfgUfcuuCfaAfcsAfsa
AD-579285	A-117428	621	CfsusAfgAfcCfuGfUfUfuUfgCfuUfuUfgUfL96	3602	A-117429	622	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60928	A-122701	623	CfsusAfgAfcCfuGfUfUfuUfgCfuUfUfuUfgAfL96	3602	A-122702	624	usCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60929	A-122703	625	GfsusAfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122704	626	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfcsusu
AD-60930	A-122705	627	GfsasAfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122706	628	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60931	A-122707	629	GfsasUfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfcsusu	3602	A-122708	630	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfcsusu
AD-60932	A-122707	631	GfsasUfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122709	632	asCfsaAfaAfgCfaAfaacAfgGfuCfaUfcsasa
AD-60933	A-122710	633	CfsasUfcAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122711	634	asCfsaAfaAfgCfaAfaacAfgGfuaUfgsasa
AD-60934	A-122712	635	CfsusUfcUfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122713	636	asCfsaAfaAfgCfaAfaacAfgGfaAfgsasa
AD-60927	A-122714	637	CfsusAfcUfgCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-122715	638	asCfsaAfaAfgCfaAfaacAfgGfuCfaGfuAfgsasa
AD-579285	A-117428	639	CfsusAfgAfcCfuGfUfUfuUfgCfuUfUfuUfgUfL96	3602	A-117429	640	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-60906	A-117428	641	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-122309	642	asCfsaAfaAfgCf(Ayh)AfaacAfgGfuCfuAfgsasa
AD-60907	A-117428	643	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-122310	644	asCfsaAfaAfgCfa(Ayh)aacAfgGfuCfuAfgsasa
AD-60908	A-117428	645	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-122311	646	asCfsaAfaAfgCfaAf(Ayh)acAfgGfuCfuAfgsasa
AD-60909	A-117428	647	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-122312	648	asCfsaAfaAfgCfaCfa(Ayh)cAfgGfuCfuAfgsasa
AD-60910	A-117428	649	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-122313	650	asCfsaAfaAfgCf(Ayh)AfaacAf(Gyh)GfuCf(Uyh)Afgsasa
AD-60911	A-122307	651	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-117429	652	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60912	A-122308	653	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-117429	654	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60913	A-122307	655	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122309	656	asCfsaAfaAfgCf(Ayh)AfaacAfgGfuCfuAfgsasa
AD-60914	A-122307	657	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122310	658	asCfsaAfaAfgCfa(Ayh)aacAfgGfuCfuAfgsasa
AD-60915	A-122307	659	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122311	660	asCfsaAfaAfgCfaAf(Ayh)acAfgGfuCfuAfgsasa
AD-579285	A-117428	661	CfsusAfgAfcCfuGfUfuUfgCfuUfuUfgUfL96	3602	A-117429	662	asCfsaAfaAfgCfaAfaacAfgGfuCfuAfgsasa
AD-60916	A-122307	663	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122312	664	asCfsaAfaAfgCfaCfa(Ayh)cAfgGfuCfuAfgsasa
AD-60917	A-122307	665	Cfsus(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122313	666	asCfsaAfaAfgCf(Ayh)AfaacAf(Gyh)GfuCf(Uy)Afgsasa
AD-60918	A-122308	667	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122309	668	asCfsaAfaAfgCf(Ayh)AfaacAfgGfuCfuAfgsasa
AD-60919	A-122308	669	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122310	670	asCfsaAfaAfgCfa(Ayh)aacAfgGfuCfuAfgsasa

Duplex ID	Sense ID	SEQ ID NO:	Sense Sequence (5' to 3')	Start In NM_174936.3	Antisense ID	SEQ ID NO:	Antisense Sequence (5' to 3')
AD-60920	A-122308	671	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122311	672	asCfsaAfaAfgCfaAf(Ayh)acAfgGfuCfuAfgsasa
AD-60921	A-122308	673	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122312	674	asCfsaAfaAfgCfaAfa(Ayh)cAfgGfuCfuAfgsasa
AD-60922	A-122308	675	(Cyh)u(Ayh)(Gyh)(Ayh)(Cyh)CfuGfUfUfuUf(Gyh)Cf(Uyh)Uf(Uyh)Uf(Gyh)UfL96	3602	A-122313	676	asCfsaAfaAfgCf(Ayh)AfaacAf(Gyh)GfuCf(Uyh)Afgsasa
AD-58900		677	CfsasAfgCfaGfaCfAfUfufaUfcUfuUfuUfuUfL96	3602		678	asAfsaAfaGfaUfaAfaugUfcUfgCfuUfgscsu
AD-59849	A-121244	679	CfsusAfgAfcCfuGfUfUfuiUfgcuuuuguL96	3602		680	asCfsaAfaagCfaAfaacAfgGfucuAfgsasa
AD-60688	A-120188	681	csusagacCfuGfuuuugcuuuuguL96	3602		682	asCfsaAfaagCfaAfaacAfgGfucuAfgsasa
AD-60212	A-122088	683	csusagacCfuGfudTuugcuuuuguL96	3602		684	asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa

Example 2: Phase I Clinical Trial of AD-60212

A Phase I, randomized, single-blind, placebo-controlled study, including, a single ascending dose (SAD) arm and a multi-ascending dose (MAD) arm, was conducted in subjects with elevated low-density lipoprotein cholesterol (LDLc or LDL-C), on or off statins, to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of subcutaneously administered AD-60212.

More specifically, in the SAD phase of the study, the ability of a single subcutaneous fixed dose of 25 mg, 100 mg, 300 mg, 500 mg, or 800 mg of AD-60212 (ALN-PCSsc) to lower both PCSK9 protein and LDL-C in healthy volunteer subjects with baseline LDL-C ≥100mg/dl (≥2.6 mmol/L) and fasting triglycerides < 400 mg/dl (<4.5 mmol/L) was tested. In the MAD phase of the study, subjects with LDL-C ≥100mg/dl, and fasting triglycerides < 400 mg/dl (<4.5 mmol/L) on and off of a stable dose of statin for ≥30 days prior to screening were treated with multiple subcutaneous injections of AD-60212 to test the ability of AD-60212 to lower both PCSK9 protein and LDL-C. Subjects in the multiple administration arm of the study were administered a single 125 mg fixed dose of AD-60212 once every week for four weeks (125 mg qW x 4), or a single 250 mg fixed dose of AD-60212 once every two weeks for one month (250 mg q2W x 2), or a single 300 mg fixed dose of AD-60212 once every month for two months (300 mg qM x 2) without statin therapy, or a single 300 mg fixed dose of AD-60212 once every month for two months (300 mg qM x 2) with statin therapy, or a single 500 mg fixed dose of AD-60212 once every month for two months (500 mg qM x 2) without statin therapy, or a single 500 mg fixed dose of AD-60212 once every month for two months (500 mg qM x 2) with statin therapy.

Plasma PCSK9 protein levels were determined using an ELISA assay and serum LDL-C levels were determined directly by β-quantification (Medpace Reference Laboratories, Leuven, Belgium). The levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), non-HDL-C (total cholesterol minus HDL-C), apolipoprotein B, lipoprotein (a) and triglyceride were also determined.

The cohort demographics and the baseline characteristics of the subjects in the SAD phase of the study are provided in Table 2A and the cohort demographics and the baseline characteristics of the subjects in the MAD phase of the study are provided in Table 2B.

The unmodified sense and antisense sequences of AD-60212 are:

Sense – 5'- CUAGACCUGUUUGCUUUUGU – 3'(SEQ ID NO: 686); and

Antisense – 5'- ACAAAAGCAAAACAGGUCUAGAA – 3' (SEQ ID NO: 685).

The modified sense and antisense sequences of AD-60212 are:

Sense – 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687); and

Antisense – 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688).

SAD Phase

AD-60212 was well tolerated at all dose levels in the SAD phase and there were no treatment discontinuations due to adverse events (AEs) and no serious AEs were reported.

The knockdown of PCSK9 protein levels in the single dose cohort, shown as a percent mean PCSK9 knockdown relative to baseline, is shown in Figure 1, and the lowering of LDL-C levels in the single dose cohort, shown as a percent mean LDL-C lowering relative to baseline, is shown in Figure 2. Table 3 provides the mean (SD) percent change from baseline in the protein level of PCSK9, the level of LDL-C, the level of total cholesterol, the level of HDL-C, the level of non-HDL-C, the level of apolipoprotein B, the level of triglycerides, and the level of apolipoprotein a at days 84 and 180 post-dose in the SAD phase.

The data demonstrate that administration of AD-60212 reduced PCSK9 levels in a dose-dependent manner up to 300 mg. Doses ≥300 mg produced similar, sustained reductions in PCSK9 levels that were maintained over a period of at least 6 months. PCSK9 levels returned to baseline (mean of last three measurements ≥80% of baseline) by day 180 in the 25-mg and 100-mg dose cohorts. In subjects receiving doses ≥300 mg (n=12), the maximum individual relative reduction from baseline in PCSK9 levels was 89% (800-mg dose, day 112). The mean maximal percent reduction (mean percent reduction at individual nadir) was 82% and was observed in the 800-mg dose cohort. Change from baseline in PCSK9 levels in subjects receiving ALN-PCSsc 300–800 mg (n=2–6 per dose group), was significantly greater than in placebo-treated subjects (P≤0.011) for all 11 measurement points from day 7±1 post-treatment through day 112 post-treatment.

The data further demonstrate that AD-60212 administration resulted in dose-dependent LDL-C reductions up to 300 mg, at which near maximal reductions were achieved. LDL-C reductions were similar across the 300–800 mg dose range. In subjects receiving these doses (n=12), the maximum individual decrease from baseline in LDL-C was 78% (500-mg dose; day 56). The mean maximal and maximal least-squares mean (LSM) percent reductions were 59% and were observed in the 500 and 800-mg cohorts. LDL-C levels returned towards baseline levels by 180 days after the last administration of the 25-mg and 100-mg doses. LDL-C reduction was maintained until at least day 180 after doses ≥300 mg. LDL-C reduction from baseline in subjects receiving ALN-PCSsc 300–800 mg (n=3–6) was statistically significant compared with placebo (P≤0.037) in all 10 determinations from day 14 ± 2 after treatment through day 112 after treatment.

Decreases in total cholesterol, non-HDL-C, apolipoprotein B and lipoprotein (a) concentrations were also noted in AD-60212-treated subjects. Reductions in these parameters were statistically significant compared with placebo for the majority of comparisons.

MAD Phase

AD-60212 was also well tolerated at all dose levels in the MAD phase and there were no treatment discontinuations due to adverse events (AEs) and no serious AEs were reported.

The knockdown of PCSK9 protein levels in the multiple dose cohort, shown as a percent mean PCSK9 knockdown relative to baseline, is shown in Figure 3, and the lowering of LDL-C levels in the multiple dose cohort, shown as a percent mean LDL-C lowering relative to baseline, is shown in Figure 4. Table 4 provides the mean (SD) percent change from baseline in the protein level of PCSK9, the level of LDL-C, the level of total cholesterol, the level of HDL-C, the level of non-HDL-C, the level of apolipoprotein B, the level of triglycerides, and the level of apolipoprotein a at days 84 and 180 post-dose in the SAD phase.

The data demonstrate that PCSK9 protein levels were reduced following administration of AD-60212 with all treatment regimens studied. Reductions were similar across all multiple dose cohorts and the reductions were maintained for at least 6 months after the last dose. Consistent with published literature (Khera AV,*et al.* (2015) *Am J Cardiol* 115:178-82; Guo YL, *et al.* (2013) *Clin Drug Investig* 33:877-83), baseline values of PCSK9 were higher in subjects receiving stable doses of statins. Reductions in PCSK9 were independent of baseline PCSK9 levels and similar in subjects irrespective of statin therapy. The maximum individual reduction from baseline in PCSK9 was 94% (500 mg QMx2 co-administered with statin, day 63). The mean maximal percent reduction was 89%, observed in subjects receiving the 500-mg dose co-treated with a statin. Change in PCSK9 concentrations from baseline in subjects receiving multiple doses of AD-60212 as monotherapy (*i.e.,* without statins; n=3–6 per dose group), was significantly greater than placebo ($P \leq 0.002$) for all 15 measurement points from day 4 post-treatment through day 126.

The data further demonstrate that similar sustained LDL-C reductions were achieved with all multiple dose AD-60212 treatment regimens.. LDL-C reduction was independent of baseline LDL-C levels and similar with and without statin co-therapy. The maximum individual LDL-C reduction was 83% (500 mg QMx2 co-administered with statin, day 29). The mean maximal percent reduction in LDL-C was 64% with a LSM reduction of 60% observed in the cohort receiving the 300-mg dose without statin. LDL-C lowering in all MD cohorts persisted for at least 6 months.

Change in LDL-C from baseline in AD-60212 monotherapy subjects (n=3–6) differed significantly from placebo ($P \leq 0.05$) over periods ranging from ~8 to ~17 weeks depending on the treatment regimen.

Decreases in total cholesterol, non-HDL-C, apolipoprotein B and lipoprotein (a) concentrations were also noted in ALN-PCSsc-treated subjects. Reductions in these parameters were statistically significant compared with placebo for the majority of comparisons.

In summary, subcutaneous administration of AD-60212 targeting PCSK9 to reduce LDL-C levels, was well tolerated in single doses of 25 to 800 mg, and in MD regimens of 2–4 doses totaling 500–1000 mg over a 28-day period.

As shown in Figures 1and 2 and Table 3, a single subcutaneous injection of a fixed dose (≥300 mg of AD-60212 resulted in durable knockdown of PCSK9 and lowering of LDL-C for over 6 months after a single dose. There was up to 89% maximal PCSK9 knockdown, with a mean maximal PCSK9 reduction of 82%, and up to 78% maximal reduction LDL-C lowering, with a mean maximal LDL-C reduction of 59% after administration of a single fixed dose of AD-60212. In addition, LDL-C was significantly (P<0.001) reduced by a mean of 44% at day 140 after a single dose.

As shown in Figures 3 and 4 and Table 4, two monthly fixed doses of AD-60212 resulted in up to 94% maximal knockdown of PCSK9, with a mean maximal PCSK9 reduction of 89%, and up to 83% maximal reduction of LDL-C, with a mean maximal LDL-C reduction of 64%, with or without concomitant statin administration.

These data demonstrate that single doses of AD-60212 (≥300 mg) and all multiple doses demonstrated herein were associated with highly sustained reductions of circulating concentrations of both PCSK9 and LDL-C. At these doses, the effect on PCSK9 and LDL-C remained significantly reduced for at least 180 days post-treatment, such that PCSK9 reductions of up to 76%, and LDL-C reductions of up to 48% were still apparent 6 months after the last AD-60212 injection, and demonstrated remarkably little variation over the 6-month post-dose period. Additive serum LDL-C lowering was attained with AD-60212 when added to statin therapy, and the combination therapy did not impact the safety and tolerability of either agent.

In both the SAD and MAD phases, decreases in total cholesterol, non-HDL-C, apolipoprotein B and lipoprotein (a) concentrations were observed in AD-60212 -treated subjects. Reductions in these parameters were statistically significant compared with placebo for the majority of comparisons.

Table 2A. SAD Cohort Demographics and Baseline Characteristics.

	Single ascending dose phase ALN-PCSsc							Placebo	
	Placebo (n=6)	25 mg (n=3)	100 mg (n=3)	300 mg (n=3)	500 mg (n=3)	800 mg (n=6)	Overall (n=24)	With Statin (n=4)	No Statin (n=8)
Age, years Mean (SD)	48 (14.2)	47 (14.2)	48 (6.2)	48 (12.7)	39 (14.0)	49 (6.7)	47 (10.7)	58 (3.0)	51 (14.2)
Sex, n (%)									
Male	2 (33.3%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	5 (83.3%)	19 (79.2%)	2 (50.0%)	6 (75.0%)
Race, n (%)									
White	4 (66.7%)	2 (66.7%)	3 (100%)	1 (33.3%)	3 (100%)	3 (50.0%)	16 (66.7%)	4 (100%)	7 (87.5%)
Black or African American	2 (33.3%)	1 (33.3%)	0	1 (33.3%)	0	0	4 (16.7%)	0	0
Asian	0	0	0	1 (33.3%)	0	1 (16.7%)	2 (8.3%)	0	0
Other	0	0	0	0	0	2 (33.3%)	2 (8.3%)	0	1 (12.5%)
Body weight, kg Mean (SD)	70.6 (12.04)	84.5 (2.11)	77.3 (6.66)	81.2 (11.04)	71.6 (7.93)	74.0 (6.01)	75.5 (9.16)	74.3 (5.07)	77.6 (10.31)
Height, cm Mean (SD)	168 (10.6)	175 (2.3)	174 (5.1)	173 (9.6)	175 (3.1)	169 (5.5)	172 (7.2)	168 (10.5)	171 (9.3)
BMI, kg/m^2 Mean (SD)	24.9 (3.17)	27.7 (0.21)	25.5 (2.10)	27.0 (1.29)	23.4 (3.01)	25.9 (1.60)	25.6 (2.39)	26.5 (2.72)	26.7 (2.64)

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| | Placebo | | ALN-PCSsc | | | | | | Overall |
	With Statin (n=4)	No Statin (n=8)	300 mg QMx2 With Statin (n=4)	300 mg QMx2 No Statin (n=6)	500 mg QMx2 With Statin (n=5)	500 mg QMx2 No Statin (n=6)	125 mg QWx4 No Statin (n=6)	250 mg Q2Wx2 No Statin (n=6)	(n=45)
Age, years Mean (SD)	58 (3.0)	51 (14.2)	52 (21.6)	47 (8.7)	56 (11.5)	42 (16.1)	55 (9.4)	61 (6.3)	52 (12.7)
Sex, n (%)									
Male	2 (50.0%)	6 (75.0%)	2 (50.0%)	6 (100%)	2 (40.0%)	3 (50.0%)	4 (66.7%)	4 (66.7%)	29 (64.4%)
Race, n (%)									
LDL-C, mmol/L Mean (SD)	3.4 (0.50)	4.6 (1.31)	3.9 (0.92)	4.2 (0.95)	3.1 (0.44)	4.1 (0.74)	3.8 (0.85)	3.7 (2.32)	3.4 (0.54)
TG, mmol/L Mean (SD)	0.8 (0.14)	1.3 (0.67)	2.0 (1.16)	1.5 (0.55)	1.8 (0.95)	1.3 (0.24)	1.4 (0.65)	1.7 (0.53)	1.4 (0.43)
PCSK9, µg/L Mean (SD)	278.95 (99.53)	342.65 (67.89)	233.77 (39.17)	253.82 (22.36)	263.23 (24.98)	279.62 (66.90)	276.32 (68.28)	460.69 (56.295)	276.23 (58.69)

BMI = body mass index; LDL-C = low-density lipoprotein cholesterol; PCSK9 = proprotein convertase subtilisin/kexin type 9; QMx2 = 2 monthly doses; QWx4 = 4 weekly doses; Q2Wx2 = 2 biweekly doses; SD = standard deviation; TG = triglycerides.

To convert values for cholesterol to mg/dL multiply by 38.67. To convert values for TG to mg/dL multiply by 88.57.

Table 2B. MAD Cohort Demographics and Baseline Characteristics.

White	4 (100%)	7 (87.5%)	3 (75.0%)	6 (100%)	3 (60.0%)	5 (83.3%)	5 (83.3%)	3 (50.0%)	36 (80.0%)
Black or African American	0	0	0	0	1 (20.0%)	0	0	1 (16.7%)	2 (4.4%)
Asian	0	0	1 (25.0%)	0	1 (20.0%)	1 (16.7%)	1 (16.7%)	0	4 (8.9%)
Other	0	1 (12.5%)	0	0	0	0	0	2 (33.3%)	3 (6.7%)
Body weight, kg Mean (SD)	74.3 (5.07)	77.6 (10.31)	85.0 (22.04)	77.8 (15.19)	71.9 (11.03)	64.9 (7.86)	73.1 (7.07)	83.2 (8.12)	75.8 (12.03)
Height, cm Mean (SD)	168 (10.5)	171 (9.3)	176 (12.5)	175 (7.4)	167 (11.7)	168 (5.3)	167 (6.9)	176 (10.1)	171 (9.2)
BMI, kg/m^2 Mean (SD)	26.5 (2.72)	26.7 (2.64)	27.1 (3.59)	25.2 (2.95)	25.7 (1.97)	23.0 (2.34)	26.2 (2.72)	27.0 (1.93)	25.9 (2.72)
LDL-C, mmol/L Mean (SD)	3.7 (2.32)	3.4 (0.54)	3.7 (0.79)	3.7 (0.52)	2.7 (0.51)	3.2 (1.29)	3.6 (0.48)	3.8 (0.37)	3.5 (0.92)
TG, mmol/L Mean (SD)	1.7 (0.53)	1.4 (0.43)	1.5 (0.98)	1.5 (1.02)	1.1 (0.50)	1.0 (0.23)	1.0 (0.29)	1.8 (0.78)	1.4 (0.66)
PCSK9, µg/L Mean (SD)	460.69 (56.295)	276.23 (58.69)	460.69 (209.435)	311.47 (59.85)	433.44 (107.28)	288.07 (69.07)	380.03 (50.63)	288.73 (53.53)	348.34 (103.99)

BMI = body mass index; LDL-C = low-density lipoprotein cholesterol; PCSK9 = proprotein convertase subtilisin/kexin type 9; QMx2 = 2 monthly doses; QWx4 = 4 weekly doses; Q2Wx2 = 2 biweekly doses; SD = standard deviation; TG = triglycerides.

To convert values for cholesterol to mg/dL multiply by 38.67. To convert values for TG to mg/dL multiply by 88.57.

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Table 3: Mean (SD) percent change from baseline in pharmacodynamic parameters in the SAD phase (Pharmacodynamic population)

			ALN-PCSsc			
	Placebo (n=6)	25 mg (n=3)	100 mg (n=3)	300 mg (n=3)	500 mg (n=3)	800 mg (n=6)
PCSK9						
Day 84						
n	5	2	3	3	3	6
Mean (SD) percent change	-0.1 (14.3)	-47.3 (7.2)	-29.9 (12.9)	-72.6 (12.1)	-68.7 (9.8)	-72.2 (8.5)
Day 180						
n	NA	NA	2	3	2	4
Mean (SD) percent change	NA	NA	-15.7 (0.2)	-47.8 (24.8)	-70.3 (6.6)	-74.3 (13.2)
Mean (SD) percent change at individual nadir[a]	-29.4 (9.53)	-54.3 (4.75)	-48.9 (27.37)	-77.9 (3.49)	-75.7 (11.75)	-82.3 (4.85)
Mean (SD) percent change at group nadir[b]	-17.5 (19.56)	-51.2 (0.56)	-41.7 (21.28)	-74.0 (0.57)	-77.7 (1.28)	-79.4 (3.27)
Time to group nadir, days	35	42	42	42	112	98
LDL-C						
Day 84						
n	5	2	3	3	3	5
Mean (SD) percent change	-7.5 (15.6)	-27.9 (11.4)	-36.6 (6.1)	-48.4 (19.0)	-47.6 (15.2)	-41.9 (12.3)

Table 3: Mean (SD) percent change from baseline in pharmacodynamic parameters in the SAD phase (Pharmacodynamic population)

				ALN-PCSsc		
	Placebo (n=6)	25 mg (n=3)	100 mg (n=3)	300 mg (n=3)	500 mg (n=3)	800 mg (n=6)
Day 180						
n	NA	NA	2	3	2	4
Mean (SD) percent change	NA	NA	-26.3 (2.1)	-47.8 (0.5)	-37.9 (21.7)	-35.2 (16.8)
Mean (SD) percent change at individual nadir[a]	-18.7 (5.61)	-34.5 (8.62)	-42.9 (15.35)	-55.0 (10.03)	-55.1 (19.93)	-59.2 (12.25)
Mean (SD) percent change at group nadir[b]	-8.6 (18.07)	-27.9 (11.43)	-38.7 (2.07)	-48.4 (18.99)	-55.1 (24.46)	-51.8 (8.44)
Time to group nadir, days	98	84	140	84	98	35
Total cholesterol						
Day 84	-1.3 (11.7)	-20.2 (9.4)	-18.2 (10.7)	-30.9 (9.4)	-24.2 (10.2)	-28.1 (11.7)
Day 180	NA	NA	-14.1 (2.9)	-30.5 (5.7)	-23.5 (11.1)	-25.0 (12.2)
HDL-C						
Day 84	11.7 (14.4)	8.3 (10.3)	19.6 (17.7)	50.5 (71.3)	6.5 (6.4)	1.9 (17.0)
Day 180	NA	NA	18.1 (26.3)	12.8 (42.5)	-2.8 (2.8)	-0.2 (16.4)
non-HDL-C						
Day 84	-6.6 (12.2)	-25.5 (11.3)	-28.8 (7.5)	-47.2 (19.2)	-34.1 (12.6)	-36.0 (12.6)
Day 180	NA	NA	-21.2 (3.6)	-38.0 (12.6)	-29.5 (13.6)	-30.4 (13.4)
Apolipoprotein B						
Day 84	-10.0 (15.6)	-18.2 (9.7)	-28.1 (15.6)	-45.5 (20.5)	-36.0 (11.7)	-44.5 (11.8)
Day 180	NA	NA	-30.5 (7.6)	-37.6 (12.2)	-29.2 (18.8)	-27.7 (13.6)
Triglycerides						

Table 3: Mean (SD) percent change from baseline in pharmacodynamic parameters in the SAD phase (Pharmacodynamic population)

	Placebo (n=6)	ALN-PCSsc				
		25 mg (n=3)	100 mg (n=3)	300 mg (n=3)	500 mg (n=3)	800 mg (n=6)
Day 84	-12.4 (7.9)	-9.0 (19.7)	-9.6 (20.2)	-25.1 (29.2)	15.1 (28.1)	24.6 (48.2)
Day 180	NA	NA	-18.7 (35.5)	45.0 (105.8)	-8.6 (10.1)	-7.4 (23.2)
Lipoprotein (a)						
Day 84	6.7 (25.7)	-2.8 (29.0)	-20.1 (3.5)	-33.8 (46.7)	-30.4 (27.0)	-22.1 (20.8)
Day 180	NA	NA	6.6 (23.7)	-37.9 (35.8)	-31.1 (26.7)	-2.5 (18.9)

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; NA = not applicable; PCSK9 = proprotein convertase subtilisin/kexin type 9;

SD = standard deviation.

[a]Individual nadir values defined as the largest post-dose percent reduction from baseline value per subject. These values were then summarized.

[b]Group nadir is defined as the largest mean post-dose percent change from baseline value during the study.

Table 4: Mean (SD) percent change from baseline in pharmacodynamic parameters in the MAD phase (Pharmacodynamic population)

	Placebo		ALN-PCSsc					
	With Statin (n=3)	No Statin (n=8)	300 mg QMx2 With Statin (n=3)	300 mg QMx2 No Statin (n=6)	500 mg QMx2 With Statin (n=5)	500 mg QMx2 No Statin (n=6)	125 mg QWx4 No Statin (n=6)	250 mg Q2Wx2 No Statin (n=6)
PCSK9								
84 days after last dose								
n	3	6	3	6	5	6	6	6
Mean (SD) percent change	-0.5 (33.4)	1.3 (36.7)	-78.1 (3.9)	-70.6 (10.9)	-82.6 (9.5)	-74.2 (8.3)	-75.0 (7.5)	-78.0 (6.8)
180 days after last dose								
n	NA	NA	1	6	4	6	6	6
Mean (SD) percent change	NA	NA	-69.7 (NC)	-62.6 (10.7)	-75.9 (10.8)	-72.3 (14.3)	-63.3 (14.5)	-67.4 (9.9)
Mean (SD) percent change at individual nadir[a]	-42.4 (3.76)	-25.3 (20.51)	-86.1 (2.06)	-80.4 (4.92)	-88.5 (3.67)	-81.5 (5.73)	-83.8 (2.13)	-82.7 (2.81)
Mean (SD) percent change at group nadir[b]	-21.2(8.9)	-6.1 (NC)	-83.6 (4.06)	-73.1 (6.31)	-85.2 (1.83)	-79.9 (5.35)	-80.3 (4.73)	-79.4 (3.83)
Time to group nadir, days	28	91	56	56	84	84	77	35
LDL-C								
84 days after last dose								
n	3	5	3	6	5	6	6	6
Mean (SD) percent change	0.9 (33.3)	-7.0 (11.6)	-44.7 (21.2)	-48.8 (9.0)	-38.9 (13.6)	-48.5 (14.2)	-41.8 (8.8)	-50.0 (10.5)
180 days after last dose								
n	NA	NA	1	6	4	6	6	6

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Table 4: Mean (SD) percent change from baseline in pharmacodynamic parameters in the MAD phase (Pharmacodynamic population)

	Placebo		ALN-PCSsc					
	With Statin (n=3)	No Statin (n=8)	300 mg QMx2 With Statin (n=3)	300 mg QMx2 No Statin (n=6)	500 mg QMx2 With Statin (n=5)	500 mg QMx2 No Statin (n=6)	125 mg QWx4 No Statin (n=6)	250 mg Q2Wx2 No Statin (n=6)
Mean (SD) percent change	NA	NA	-30.0 (NC)	-44.3 (12.8)	-44.2 (26.2)	-45.3 (16.1)	-34.5 (5.8)	-42.1 (16.6)
Mean (SD) percent change at individual nadir[a]	-27.7 (13.19)	-19.2 (9.68)	-53.8 (19.78)	-64.4 (13.22)	-59.9 (18.14)	-56.2 (14.59)	-52.1 (4.75)	-60.4 (11.02)
Mean (SD) percent change at group nadir[b]	-18.4 (17.7)	-16.3 (NC)	-46.7 (18.29)	-55.7 (13.20)	-48.9 (23.77)	-51.9 (14.97)	-44.8 (4.07)	-54.8 (7.77)
Time to group nadir, days	35	105	70	70	140	140	63	49
Total cholesterol								
84 days after last dose	2.9 (25.1)	-11.8 (11.3)	-24.2 (13.4)	-39.9 (7.4)	-28.6 (16.1)	-25.8 (9.3)	-25.9 (5.4)	-32.0 (7.4)
180 days after last dose	NA	NA	-13.9 (NA)	-26.0 (6.5)	-25.0 (19.6)	-24.2 (12.8)	-22.2 (4.7)	-26.4 (13.9)
HDL-C								
84 days after last dose	10.6 (11.8)	-2.1 (15.1)	11.2 (9.4)	13.5 (15.6)	5.2 (15.9)	13.1 (15.9)	7.3 (3.9)	7.0 (15.8)
180 days after last dose	NA	NA	20.5 (NA)	7.5 (7.7)	3.8 (10.6)	6.0 (12.7)	3.5 (6.5)	10.2 (11.0)
non-HDL-C								
84 days after last dose	1.3 (36.5)	-15.1 (11.2)	-35.2 (10.7)	-55.3 (12.7)	-43.4 (19.1)	-43.6 (11.8)	-37.4 (9.6)	-42.5 (9.2)
	NA	NA	-25.5 (NA)	-35.5 (8.0)	-36.4 (22.0)	-37.7 (15.4)	-31.1 (4.9)	-36.6 (16.3)
Apolipoprotein B								
84 days after last dose	-6.1 (31.7)	-15.3 (11.0)	-36.8 (9.7)	-51.5 (10.7)	-40.1 (14.0)	-45.3 (11.9)	-36.4 (10.1)	-42.7 (9.9)

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Table 4: Mean (SD) percent change from baseline in pharmacodynamic parameters in the MAD phase (Pharmacodynamic population)

	Placebo		ALN-PCSsc					
	With Statin (n=3)	No Statin (n=8)	300 mg QMx2 With Statin (n=3)	300 mg QMx2 No Statin (n=6)	500 mg QMx2 With Statin (n=5)	500 mg QMx2 No Statin (n=6)	125 mg QWx4 No Statin (n=6)	250 mg Q2Wx2 No Statin (n=6)
180 days after last dose	NA	NA	-24.1 (NA)	-35.1 (10.1)	-34.9 (21.3)	-37.4 (14.8)	-24.4 (3.1)	-36.5 (15.7)
Triglycerides								
84 days after last dose	1.5 (45.7)	-8.1 (33.8)	-8.8 (6.5)	-39.3 (13.8)	-16.6 (15.2)	-0.1 (24.5)	-7.5 (19.0)	-18.0 (12.0)
180 days after last dose	NA	NA	-13.1 (NA)	7.4 (37.3)	7.2 (23.1)	6.1 (15.8)	-0.7 (28.9)	21.3 (48.7)
Lipoprotein (a)								
84 days after last dose	3.2 (20.9)	-14.7 (18.6)	-17.9 (42.5)	-19.4 (24.9)	-28.9 (28.0)	-27.6 (15.6)	-27.4 (8.9)	-25.3 (12.9)
180 days after last dose	NA	NA	-12.2 (NA)	-15.9 (26.6)	-23.7 (26.4)	-27.7 (23.7)	-29.0 (15.3)	-28.9 (12.6)

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; NA = not applicable; NC = not calculated; PCSK9 = proprotein convertase subtilisin/kexin type 9; QMx2 = 2 monthly doses; QWx4 = 4 weekly doses; QWx4 = 4 weekly doses; Q2Wx2 = 2 biweekly doses; SD = standard deviation.

[a]Individual nadir values defined as the largest post-dose percent reduction from baseline value per subject. These values were then summarized.

[b]Group nadir is defined as the largest mean post-dose percent change from baseline value during the study.

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We claim:

1.   A method of inhibiting the expression of a PCSK9 gene in a subject,
comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a
double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense
strand forming a double stranded region, the antisense strand comprising a region of
complementarity which comprises at least 15 contiguous nucleotides differing by no more
than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1,
thereby inhibiting the expression of the PCSK9 gene in the subject.

2.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a
subject, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg
of a double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense
strand forming a double stranded region, the antisense strand comprising a region of
complementarity which comprises at least 15 contiguous nucleotides differing by no more
than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1,
thereby decreasing the level of LDLc in the subject.

3.   A method of treating a subject having a disorder that would benefit from
reduction in PCSK9 expression, comprising administering to the subject a fixed dose of about
25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense
strand forming a double stranded region, the antisense strand comprising a region of
complementarity which comprises at least 15 contiguous nucleotides differing by no more
than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1,
thereby treating the subject having a disorder that would benefit from reduction in PCSK9
expression.

4.   A method of treating a subject having hyperlipidemia, comprising
administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-
stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense
strand forming a double stranded region, the antisense strand comprising a region of
complementarity which comprises at least 15 contiguous nucleotides differing by no more
than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1,
thereby treating the subject having hperlipidemia.

5.   The method of any one of claims 1-4, wherein the fixed dose is administered to the subject at an interval of once a week.

6.   The method of any one of claims 1-4, wherein the fixed dose is administered to the subject at an interval of once every two weeks.

7.   The method of any one of claims 1-4, wherein the fixed dose is administered to the subject at an interval of once a month.

8.   The method of any one of claims 1-4, wherein the fixed dose is administered to the subject at an interval of once a quarter.

9.   The method of any one of claims 1-4, wherein the fixed dose is administered to the subject at an interval of bianually.

10.   The method of claim 5, wherein the subject is administered a fixed dose of about 25 mg to about 50 mg once a week.

11.   The method of claim 6, wherein the subject is administered a fixed dose of about 50 mg to about 100 mg once every two weeks.

12.   The method of claim 7, wherein the subject is administered a fixed dose of about 100 mg to about 200 mg once a month.

13.   The method of claim 8, wherein the subject is administered a fixed dose of about 200 mg to about 800 mg once a quarter.

14.   The method of claim 9, wherein the subject is administered a fixed dose of about 200 mg to about 800 mg biannually.

15.   A method of inhibiting the expression of a PCSK9 gene in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,
        wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and
        wherein the maintenance phase comprises administering a fixed dose of about 25 mg to about 100 mg of the RNAi agent to the subject about once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby inhibiting the expression of the PCSK9 gene in the subject.

16.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,
        wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and
        wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby decreasing the level of LDLc in the subject.

17.   A method of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,
        wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and
        wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

18.   A method of treating a subject having hyperlipidemia, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 3544-3623 of the nucleotide sequence of SEQ ID NO:1, thereby treating the subject having hperlipidemia.

19.   The method of any one of claims 1-4 and 15-18, wherein the double stranded RNAi agent is administered to the subject subcutaneously.

20.   The method of claim 19, wherein the subcutaneous administration is subcutaneous injection.

21.   The method of any one of claims 1-4 and 15-18, wherein the double stranded RNAi agent is administered to the subject intramuscularly.

22.   The method of any one of claims 1-4 and 15-18, wherein the antisense strand comprises a nucleotide sequence corresponding to the unmodified sequence of any one of the nucleotide sequences provided in Table 1.

23.   The method of any one of claims 1-4 and 15-18, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3'(SEQ ID NO: 685) .

24.   The method of any one of claims 1-4 and 15-18, wherein the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686).

25.   The method of any one of claims 1-4 and 15-18, wherein the double-stranded ribonucleic acid RNAi agent comprises at least one modified nucleotide.

26.   The method of any one of claims 1-4 and 15-18, wherein substantially of the nucleotides of the sense strand are modified nucleotides.

27.   The method of any one of claims 1-4 and 15-18, wherein substantially all of the nucleotides of the antisense strand are modified nucleotides.

28.   The method of any one of claims 1-4 and 15-18, wherein all of the nucleotides of the sense strand are modified nucleotides.

29.   The method of any one of claims 1-4 and 15-18, wherein all of the nucleotides of the antisense strand are modified nucleotides.

30.   A method of inhibiting the expression of a PCSK9 gene in a subject, comprising administering to the subject a single fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby inhibiting expression of the PCSK9 gene in the subject.

31.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3'(SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby decreasing the level of LDLc in the subject.

32.   A method of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,
        wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU

– 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

33.   A method of treating a subject having hyperlipidemia, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having hyperlipidemia.

34.   A method of inhibiting the expression of a PCSK9 gene in a subject, comprising administering to the subject a single fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby inhibiting expression of the PCSK9 gene in the subject.

35.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby decreasing the level of LDLc in the subject.

36.   A method of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

37.   A method of treating a subject having hyperlipidemia, comprising administering to the subject a fixed dose of about 25 mg to about 800 mg of a double-stranded ribonucleic acid (RNAi) agent,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby treating the subject having hyperlipidemia.

38.   The method of any one of claims 30-37, wherein the fixed dose is administered to the subject at an interval of once a week.

39.   The method of any one of claims 30-37, wherein the fixed dose is administered to the subject at an interval of once every two weeks.

40.   The method of any one of claims 30-37, wherein the fixed dose is administered to the subject at an interval of once a month.

41.   The method of any one of claims 30-37, wherein the fixed dose is administered to the subject at an interval of once a quarter.

42.   The method of any one of claims 30-37, wherein the fixed dose is administered to the subject at an interval of bianually.

43.   The method of claim 38, wherein the subject is administered a fixed dose of about 25 mg to about 50 mg once a week.

44.   The method of claim 39, wherein the subject is administered a fixed dose of about 50 mg to about 100 mg once every two weeks.

45.   The method of claim 40, wherein the subject is administered a fixed dose of about 100 mg to about 200 mg once a month.

46.   The method of claim 41, wherein the subject is administered a fixed dose of about 200 mg to about 800 mg once a quarter.

47.   The method of claim 42, wherein the subject is administered a fixed dose of about 200 mg to about 800 mg biannually.

48.   A method of inhibiting the expression of a PCSK9 gene in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby inhibiting expression of the PCSK9 gene in the subject.

49.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby decreasing the level of LDLc in the subject.

50.   A method of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUTUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

51.   A method of treating a subject having hyperlipidemia, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject , and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, thereby treating the subject having hyperlipidemia.

52.   A method of inhibiting the expression of a PCSK9 gene in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 800 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby inhibiting expression of the PCSK9 gene in the subject.

53.   A method of decreasing the level of low densiy lipoprotein (LDLc) in a subject, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby decreasing the level of LDLc in the subject.

5   54.   A method of treating a subject having a disorder that would benefit from reduction in PCSK9 expression, comprising administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to

10   about 600 mg of the RNAi agent to the subject, and

wherein the maintenance phase comprises administering to the subject a fixed dose of about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the

15   nucleotide sequence 5'- ACAAAAGCAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9

20   antibody, or antigen-binding fragment thereof,

thereby treating the subject having a disorder that would benefit from reduction in PCSK9 expression.

          55.   A method of treating a subject having hyperlipidemia, comprising

25   administering to the subject a double-stranded ribonucleic acid (RNAi) agent in a dosing regimen that includes a loading phase followed by a maintenance phase,

wherein the loading phase comprises administering a fixed dose of about 200 mg to about 600 mg of the RNAi agent to the subject , and

wherein the maintenance phase comprises administering to the subject a fixed dose of

30   about 25 mg to about 100 mg of the RNAi agent once a quarter,

wherein the double-stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises the nucleotide sequence 5'- ACAAAAGCAAACAGGUCUAGAA - 3' (SEQ ID NO: 685) and the sense strand comprises the nucleotide sequence 5'- CUAGACCUGUUUGCUUUUGU

35   – 3' (SEQ ID NO: 686), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

administering to the subject a therapeutically effective amount of an anti-PCSK9 antibody, or antigen-binding fragment thereof,

thereby treating the subject having hyperlipidemia.

56.   The method of any one of claims 1-4, 15-18, 30-37, and 48-55, wherein the subject is a human.

57.   The method of any one of claims 3, 17, 32, 36, 50, and 54, wherein the disorder that would benefit from reduction in PCSK9 expression is hypercholesterolemia.

58.   The method of any one of claims 4, 18, 33, 37, 51, and 55, wherein the hyperlipidemia is hypercholesterolemia.

59.   The method of any one of claims 30-37 and 48-55, wherein the double stranded RNAi agent is administered to the subject subcutaneously.

60.   The method of claim 59, wherein said subcutaneous administration is subcutaneous injection.

61.   The method of any one of claims 30-37 and 44-55, wherein the double stranded RNAi agent is administered intramuscularly.

62.   The method of any one of claims 23, 24, 30-37 and 48-55, wherein the sense strand comprises the nucleotide sequence of 5'- csusagacCfuGfudTuugcuuuugu – 3' (SEQ ID NO: 687) and the antisense strand comprises the nucleotide sequence of 5'- asCfsaAfAfAfgCfaAfaAfcAfgGfuCfuagsasa – 3' (SEQ ID NO: 688) (AD-60212),

wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, or U; Af, Gf, Cf or Uf are 2'-fluoro A, G, C or U; dT is 2`-deoxythymidine; and s is a phosphorothioate linkage.

63.   The method of any one of claims 1-4, 15-18, 30-37, and 48-55, wherein the double-stranded ribonucleic acid RNAi agent further comprises a ligand.

64.   The method of claim 63, wherein the ligand is conjugated to the 3' end of the sense strand of the double-stranded ribonucleic acid RNAi agent.

65.   The method of claim 63, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

66.   The method of claim 65, wherein the ligand is
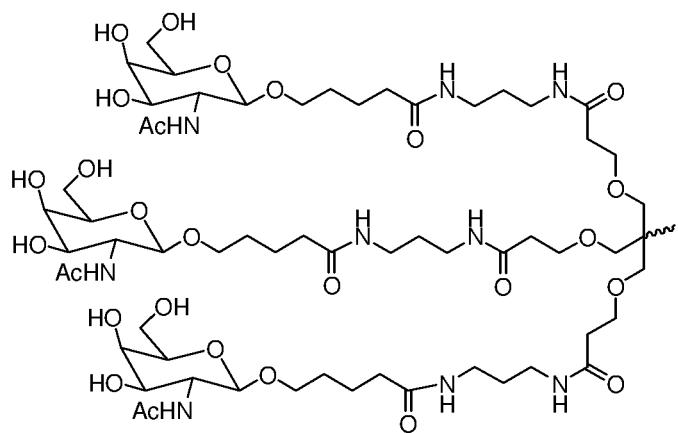
67.   The method of claim 65, wherein the double-stranded ribonucleic acid RNAi agent is conjugated to the ligand as shown in the following schematic
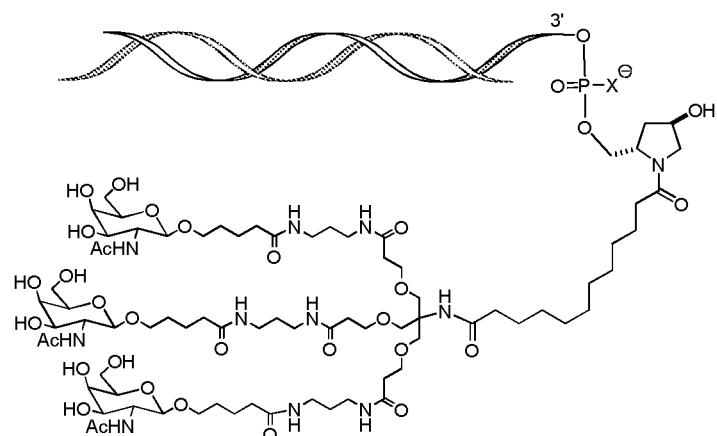
and, wherein X is O or S.

68.   The method of claim 67, wherein the X is O.

69.   The method of any one of claims 1-4, 15-18, 30-37, and 48-55, wherein PCSK9 expression is inhibited by at least about 30%.

70.   The method of any one of claims 1-4, 15-18, 30-37, and 48-55, further comprising determining an LDLR genotype or phenotype of the subject.

71.   The method of any one of claims 1-4, 15-18, 30-37, and 48-55, wherein administering results in a decrease in serum cholesterol in the subject and/or a decrease in PCSK9 protein accumulation.

72. The method of any one of claims 1-4, 15-18, 30-37, and 48-55, further comprising determining the serum cholesterol level in the subject.

73. The method of any one of claims 1-4, 15-18, 30-37, and 48-55, further comprising administering an additional therapeutic agent to the subject.

74. The method of claim 73, wherein the additional therapeutic agent is a statin.

75. The method of claim 73, wherein the additional therapeutic agent is an anti-PCSK9 antibody.

76. The method of claim 75, wherein the anti-PCSK9 antibody is selected from the group consisting of alirocumab (Praluent), evolocumab (Repatha), and bococizumab.

77. The method of any one of claims 1-4, 15-18, 30-37, and 48-55, wherein the RNAi agent is administered as a pharmaceutical compositon.

78. The method of claim 77, wherein the RNAi agent is administered in an unbuffered solution.

79. The method of claim 78, wherein the unbuffered solution is saline or water.

80. The method of claim 69, wherein the siRNA is administered with a buffer solution.

81. The method of claim 80, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

82. The method of claim 80, wherein the buffer solution is phosphate buffered saline (PBS).

83. A kit for performing the method of any one of claims 1-4, 15-18, 30-37, and 48-55, comprising
    a) the RNAi agent, and
    b) instructions for use, and
    c) optionally, means for administering the RNAi agent to the subject.

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FIG. 1

FIG. 2

Days since study treatment

Mean (+/- SEM) % LDC-C Change From Baseline

Cohort
△ Placebo (n=6)
◮ 300 mg ALN-PCSsc (n=3)
△ 25 mg ALN-PCSsc (n=3)
◮ 500 mg ALN-PCSsc (n=3)
▲ 100 mg ALN-PCSsc (n=3)
△ 800 mg ALN-PCSsc (n=6)

Days since first study treatment

Mean (+/- SEM) % PCSK9 Change From Baseline

Cohort △ Placebo (n=8)
◮ 300 mg ALN-PCSsc (n=6)
◮ 500 mg ALN-PCSsc (n=6)
◮ 125 mg QWx4 ALN-PCSsc (n=6)

△ Placebo plus statin co-medication (n=3)
▲ 300 mg ALN-PCSsc plus statin co-medication (n=3)
◮ 500 mg ALN-PCSsc plus statin co-medication (n=5)
△ 250 mg Q2Wx2 ALN-PCSsc (n=6)

FIG. 3

FIG. 4

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/7115 A61K31/713 A61P3/06 A61P3/00
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)

A61K A61P

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, FSTA, 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	Kevin Fitzgerald: "A Subcutaneous, Potent and Durable RNAi Platform Targeting Metabolic Diseases, Genes PCSK9, ApoCIII and ANGPLT3", , 1 March 2014 (2014-03-01), XP055314837, Retrieved from the Internet: URL:http://www.alnylam.com/web/assets/ALNY-CardioMetabolicPrograms-ATVB-May2014.pdf pages 3-11 ----- -/--	1-83

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 November 2016	16/11/2016

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	Anna Borodovsky: "Abstract 11936: Development of Monthly to Quarterly Subcutaneous Administration of RNAi Therapeutics Targeting the Metabolic Diseases Genes PCSK9, ApoC3 and ANGPTL3 ¦ Circulation", , 25 November 2014 (2014-11-25), XP055314834, Retrieved from the Internet: URL:http://circ.ahajournals.org/content/13 0/Suppl_2/A11936 [retrieved on 2016-10-28] the whole document -----	1-83
X	WO 2014/089313 A1 (ALNYLAM PHARMACEUTICALS [US]) 12 June 2014 (2014-06-12) page 188, lines 1-6 page 190, lines 4-15 page 90, lines 21-33 page 10, lines 19-23 page 95, line 13-page 97, line 20; claims 1-85; figure 19; example 7; table 11; compounds AD-60212; sequences 1665,1666 -----	1-83
A	Kevin Fitzgerald ET AL: "Eff ect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial", , 3 October 2013 (2013-10-03), XP055314131, Retrieved from the Internet: URL:http://ac.els-cdn.com/S014067361361914 5/1-s2.0-S0140673613619145-main.pdf?_tid=9 34e5128-9b71-11e6-b0c6-00000aacb35d&acdnat =1477482458_13f4512706bdde225e2895b7dd2029 d6 [retrieved on 2016-10-26] abstract ----- -/--	1-83

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Kevin Fitzgerald ET AL: "A Subcutaneously Administered Investigational RNAi Therapeutic (ALN-PCSsc), Targeting PCSK9 for the Treatment of Hypercholesterolemia: Initial Phase 1 Study Results D", , 30 August 2015 (2015-08-30), XP055314809, Retrieved from the Internet: URL:http://www.alnylam.com/web/assets/ALN-PCSsc-Phase-1_Presentation_08302015.pdf [retrieved on 2016-10-28] pages 1-23 -& Anonymous: "Positive Initial Clinical Data from Phase 1 Trial with ALN-PCSsc - Alnylam", , 30 August 2015 (2015-08-30), XP55314896, Retrieved from the Internet: URL:http://www.alnylam.com/capella/present ations/positive-initial-phase-1-data-aln-p cssc-esc2015/ [retrieved on 2016-10-28] the whole document -----	1-83
X,P	Kevin Fitzgerald ET AL: "ALN-PCSsc, an RNAi Investigational Agent That Inhibits PCSK9 Synthesis With the Potential for Effective Bi-Annual Dosing: Interim Results", , 11 November 2015 (2015-11-11), XP055314803, Retrieved from the Internet: URL:http://www.alnylam.com/web/assets/AHA_ PCS-Ph-1_111115.pdf [retrieved on 2016-10-28] pages 1-19 -& Anonymous: "New Clinical Data from Phase 1 Trial with ALN-PCSsc Confirms Potential for Bi-Annual Dosing - Alnylam", , 11 November 2015 (2015-11-11), XP55314805, Retrieved from the Internet: URL:http://www.alnylam.com/capella/present ations/data-from-phase-1-trial-with-aln-pc ssc-confirms-potential-for-bi-annual-dosin g/ [retrieved on 2016-10-28] abstract ----- -/--	1-83

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Anonymous: "ALN-PCS in healthy volunteers: The NCT02314442 study (phase I)", , 29 August 2015 (2015-08-29), XP055314190, Retrieved from the Internet: URL:https://integrity.thomson-pharma.com/integrity/xmlxsl/pk_csl_list.xml_csl_ref_popup_pr?p_origen=QCK&p_tsearch=&p_orden=&p_type=FICHA&p_sess_index=973191&p_id=305219 [retrieved on 2016-10-26] abstract -----	1-83

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		CN	104854242 A	19-08-2015
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		JP	2016506240 A	03-03-2016
		KR	20150091097 A	07-08-2015
		US	2016017335 A1	21-01-2016
		WO	2014089313 A1	12-06-2014

摘要

本发明涉及抑制对象中 PCSK9 基因表达的方法，以及使用靶向 PCSK9 基因的 RNAi 试剂例如双链 RNAi 试剂来治疗患有脂质紊乱，例如高脂血症的对象的治疗性和预防性方法。

摘要