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(54) Benævnelse: **ANGIOTENSINOGEN (AGT) IRNA COMPOSITIONS AND METHODS OF USE THEREOF**

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DESCRIPTION

Related Applications

[0001] This application is related to U.S. Provisional Application No. 62/671,094, filed on May 14, 2018, U.S. Provisional Application No. 62/727,141, filed on September 5, 2018, and U.S. Provisional Application No. 62/816,996, filed on March 12, 2019.

Background

[0002] The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the regulation of blood pressure. The RAAS cascade begins with the release of angiotensinogen from the liver, and renin by the juxtaglomerular cells of the kidney into the circulation. Renin secretion is stimulated by several factors, including Na+ load in the distal tubule, β-sympathetic stimulation, or reduced renal perfusion. Active renin in the plasma cleaves angiotensinogen (produced by the liver) to angiotensin I, which is then converted by circulating and locally expressed angiotensin-converting enzyme (ACE) to angiotensin II. Most of the effects of angiotensin II on the RAAS are exerted by its binding to angiotensin II type 1 receptors (AT$_1$R), leading to arterial vasoconstriction, tubular and glomerular effects, such as enhanced Na+ reabsorption or modulation of glomerular filtration rate. In addition, together with other stimuli such as adrenocorticotropin, anti-diuretic hormone, catecholamines, endothelin, serotonin, and levels of Mg2+ and K+, AT$_1$R stimulation leads to aldosterone release which, in turn, promotes Na+ and K+ excretion in the renal distal convoluted tubule.

[0003] Dysregulation of the RAAS leading to, for example, excessive angiotensin II production or AT$_1$R stimulation results in hypertension which can lead to, e.g., increased oxidative stress, promotion of inflammation, hypertrophy, and fibrosis in the heart, kidneys, and arteries, and result in, *e.g.,* left ventricular fibrosis, arterial remodeling, and glomerulosclerosis.

[0004] Hypertension is the most prevalent, controllable disease in developed countries, affecting 20-50% of adult populations. Hypertension is a major risk factor for various diseases, disorders and conditions such as, shortened life expectancy, chronic kidney disease, stroke, myocardial infarction, heart failure, aneurysms (*e.g.* aortic aneurysm), peripheral artery disease, heart damage (*e.g.,* heart enlargement or hypertrophy) and other cardiovascular related diseases, disorders, or conditions. In addition, hypertension has been shown to be an important risk factor for cardiovascular morbidity and mortality accounting for, or contributing to, 62% of all strokes and 49% of all cases of heart disease. In 2017, changes in the guidelines for diagnosis, prevention, and treatment of hypertension were developed providing goals for even lower blood pressure to further decrease risk of development of diseases and disorders associated with hypertension (see, *e.g.,* Reboussin et al. Systematic Review for the 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. JAm Coll Cardiol. 2017 Nov 7. pii: S0735-1097(17)41517-8. doi: 10.1016/j.jacc.2017.11.004; and Whelton et al. (2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. JAm Coll Cardiol. 2017 Nov 7. pii: S0735-1097(17)41519-1. doi: 10.1016/j.jacc.2017.11.006). WO 2015/179724 describes double stranded RNA molecules inhibiting the expression of angiotensinogen.

[0005] Despite the number of anti-hypertensive drugs available for treating hypertension, more than two-thirds of subjects are not controlled with one anti-hypertensive agent and require two or more anti-hypertensive agents selected from different drug classes. This further reduces the number of subjects with controlled blood pressure as adherence is reduced and side-effects are increased with increasing numbers of medications.

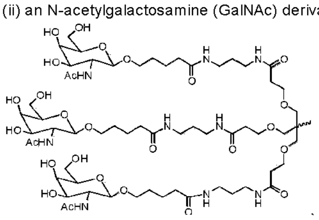
Summary of the Disclosure

[0006] The present invention is defined in the accompanying claims. Embodiments according to the invention are presented in the following numbered paragraphs:

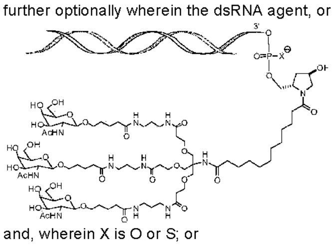
1. (1). A double stranded ribonucleic acid (dsRNA) agent, or salt thereof, for inhibiting expression of angiotensinogen (AGT), wherein the dsRNA agent, or salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence 5'-gsuscaucCfaCfAfAfugagaguaca-3' (SEQ ID NO:482), and the antisense strand comprises the nucleotide sequence 5'- usGfsuac(Tgn)cucauugUfgGfaugacsgsa-3' (SEQ ID NO:666),
   wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U, respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U, respectively; s is a phosphorothioate linkage; and (Tgn) is a thymidine-glycol nucleic acid (GNA) S-Isomer, and further comprising a ligand which is an N-acetylgalactosamine (GalNAc) derivative.
2. (2). The dsRNA agent, or salt thereof, of paragraph 1, wherein the ligand is:

   (i) conjugated to the 3' end of the sense strand of the dsRNA agent, or salt thereof; and/or

   (ii) an N-acetylgalactosamine (GalNAc) derivative that is

   further optionally wherein the dsRNA agent, or salt thereof, is conjugated to the ligand as shown in the following schematic

   and, wherein X is O or S; or

   (iv) one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.
3. (3). An isolated cell containing the dsRNA agent, or salt thereof, of paragraph 1 or 2.

4. (4). A pharmaceutical composition for inhibiting expression of a gene encoding AGT comprising the dsRNA agent, or salt thereof, of paragraph 1 or 2.
5. (5). A pharmaceutical composition comprising the dsRNA agent, or salt thereof, of paragraph 1 or 2, and a lipid formulation.
6. (6). An *in vitro* method of inhibiting expression of an AGT gene in a cell, the method comprising contacting the cell with the dsRNA agent, or salt thereof, of paragraph 1 or 2 or the pharmaceutical composition of paragraph 4 or 5, thereby inhibiting expression of the AGT gene in the cell.
7. (7). The dsRNA agent, or salt thereof, of paragraph 1 or 2 or the pharmaceutical composition of paragraph 4 or 5 for use in treating a subject having an AGT-associated disorder.
8. (8). The dsRNA agent, or salt thereof, or pharmaceutical composition for use of paragraph 7, wherein the subject has been diagnosed with an AGT-associated disorder,
   optionally wherein the AGT-associated disorder is selected from the group consisting of high blood pressure, hypertension, borderline hypertension, primary hypertension, secondary hypertension isolated systolic or diastolic hypertension, pregnancy-associated hypertension, diabetic hypertension, resistant hypertension, refractory hypertension, paroxysmal hypertension, renovascular hypertension, Goldblatt hypertension, hypertension associated with low plasma renin activity or plasma renin concentration, ocular hypertension, glaucoma, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension; hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, vasculopathy, diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiac myopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, heart failure, myocardial infarction, angina, stroke, renal disease, renal failure, systemic sclerosis, intrauterine growth restriction (IUGR) , fetal growth restriction, obesity, liver steatosis/ fatty liver, non-alcoholic Steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD); glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome.
9. (9). The dsRNA agent, or salt thereof, or pharmaceutical composition for use of paragraph 7 or 8, wherein contacting the cell with the dsRNA agent, or salt thereof, or pharmaceutical composition inhibits the expression of AGT by at least 50%, 60%, 70%, 80%, 90%, 95%.
10. (10). The dsRNA agent, or salt thereof, or the pharmaceutical composition for use of any one of paragraphs 7-9, wherein the subject:
    1. (i) has a systolic blood pressure of at least 130 mm Hg or a diastolic blood pressure of at least 80 mm Hg;
    2. (ii) has a systolic blood pressure of at least 140 mm Hg and a diastolic blood pressure of at least 80 mm Hg;
    3. (iii) is human; and/or
    4. (iv) is part of a group susceptible to salt sensitivity, is overweight, is obese, or is pregnant.
11. (11). The dsRNA agent, or salt thereof, or pharmaceutical composition for use of any one of paragraphs 7-10, wherein the dsRNA agent, or salt thereof, or pharmaceutical composition is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg, and/or subcutaneously; and/or
    an additional therapeutic agent for treatment of hypertension is administered to the subject, optionally wherein the additional therapeutic agent:
    1. (a) is selected from the group consisting of a diuretic, an angiotensin converting enzyme (ACE) inhibitor, an angiotensin II receptor antagonist, a beta-blocker, a vasodialator, a calcium channel blocker, an aldosterone antagonist, an alpha2-agonist, a renin inhibitor, an alpha-blocker, a peripheral acting adrenergic agent, a selective D1 receptor partial agonist, a nonselective alpha-adrenergic antagonist, a synthetic, a steroidal antimineralocorticoid agent, an angiotensin receptor-neprilysin inhibitors (ARNi), Entresto®, sacubitril/valsartan; or an endothelin receptor antagonist (ERA), sitaxentan, ambrisentan, atrasentan, BQ-123, zibotentan, bosentan, macitentan, and tezosentan; a combination of any of the foregoing; and a hypertension therapeutic agent formulated as a combination of agents; or
    2. (b) comprises an angiotensin II receptor antagonist, further optionally wherein the angiotensin II receptor antagonist is selected from the group consisting of losartan, valsartan, olmesartan, eprosartan, and azilsartan.
12. (12). A kit comprising the dsRNA agent, or salt thereof, of paragraph 1 or 2 or the pharmaceutical composition of paragraph 4 or 5.

[0007] The present disclosure provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding angiotensinogen (AGT). The AGT may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

[0008] In an aspect, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of angiotensinogen (AGT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 19 contiguous nucleotides from the nucleotide sequence of any one of nucleotides 635-658, 636-658, 642-667, 642-664, 645-667, 1248-1273, 1248-1272, 1248-1270, 1250-1272, 1251-1273, 1580-1602, 1584-1606, 1587-1609, 1601-1623, 1881-1903, 2074-2097, 2074-2096, 2075-2097, 2080-2102, 2272-2294, 2276-2298, 2281-2304, 2281-2303, or 2282-2304 of SEQ ID NO:1 and the antisense strand comprises at least 19 contiguous nucleotides from the nucleotide sequence of SEQ ID NO:2.

[0009] In certain options, the sense strand comprises at least 21 contiguous nucleotides of any one of nucleotides 635-658, 636-658, 642-667, 642-664, 645-667, 1248-1273, 1248-1272, 1248-1270, 1250-1272, 1251-1273, 1580-1602, 1584-1606, 1587-1609, 1601-1623, 1881-1903, 2074-2097, 2074-2096, 2075-2097, 2080-2102, 2272-2294, 2276-2298, 2281-2304, 2281-2303, or 2282-2304 of SEQ ID NO:1. In certain options, the antisense strand comprises at least 21 contiguous nucleotides from the nucleotide sequence of SEQ ID NO:2.

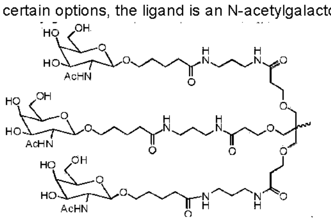
[0010] In certain options, the antisense strand comprises at least 19 contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385. In certain options, the sense strand comprises at least 19 contiguous nucleotides from any one of the sense strand nucleotide sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385. In certain options, the sense and antisense strands comprise nucleotide sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385.

[0011] In certain options, the antisense strand comprises at least 19 contiguous nucleotides from nucleotide sequence of the antisense strand of AD-85481 (5'-UGUACUCUCAUUGUGGAUGACGA-3' (SEQ ID NO: 9)). In certain options, the sense strand comprises at least 19 contiguous nucleotides from the nucleotide sequences of the sense strand of AD-85481 (5'-GUCAUCCACAAUGAGAGUACA-3' (SEQ ID NO: 10)). In certain options, the sense and antisense strands comprise the nucleotide sequences of the sense and antisense strands of AD-85481 (5'-UGUACUCUCAUUGUGGAUGACGA-3' (SEQ ID NO: 9) and 5'-GUCAUCCACAAUGAGAGUACA-3' (SEQ ID NO: 10)).

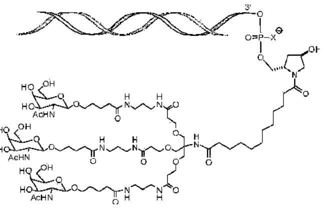
[0012] In certain options, the dsRNA agent comprises at least one modified nucleotide. In certain options, substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification. In certain options, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification. In certain options, at least one of the modified nucleotides is selected from the group of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof. In certain options, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C- allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, GNA, and combinations thereof. In certain options, the modifications on the nucleotides are 2'-O-methyl or 2'-fluoro modifications. In certain options, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof. In certain options, at least one of the nucleotide modification is a thermally destabilizing nucleotide modification. In certain options, the thermally destabilizing nucleotide modification is selected from the group consisting of of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA)

[0013] In certain options, the double stranded region is 19- 21 nucleotides in length. In certain options, the double stranded region is 21 nucleotides in length. In certain options, each strand of the dsRNA agent is independently no more than 30 nucleotides in length. In certain options, at least one strand of the dsRNA agent comprises a 3' overhang of at least 1 nucleotide or at least 2 nucleotides.

[0014] In certain options, dsRNA agent further comprises a ligand. In certain options, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent. In certain options, the ligand is an N-acetylgalactosamine (GalNAc) derivative, e.g., wherein the ligand is



[0015] In certain options, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S, e.g., wherein the X is O.

[0016] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand comprises a region of complementarity to an mRNA encoding human AGT, wherein the region of complementarity comprises at least 19 nucleotides one of the antisense strand sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385. In certain options, the antisense strand comprises a region of complementarity to an mRNA encoding human AGT, wherein the region of complementarity comprises any one of the antisense strand sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385. In certain options, the region of complementarity consists of any one of the antisense strand sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385.

[0017] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand comprises the chemically modified nucleotide sequence of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385.

[0018] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand comprises the chemically modified nucleotide sequence of the duplex AD-85481 (5'-usGfsuac(Tgn)cucauugUfgGfaugacsgsa-3' (SEQ ID NO: 11)) wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively; Af, Cf, Gf, and Uf are 2'-O-fluoroadenosine-3'-phosphate, 2'-O-fluorocytidine-3'-phosphate, 2'-O-fluoroguanosine-3'-phosphate, and 2'-O-fluorouridine-3'-phosphate, respectively; dT is a deoxy-thymine; s is a phosphorothioate linkage; and (Tgn) is thymidine-glycol nucleic acid (GNA) S-isomer.

[0019] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand and the sense strand comprise the chemically modified nucleotide sequences of a duplex selected from the group consisting of AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, AD-85655, AD-126306, AD-126307, AD-126308, AD-126310, AD133360, AD-133361, AD-133362, AD-133374, and AD-133385.

[0020] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand and the sense strand comprise the chemically modified nucleotide sequences of the duplex AD-85481 (5'-usGfsuac(Tgn)cucauugUfgGfaugacsgsa-3' (SEQ ID NO: 11) and 5'-gsuscaucCfaCfAfAfugagaguaca-3' (SEQ ID NO: 12)) wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively; Af, Cf, Gf, and Uf are 2'-O-fluoroadenosine-3'-phosphate, 2'-O-fluorocytidine-3'-phosphate, 2'-O-fluoroguanosine-3'-phosphate, and 2'-O-fluorouridine-3'-phosphate, respectively; dT is a deoxy-thymine; s is a phosphorothioate linkage; and (Tgn) is thymidine-glycol nucleic acid (GNA) S-isomer; and wherein the 3'-end of the sense strand is optionally conjugated to an N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (L96) ligand.

[0021] In certain options, the disclosure provides a dsRNA agent, wherein the antisense strand and the sense strand consist of the chemically modified nucleotide sequences of the duplex AD-85481 (5'-usGfsuac(Tgn)cucauugUfgGfaugacsgsa-3' (SEQ ID NO: 11) and 5'-gsuscaucCfaCfAfAfugagaguaca-3' (SEQ ID NO: 12)), wherein the 3'-end of the sense strand is conjugated to an N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (L96) ligand, wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively; Af, Cf, Gf, and Uf are 2'-O-fluoroadenosine-3'-phosphate, 2'-O-fluorocytidine-3'-phosphate, 2'-O-fluoroguanosine-3'-phosphate, and 2'-O-fluorouridine-3'-phosphate, respectively; dT is a deoxy-thymine; s is a phosphorothioate linkage; and (Tgn) is thymidine-glycol nucleic acid (GNA) S-isomer.

[0022] In certain options, the double stranded region of the dsRNA agent is about 19-30 nucleotide pairs in length, about 19-25 nucleotide pairs in length, about 23-27 nucleotide pairs in length, about 19-23 nucleotide pairs in length, about 21-23 nucleotide pairs in length.

[0023] In certain options, each strand of the dsRNA agent is independently 19-30 nucleotides in length.

[0024] In certain options, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

[0025] In certain options, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage. In certain options, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. In certain options, the strand is the antisense strand. In certain options, the strand is the sense strand. In certain options, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. In certain options, the strand is the antisense strand. In certain options, the strand is the sense strand. In certain options, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In certain options, the strand is the antisense strand.

[0026] In certain options, the dsRNA agent at the 1 position of the 5'-end of the antisense strand of the duplex comprises a base pair that is an AU base pair.

[0027] In certain options, the dsRNA agent comprises a sense strand has a total of 21 nucleotides and an antisense strand has a total of 23 nucleotides.

[0028] In an aspect, the disclosure provides a cell containing the dsRNA agent of the disclosure.

[0029] In an aspect, the disclosure provides a pharmaceutical composition for inhibiting expression of a gene encoding AGT comprising the dsRNA agent of the disclosure. In certain options, the pharmaceutical composition comprises the dsRNA agent and a lipid formulation.

[0030] In an aspect, the disclosure provides a method of inhibiting expression of an AGTgene in a cell, the method comprising:

1. (a) contacting the cell with the dsRNA agent or a pharmaceutical composition of the disclosure; and
2. (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the AGT gene, thereby inhibiting expression of the AGT gene in the cell.

[0031] In certain options, the cell is within a subject. In certain options, the subject is a human. In certain options, the subject has been diagnosed with an AGT-associated disorder.

[0032] In certain options, the AGT -associated disorder is selected from high blood pressure, hypertension, borderline hypertension, primary hypertension, secondary hypertension isolated systolic or diastolic hypertension, pregnancy-associated hypertension, diabetic hypertension, resistant hypertension, refractory hypertension, paroxysmal hypertension, renovascular hypertension, Goldblatt hypertension, ocular hypertension, glaucoma, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension; hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, vasculopathy, diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiac myopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, heart failure, myocardial infarction, angina, stroke, renal disease, renal failure, systemic sclerosis, intrauterine growth restriction (IUGR) , fetal growth restriction, obesity, liver steatosis/ fatty liver, non-alcoholic Steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD); glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome.

[0033] In certain options, the subject has a systolic blood pressure of at least 130 mm Hg or a diastolic blood pressure of at least 80 mm Hg. In certain options, the subject has a systolic blood pressure of at least 140 mm Hg and a diastolic blood pressure of at least 80 mm Hg. In certain options, the subject is part of a group susceptible to salt sensitivity, is overweight, is obese, or is pregnant.

[0034] In certain options, contacting the cell with the dsRNA agent inhibits the expression of AGT by at least 50%, 60%, 70%, 80%, 90%, 95% (*e.g.,* as compared to the level of expression of AGT prior to first contacting the cell with the dsRNA agent; e.g., prior to administration of a first dose of the dsRNA agent to the subject). In certain options, inhibiting expression of AGT decreases an AGT protein level in a subject serum sample(s) by at least 50%, 60%, 70%, 80%, 90%, or 95%, *e.g.,* as compared to the level of expression of AGT prior to first contacting the cell with the dsRNA agent.

[0035] In an aspect, the disclosure provides a method of treating a an AGT-associated disorder in a subject, comprising administering to the subject the dsRNA agent or the pharmaceutical composition of the disclosure, thereby treating the AGT-associated disorder in the subject. In certain options, the subject has a systolic blood pressure of at least 130 mm Hg or a diastolic blood pressure of at least 80 mm Hg. In certain options, the subject has a systolic blood pressure of at least 140 mm Hg and diastolic blood pressure of at least 80 mm Hg. In certain options, the subject is human. In certain options, subject is part of a group susceptible to salt sensitivity, is overweight, is obese, or is pregnant.

[0036] In certain options of the disclosure, the dsRNA agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg. In certain options, the dsRNA agent is administered to the subject subcutaneously. In certain options, the level of AGT is measured in the subject. In certain options, the level of AGT in the subject is an AGT protein level in a subject blood sample(s), serum sample(s), or urine sample(s).

[0037] In certain options, an additional therapeutic agent for treatment of hypertension is administered to the subject. In certain options, the additional therapeutic agent is selected from the group consisting of of a diuretic, an angiotensin converting enzyme (ACE) inhibitor, an angiotensin II receptor antagonist, a beta-blocker, a vasodialator, a calcium channel blocker, an aldosterone antagonist, an alpha2-agonist, a renin inhibitor, an alpha-blocker, a peripheral acting adrenergic agent, a selective D1 receptor partial agonist, a nonselective alpha-adrenergic antagonist, a synthetic, and steroidal antimineralocorticoid agent; or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents. In certain options, the additional therapeutic agent comprises an angiotensin II receptor antagonist, *e.g.*, losartan, valsartan, olmesartan, eprosartan, and azilsartan. In certain options, the additional therapeutic agent is an angiotensin receptor-neprilysin inhibitor (ARNi), *e.g.*, Entresto®, sacubitril/valsartan; or an endothelin receptor antagonist (ERA), *e.g.*, sitaxentan, ambrisentan, atrasentan, BQ-123, zibotentan, bosentan, macitentan, and tezosentan.

[0038] The disclosure also provides uses of the dsRNA agents and the pharmaceutical compositions provided herein for treatment of an AGT-associated disorder. In certain options, the uses include any of the methods provided by the disclosure.

[0039] The disclosure provides kits comprising a dsRNA agent of the disclosure. In certain options, the disclosure provides kits for practicing a method of the disclosure.

Brief Description of the Drawings

[0040]

Figure 1A is a graph showing serum AGT protein levels in cynomolgus monkeys (n = 3 per group) treated with a single 3 mg/kg dose of the indicated siRNAs. AGT levels are shown as a percent of AGT level prior to treatment.

Figure 1B is a graph showing serum AGT protein levels in cynomolgus monkeys (n = 3 per group) treated with a single 0.3 mg/kg, 1 mg/kg, or 3 mg/kg dose of AD-85481 or AD-67327 on day 1. AGT levels are shown as a percent of AGT level prior to treatment.

Figure 1C is a graph showing serum AGT protein levels in cynomolgus monkeys (n = 3 per group) administered a 1 mg/kg dose of AD-85481 or AD-67327 once every four weeks for three doses. AGT levels are shown as a percent of AGT level prior to treatment.

Figures 2A-2G show the results of a various parameters in a study of spontaneously hypertensive rats (n=9 per group) treated with a vehicle, valsartan (31 mg/kg/day), a rat specific AGT-siRNA (10 mg/kg q2w), captopril (100 mg/kg/day), valsartan and captopril, or valsartan and AGT-siRNA.

Figure 2A shows plasma AGT levels at the start (solid bars) and end (stippled bars) of the study (at four weeks).

Figure 2B shows daily blood pressure readings compared to baseline.

Figure 2C is a graph showing heart weight: tibial length ratios.

Figure 2D is a graph showing plasma renin activity level at the start (solid bars) and end (stippled bars) of the study (at four weeks).

Figure 2E is a graph of heart weight:tibial length graphed against mean arterial pressure (MAP) in mm Hg.

Figure 2F is a graph of cardiomyocyte size.

Figure 2G is a graph of N-terminal pro b-type natriuretic peptide (NT-proBNP) levels.

Figure 3 is a graph showing urine AGT levels in the spontaneously hypertensive rat study.

Figure 4A is a graph showing the level of blood Ang I in the spontaneous hypertensive rat study.

Figure 4B is a graph showing the level of blood Ang II in the spontaneous hypertensive rat study.

Figure 4C is a graph showing the ratio of blood Ang II to blood Ang I in the spontaneous hypertensive rat study.

Figure 5A is a graph showing the level of renal Ang I in the spontaneous hypertensive rat study.

Figure 5B is a graph showing the level of renal Ang II in the spontaneous hypertensive rat study.

Figure 5C is a graph showing the ratio of renal Ang II to renal Ang I in the spontaneous hypertensive rat study.

Figure 6A is a graph showing the level of angiotensin receptor 1a in the kidney cortex and medulla in the spontaneous rat hypertensive study.

Figure 6B is a graph showing the level of angiotensin 1b receptor in the kidney cortex and medulla in the spontaneous rat hypertensive study.

Figure 6C is a graph showing the level of ACE in the kidney cortex and medulla in the spontaneous rat hypertensive study.

Figure 7 is a graph showing urinary volume at baseline and at 4 weeks after the start of treatment in the spontaneous rat hypertensive study.

Figure 8A is a graph showing average body weights of high fat fed diet induced obesity (DIO) mice or normal chow fed mice (n = 5 per group) treated with either an AGT dsRNA agent or PBS.

Figure 8B is a graph showing terminal liver, adipose, and muscle weights (n = 5 per group) of high fat fed diet induced obesity (DIO) mice or normal chow fed mice treated with either an AGT dsRNA agent or PBS.

Figure 9A is a graph showing the changes in plasma glucose levels (mg/dL) in high fat fed diet induced obesity (DIO) mice or normal chow fed mice (n = 5 per group) treated with either an AGT dsRNA agent or PBS at week 0 prior to first treatment dose.

Figure 9B is a graph showing the plasma glucose levels (mg/dL) in high fat fed diet induced obesity (DIO) mice or normal chow fed mice (n = 5 per group) treated with either an AGT dsRNA agent or PBS at week 6 of the experiment.

Figure 9C is a graph showing the plasma glucose levels (mg/dL) in high fat fed diet induced obesity (DIO) mice or normal chow fed mice (n = 5 per group) treated with either an AGT dsRNA agent or PBS at week 12 of the experiment.

Figure 10A is a graph showing average body weights of high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice.

Figure 10B is a graph showing average cumulative weight gain of high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice.

Figures 11A-11C are graphs showing serum liver enzymes in high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice at week 20 of the experiment.

Figure 11A is a graph showing alanine transaminase (ALT) levels in high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice.

Figure 11B is a graph showing aspartate transaminase (AST) levels in high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice.

Figure 11C is a graph showing glutamate dehydrogenase (GLDH) levels in high fat high fructose (HF HFr) fed mice treated with either an AGT dsRNA agent or PBS, or normal chow fed (LFD) mice.

Detailed Description of the Disclosure

[0041] The present disclosure provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an AGT gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (AGT gene) in mammals.

[0042] The iRNAs of the disclosure have been designed to target the human AGT gene, including portions of the gene that are conserved in the AGT orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the disclosure improved efficacy, stability, potency, durability, and safety.

[0043] Accordingly, the present disclosure provides methods for treating and preventing an AGT-associated disorder, *e.g.*, hypertension, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an AGT gene.

[0044] The iRNAs of the disclosure include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.,* 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, which region is substantially complementary to at least part of an mRNA transcript of an AGT gene.

[0045] In certain options, one or both of the strands of the double stranded RNAi agents of the disclosure is up to 66 nucleotides in length, 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 an AGT gene. In some options, such iRNA agents having longer length antisense strands preferably may 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.

[0046] The use of iRNAs of the disclosure enables the targeted degradation of mRNAs of the corresponding gene (AGT gene) in mammals. Using *in vitro* and *in vivo* assays, the present inventors have demonstrated that iRNAs targeting an AGT gene can mediate RNAi, resulting in significant inhibition of expression of AGT. Inhibition of expression of AGT in such a subject will prevent or treat development of a AGT-associated disorder, *e.g.*, hypertension. Thus, methods and compositions including these iRNAs are useful for preventing and treating a subject susceptible to or diagnosed with an AGT-associated disorder, *e.g.*, hypertension. The methods and compositions herein are useful for reducing the level of AGT in a subject.

[0047] The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an AGT gene as well as compositions, uses, and methods for treating subjects that would benefit from reduction of the expression of an AGT gene, *e.g.*, subjects susceptible to or diagnosed with an AGT-associated disorder, *e.g.*, hypertension.

I. Definitions

[0048] In order that the present disclosure 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 disclosure.

[0049] 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.

[0050] The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

[0051] 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."

[0052] 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 options, about means ±10%. In certain options, 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.

[0053] 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 19 nucleotides of a 21 nucleotide nucleic acid molecule" means that 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.

[0054] As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or integers, 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, ranges include both the upper and lower limit.

[0055] In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

[0056] As used herein, "angiotensinogen," used interchangeably with the term "AGT" refers to the well-known gene and polypeptide, also known in the art as Serpin Peptidase Inhibitor, Clade A, Member 8; Alpha-1 Antiproteinase; Antitrypsin; SERPINA8; Angiotensin I; Serpin A8; Angiotensin II; Alpha-1 Antiproteinase angiotensinogen; antitrypsin; pre-angiotensinogen2; ANHU; Serine Proteinase Inhibitor; and Cysteine Proteinase Inhibitor.

[0057] The term "AGT" includes human AGT, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI: 188595658 (NM_000029.3; SEQ ID NO:1); *Macaca fascicularis* AGT, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI: 90075391 (AB170313.1: SEQ ID NO:3); mouse (*Mus musculus*) AGT, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI: 113461997 (NM_007428.3; SEQ ID NO:5); and rat AGT (*Rattus norvegicus*) AGT the amino acid and complete coding sequence of which may be found in for example, for example GenBank Accession No. GI:51036672 (NM_134432; SEQ ID NO:7).

[0058] Additional examples of AGT mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

[0059] The term"AGT," as used herein, also refers to naturally occurring DNA sequence variations of the AGT gene, such as a single nucleotide polymorphism (SNP) in the AGT gene. Exemplary SNPs may be found in the dbSNP database available at www.ncbi.nlm.nih.gov/projects/SNP/snp-_ref.cgi?geneId=183. Non-limiting examples of sequence variations within the AGT gene include, for example, those described in U.S. Patent No. 5,589,584. For example, sequence variations within the AGT gene may include as a C→T at position -532 (relative to the transcription start site); a G→A at position -386; a G→A at position -218; a C→T at position -18; a G→A and a A→C at position -6 and -10; a C→T at position +10 (untanslated); a C→T at position +521 (T174M); a T→C at position +597 (P199P); a T→C at position +704 (M235T; also see, *e.g.,* Reference SNP (refSNP) Cluster Report: rs699, available at www.ncbi.nlm.nih.gov/SNP); a A→G at position +743 (Y248C); a C→T at position +813 (N271N); a G→A at position +1017 (L339L); a C→A at position +1075 (L359M); and/or a G→A at position +1162 (V388M).

[0060] As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an AGT gene, including mRNA that is a product of RNA processing of a primary transcription product. 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 an AGT gene. In one option, the target sequence is within the protein coding region of AGT.

[0061] The target sequence may be from about 19-36 nucleotides in length, *e.g.*, preferably about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 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 disclosure.

[0062] 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.

[0063] "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 2). 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 disclosure 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 disclosure.

[0064] 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 an AGT gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

[0065] In one option, an RNAi agent of the disclosure includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, an AGT 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 disclosure 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.,* an AGT gene. Accordingly, the term "siRNA" is also used herein to refer to an iRNA as described above.

[0066] In certain options, the RNAi agent may be a single-stranded siRNA (ssRNAi) 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. 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.

[0067] In certain options, an "iRNA" for use in the compositions, uses, and methods of the disclosure is a double stranded RNA and is referred to herein as a "double stranded RNA 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.,* an AGT gene. In some options of the disclosure, 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.

[0068] 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 or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the disclosure 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 "iRNA" or "RNAi agent" for the purposes of this specification and claims.

[0069] 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 19 to 36 base pairs in length, e.g., about 19-30 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 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 disclosure.

[0070] 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 options, the hairpin loop can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some options, the hairpin loop can be 10 or fewer nucleotides. In some options, the hairpin loop can be 8 or fewer unpaired nucleotides. In some options, the hairpin loop can be 4-10 unpaired nucleotides. In some options, the hairpin loop can be 4-8 nucleotides.

[0071] Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, 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.

[0072] In certain options, an iRNA agent of the disclosure is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, an AGT gene, to direct cleavage of the target RNA.

[0073] In some options, an iRNA of the disclosure is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, an AGT target mRNA sequence, to direct the cleavage of the target RNA.

[0074] As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double stranded iRNA. 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 the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

[0075] In certain options, the antisense 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 or the 5'-end. In certain options, 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, 10-25 nucleotides, 10-20 nucleotides, or 10-15 nucleotides in length. In certain options, an extended overhang is on the sense strand of the duplex. In certain options, an extended overhang is present on the 3'end of the sense strand of the duplex. In certain options, an extended overhang is present on the 5'end of the sense strand of the duplex. In certain options, an extended overhang is on the antisense strand of the duplex. In certain options, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain options, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain options, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate. In certain options, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

[0076] "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNA agent, *i.e.,* no nucleotide overhang. A "blunt ended" double stranded RNA agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the disclosure include RNAi agents with no nucleotide overhang at one end (*i.e.,* agents with one overhang and one blunt end) or with no nucleotide overhangs at either end. Most often such a molecule will be double-stranded over its entire length.

[0077] 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.*, an AGT 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.*, an AGT 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, or 3 nucleotides of the 5'- or 3'-end of the iRNA. In some options, a double stranded RNA agent of the disclosure includes a nucleotide mismatch in the antisense strand. In some options, a double stranded RNA agent of the disclosure includes a nucleotide mismatch in the sense strand. In some options, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another option, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA.

[0078] 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.

[0079] As used herein, "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.

[0080] 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 options, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some options, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some options, 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.

[0081] 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.

[0082] 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.

[0083] "Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs 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.

[0084] 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 a double stranded RNA agent and a target sequence, as will be understood from the context of their use.

[0085] 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 an AGT gene). For example, a polynucleotide is complementary to at least a part of an AGT mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an AGT gene.

[0086] Accordingly, in some options, the sense strand polynucleotides and the antisense polynucleotides disclosed herein are fully complementary to the target AGT sequence. In other options, the sense strand polynucleotides or the antisense polynucleotides disclosed herein are substantially complementary to the target AGT sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1 and 2, or a fragment of any one of SEQ ID NOs:1 and 2, such as at least 90%, or 95% complementary; or 100% complementary.

[0087] Accordingly, in some options, the antisense strand polynucleotides disclosed herein are fully complementary to the target AGT sequence. In other options, the antisense strand polynucleotides disclosed herein are substantially complementary to the target AGT sequence and comprise a contiguous nucleotide sequence which is at least about 90% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:1, or a fragment of SEQ ID NO:1, such as about 90%, or about 95%, complementary. In certain options, the fragment of SEQ ID NO: 1 is selected from the group of nucleotides 632-658, 635-658, 636-658, 1248-1273, 1248-1270, 1250-1272, 1251-1273, 1580-1602, 1584-1606, 1587-1609, 1601-1623, 1881-1903, 2074-2097, 2074-2096, 2075-2097, 2080-2102, 2272-2294, 2276-2298, 2281-2304, 2281-2303, or 2282-2304 of SEQ ID NO: 1. In preferred options, the duplex does not consist of the sense strand consisting of uscsucccAfcCfUfUfuucuucuaauL96 (SEQ ID NO: 13) and the antisense strand consisting of asUfsuagAfagaaaagGfuGfggagascsu (SEQ ID NO: 14).

[0088] In some options, an iRNA of the disclosure includes an antisense strand that is substantially complementary to the target AGT sequence and comprises a contiguous nucleotide sequence which is at least about 90% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the sense strands in Table 3, Table 5, or Table 6 or a fragment of any one of the sense strands in Table 3, Table 5, or Table 6, such as about 90%, 95%, or 100% complementary.

[0089] In some options, an iRNA of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target AGT sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 90% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the antisense strands in Table 3, 5, or 6, or a fragment of any one of the antisense strands in Table 3 or 5, such as about 90%, 95%, or 100%.

[0090] In certain options, the sense and antisense strands in Table 3 or Table 5 are selected from duplexes AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, and AD-85655.

[0091] In general, an "iRNA" includes 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 a dsRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

[0092] In an aspect of the disclosure, an agent for use in the methods and compositions of the disclosure is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide 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 oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

[0093] The phrase "contacting a cell with an iRNA," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the individual performing the method, or alternatively, the iRNA may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

[0094] Contacting a cell *in vitro* may be done, for example, by incubating the cell with the iRNA. Contacting a cell *in vivo* may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc, that directs the iRNA to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an iRNA and subsequently transplanted into a subject.

[0095] In certain options, contacting a cell with an iRNA includes "introducing" or "delivering the iRNA into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

[0096] The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*, an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069.

[0097] As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In an option, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in AGT expression; a human at risk for a disease or disorder that would benefit from reduction in AGT expression; a human having a disease or disorder that would benefit from reduction in AGT expression; or human being treated for a disease or disorder that would benefit from reduction in AGT expression as described herein. The diagnostic criteria for an AGT-associated disorder, *e.g.*, hypertension, are provided below. In some options, the subject is a female human. In other options, the subject is a male human. In certain options, the subject is part of a group susceptible to salt sensitivity, *e.g.*, black or an older adult (≥65 years of age). In certain options, the subject is overweight or obese, *e.g.*, a subject that suffers from central obesity. In certain options, the subject is sedentary. In certain options, the subject is pregnant.

[0098] As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result, such as reducing at least one sign or symptom of an AGT-associated disorder, e.g., hypertension in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted AGT expression, *e.g.*, angiotensin II type 1 receptor activation (AT_1R) (*e.g.*, hypertension, chronic kidney disease, stroke, myocardial infarction, heart failure, aneurysms, peripheral artery disease, heart disease, increased oxidative stress, *e.g.*, increased superoxide formation, inflammation, vasoconstriction, sodium and water retention, potassium and magnesium loss, renin suppression, myocyte and smooth muscle hypertrophy, increased collagen sysnthesis, stimulation of vascular, myocardial and renal fibrosis, increased rate and force of cardiac contractions, altered heart rate, *e.g.*, increased arrhythmia, stimulation of plasminogen activator inhibitor 1 (PAI1), activation of the sympathetic nervous system, and increased endothelin secretion), symptoms of pregnancy-associated hypertension (*e.g.*, preeclampsia, and eclampsia), including, but not limited to intrauterine growth restriction (IUGR) or fetal growth restriction, symptoms associated with malignant hypertension, symptoms associated with hyperaldosteronism; diminishing the extent of unwanted AT_1R activation; stabilization (*i.e.,* not worsening) of the state of chronic AT_1R activation; amelioration or palliation of unwanted AT_1R activation (*e.g.*, hypertension, chronic kidney disease, stroke, myocardial infarction, heart failure, aneurysms, peripheral artery disease, heart disease, increased oxidative stress, *e.g.*, increased superoxide formation, inflammation, vasoconstriction, sodium and water retention, potassium and magnesium loss, renin suppression, myocyte and smooth muscle hypertrophy, increased collagen sysnthesis, stimulation of vascular, myocardial and renal fibrosis, increased rate and force of cardiac contractions, altered heart rate, *e.g.*, increased arrhythmia, stimulation of plasminogen activator inhibitor 1 (PAI1), activation of the sympathetic nervous system, and increased endothelin secretion) whether detectable or undetectable. AGT-associated disorders can also include obesity, liver steatosis/ fatty liver, *e.g.*, non-alcoholic Steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome. In certain options, hypertension includes hypertension associated with low plasma renin activity or plasma renin concentration. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

[0099] The term "lower" in the context of the level of AGT gene expression or agt protein production in a subject, or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection for the detection method in a relevant cell or tissue, *e.g.*, a liver cell, or other subject sample, *e.g.*, blood or serum derived therefrom, urine.

[0100] As used herein, "prevention" or "preventing," when used in reference to a disease or disorder, that would benefit from a reduction in expression of an AGT gene or production of agt protein, *e.g.*, in a subject susceptible to an AGT-associated disorder due to, *e.g.*, aging, genetic factors, hormone changes, diet, and a sedentary lifestyle. In certain options, the disease or disorder is *e.g.,* a symptom of unwanted AT_1R activation, such as a hypertension, chronic kidney disease, stroke, myocardial infarction, heart failure, aneurysms, peripheral artery disease, heart disease, increased oxidative stress, *e.g.*, increased superoxide formation, inflammation, vasoconstriction, sodium and water retention, potassium and magnesium loss, renin suppression, myocyte and smooth muscle hypertrophy, increased collagen synthesis, stimulation of vascular, myocardial and renal fibrosis, increased rate and force of cardiac contractions, altered heart rate, *e.g.*, increased arrhythmia, stimulation of plasminogen activator inhibitor 1 (PAI1), activation of the sympathetic nervous system, and increased endothelin secretion. AGT-associated disorders can also include obesity, liver steatosis/ fatty liver, *e.g.*, non-alcoholic Steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome. In certain options, hypertension includes hypertension associated with low plasma renin activity or plasma renin concentration. The likelihood of developing, *e.g.*, hypertension, is reduced, for example, when an individual having one or more risk factors for a hypertension either fails to develop hypertension or develops hypertension with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop an AGT-associated disorder, *e.g.*, hypertension or a delay in the time to develop hypertension by months or years is considered effective prevention. Prevention may require administration of more than one dose if the iRNA agent.

[0101] As used herein, the term "angiotensinogen-associated disease" or "AGT-associated disease," is a disease or disorder that is caused by, or associated with renin-angiotensin-aldosterone system (RAAS) activation, or a disease or disorder the symptoms of which or progression of which responds to RAAS inactivation. The term "angiotensinogen-associated disease" includes a disease, disorder or condition that would benefit from reduction in AGT expression. Such diseases are typically associated with high blood pressure. Non-limiting examples of angiotensinogen-associated diseases include hypertension, *e.g.*, borderline hypertension (also known as prehypertension), primary hypertension (also known as essential hypertension or idiopathic hypertension), secondary hypertension (also known as inessential hypertension), isolated systolic or diastolic hypertension, pregnancy-associated hypertension (*e.g.*, preeclampsia, eclampsia, and post-partum preelampsia), diabetic hypertension, resistant hypertension, refractory hypertension, paroxysmal hypertension, renovascular hypertension (also known as renal hypertension), Goldblatt hypertension, ocular hypertension, glaucoma, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension; hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, vasculopathy (including peripheral vascular disease), diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiac myopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, sleep apnea, heart failure (*e.g.*, left ventricular systolic dysfunction), myocardial infarction, angina, stroke, renal disease *e.g.*, chronic kidney disease or diabetic nephropathy optionally in the context of pregnancy, renal failure, *e.g.*, chronic renal failure, and systemic sclerosis (*e.g.*, scleroderma renal crisis). In certain options, AGT-associated disease includes intrauterine growth restriction (IUGR) or fetal growth restriction. In certain options, AGT-associated disorders also include obesity, liver steatosis/ fatty liver, *e.g.*, non-alcoholic Steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome. In certain options, hypertension includes hypertension associated with low plasma renin activity or plasma renin concentration.

[0102] Thresholds for high blood pressure and stages of hypertension are discussed in detail below.

[0103] In one option, an angiotensinogen-associated disease is primary hypertension. "Primary hypertension" is a result of environmental or genetic causes (*e.g.*, a result of no obvious underlying medical cause).

[0104] In one option, an angiotensinogen-associated disease is secondary hypertension. "Secondary hypertension" has an identifiable underlying disorder which can be of multiple etiologies, including renal, vascular, and endocrine causes, *e.g.,* renal parenchymal disease (*e.g.*, polycystic kidneys, glomerular or interstitial disease), renal vascular disease (*e.g.*, renal artery stenosis, fibromuscular dysplasia), endocrine disorders (*e.g.*, adrenocorticosteroid or mineralocorticoid excess, pheochromocytoma, hyperthyroidism or hypothyroidism, growth hormone excess, hyperparathyroidism), coarctation of the aorta, or oral contraceptive use.

[0105] In one option, an angiotensinogen-associated disease is pregnancy-associated hypertension, *e.g.*, chronic hypertension of pregnancy, gestational hypertension, preeclampsia, eclampsia, preeclampsia superimposed on chronic hypertension, HELLP syndrome, and gestational hypertension (also known as transient hypertension of pregnancy, chronic hypertension identified in the latter half of pregnancy, and pregnancy-induced hypertension (PIH)). Diagnostic criteria for pregnancy-associated hypertension are provided below.

[0106] In one option, an angiotensinogen-associated disease is resistant hypertension. "Resistant hypertension" is blood pressure that remains above goal (*e.g.*, above 130 mm Hg systolic or above 90 diastolic) in spite of concurrent use of three antihypertensive agents of different classes, one of which is a thiazide diuretic. Subjects whose blood pressure is controlled with four or more medications are also considered to have resistant hypertension.

[0107] A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any treatment. The iRNA employed in the methods of the present disclosure may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

[0108] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, 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.

[0109] 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. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

[0110] The term "sample," as used herein, includes 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, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, 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 options, 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 some options, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood or blood derived serum or plasma from the subject.

I. iRNAs of the Disclosure

[0111] The present disclosure provides iRNAs which inhibit the expression of an AGT gene. In preferred options, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an AGT gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing an AGT-associated disorder, *e.g.*, hypertension. The dsRNAi agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an AGT gene. The region of complementarity is about 19-30 nucleotides in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or 19 nucleotides in length). Upon contact with a cell expressing the AGT gene, the iRNA inhibits the expression of the AGT gene (*e.g.*, a human, a primate, a non-primate, or a rat AGT gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In preferred options, inhibition of expression is determined by the qPCR method provided in the examples, especially in Example 2 with the siRNA at a 10 nM concentration in an appropriate organism cell line provided therein. In preferred options, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.,* a mouse or an AAV-infected mouse expressing the human target gene, *e.g.,* when administered a single dose at 3 mg/kg at the nadir of RNA expression. RNA expression in liver is determined using the PCR methods provided in Example 2.

[0112] 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 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 an AGT 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, 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.

[0113] Generally, the duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

[0114] In some options, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to 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).

[0115] 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 19 to about 30 base pairs, *e.g.,* about 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 option, 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 option, a miRNA is a dsRNA. In another option, a dsRNA is not a naturally occurring miRNA. In another option, an iRNA agent useful to target AGT gene expression is not generated in the target cell by cleavage of a larger dsRNA.

[0116] A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have 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 an antisense or sense strand of a dsRNA.

[0117] A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi compounds of the disclosure 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. Similarly, single-stranded oligonucleotides of the disclosure can be prepared using solution-phase or solid-phase organic synthesis or both.

[0118] In an aspect, a dsRNA of the disclosure 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 Tables 3, 5, and 6, and the corresponding antisense strand of the sense strand is selected from the group of sequences of Table 3, 5, and 6. 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 an AGT gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in Table 5, or 6, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in Table 3, 5, or 6. In certain options, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other options, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide. In certain options, the sense or antisense strand from Table 3 or 5 is selected from AD-85481, AD-84701, AD-84703, AD-84704, AD-84705, AD-84707, AD-84715, AD-84716, AD-84739, AD-84741, AD-84746, AD-85432, AD-85434, AD-85435, AD-85436, AD-85437, AD-85438, AD-85441, AD-85442, AD-85443, AD-85444, AD-85446, AD-85447, AD-85482, AD-85485, AD-85493, AD-85496, AD-85504, AD-85517, AD-85519, AD-85524, AD-85622, AD-85623, AD-85625, AD-85626, AD-85634, AD-85635, AD-85637, and AD-85655.

[0119] It will be understood that, although the sequences in Table 3 are not described as modified or conjugated sequences, the RNA of the iRNA of the disclosure e.g., a dsRNA of the disclosure, may comprise any one of the sequences set forth in Table 3, or the sequences of Table 5 or 6 that are modified, or the sequences of Table 5 or 6 that are conjugated. In other words, the disclosure encompasses dsRNA of Table 3, 5, and 6 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

[0120] The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 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 options described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Table 3, 5, and 6, 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 Table 3, 5, and 6 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 19, 20, or more contiguous nucleotides derived from one of the sequences of Table 3, 5, and 6, and differing in their ability to inhibit the expression of an AGT 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 disclosure.

[0121] In addition, the RNAs provided in Table 3, 5, and 6 identify a site(s) in an AGT transcript that is susceptible to RISC-mediated cleavage. As such, the present disclosure 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 19 contiguous nucleotides from one of the sequences provided in Table 3, 5, and 6 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an AGT gene.

II. Modified iRNAs of the Disclosure

[0122] In certain options, the RNA of the iRNA of the disclosure *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.*, chemical modifications or conjugations known in the art and described herein. In other options, the RNA of an iRNA of the disclosure, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain options of the disclosure, substantially all of the nucleotides of an iRNA of the disclosure are modified. In other options of the disclosure, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1unmodified nucleotides are present in a strand of the iRNA.

[0123] The nucleic acids featured in the disclosure can be synthesized 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. Modifications include, for example, 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 of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the options 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 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 options, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

[0124] Modified RNA backbones include, for example, phosphorothioates, chiral 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 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.

[0125] 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; 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; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat RE39464.

[0126] 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 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.

[0127] Representative U.S. Patents that teach the preparation of the above oligonucleosides 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.

[0128] Suitable RNA mimetics are contemplated for use in iRNAs provided herein, 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 in which an RNA mimetic that has been shown to have excellent hybridization properties is referred to as a peptide 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 US 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. Additional PNA compounds suitable for use in the iRNAs of the disclosure are described in, for example, in Nielsen et al., Science, 1991, 254, 1497-1500.

[0129] Some options featured in the disclosure include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $--CH_2--NH--CH_2-$, $--CH_2--N(CH_3)--O--CH_2--$[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 options, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

[0130] 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 $O[(CH_2)_nO]_mCH_3$, $O(CH_2)._nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other options, 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 options, 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'-0-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-$O--CH_2--O--CH_2--N(CH_2)_2$. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

[0131] 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 US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patent 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.

[0132] 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, 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 increasing the binding affinity of the oligomeric compounds featured in the disclosure. 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 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.

[0133] 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, 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.

[0134] 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. 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).

[0135] In some options, the RNA of an iRNA can also be modified to include one or more bicyclic sugar moieties. 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 certain options, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some options an agent of the disclosure may 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 bicyclic sugar moiety comprising a 4'-CH_2-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 disclosure include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain options, the antisense polynucleotide agents of the disclosure 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'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4'-$C(CH_3)(CH_3)$-0-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4'-CH_2-$N(OCH_3)$-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4'-CH_2-O-$N(CH_3)$-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH_2-N(R)-O-2', wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4'-CH_2-$C(H)(CH_3)$-2' (see, *e.g.*, Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH_2-$C(=CH_2)$-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426).

[0136] Additional representative U.S. Patents and U.S. 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.

[0137] 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).

[0138] 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'-$CH(CH_3)$-O-2' bridge. In one option, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

[0139] An iRNA of the disclosure 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.

[0140] Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868.

[0141] In some options, an iRNA of the disclosure comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see Nuc. Acids Symp. Series, 52, 133-134 (2008) and Fluiter et al., Mol. Biosyst., 2009, 10, 1039).

[0142] Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Patent No. 8,314,227; and U.S. Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020.

[0143] 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.

[0144] Other modifications of the nucleotides of an iRNA of the disclosure include a 5' phosphate or 5' phosphate mimic, *e.g.*, a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511.

A. Modified iRNAs Comprising Motifs of the Disclosure

[0145] In certain aspects of the disclosure, the double stranded RNA agents of the disclosure include agents with chemical modifications as disclosed, for example, in WO2013/075035. WO2013/075035 provides motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some options, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

[0146] More specifically, when the sense strand and antisense strand of the double stranded RNA 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 a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

[0147] Accordingly, the disclosure provides double stranded RNA agents capable of inhibiting the expression of a target gene (*i.e.,* AGT gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 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.

[0148] The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be, for example, the duplex region can be 27-30 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 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

[0149] In certain options, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 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. In certain options, the overhang regions can include extended overhang regions as provided above. 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.

[0150] In certain options, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethy 1-5-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.

[0151] The 5'- or 3'- overhangs at the sense strand, antisense strand, or both strands of the dsRNAi agent may be phosphorylated. In some options, 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 some options, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some options, this 3'-overhang is present in the antisense strand. In some options, this 3'-overhang is present in the sense strand.

[0152] The dsRNAi 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'- end of the sense strand or, alternatively, at the 3'-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 dsRNAi agent 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.

[0153] In certain options, the dsRNAi 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.

[0154] In other options, the dsRNAi 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.

[0155] In yet other options, the dsRNAi 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.

[0156] In certain options, the dsRNAi 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.

[0157] 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 option, 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 certain options, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain options each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.*, in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (preferably GalNAcs).

[0158] In certain options, the dsRNAi 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.

[0159] In certain options, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi 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 which is at least 25 nucleotides in length, and the second strand is sufficiently complementary 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 dsRNAi 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 dsRNAi agent further comprises a ligand.

[0160] In certain options, the sense strand of the dsRNAi 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.

[0161] In certain options, the antisense strand of the dsRNAi 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.

[0162] For a dsRNAi agent having a duplex region of 19-23 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; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first 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 dsRNAi agent from the 5'-end.

[0163] The sense strand of the dsRNAi 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.

[0164] In some options, the sense strand of the dsRNAi 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 chemistries 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.

[0165] Like the sense strand, the antisense strand of the dsRNAi 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.

[0166] In some options, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

[0167] In other options, the wing modification on the sense strand or antisense strand of the dsRNAi 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.

[0168] When the sense strand and the antisense strand of the dsRNAi 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.

[0169] When the sense strand and the antisense strand of the dsRNAi 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.

[0170] In some options, every nucleotide in the sense strand and antisense strand of the dsRNAi 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 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.

[0171] 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 an 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.

[0172] 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 options 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.

[0173] In some options, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, 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 option, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro.

[0174] 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.

[0175] In certain options, the N_a 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.*

[0176] 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.*

[0177] In some options, the dsRNAi agent of the disclosure 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 'to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3'of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' to 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.

[0178] In some options, the dsRNAi 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.

[0179] The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

[0180] In some options, 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. Alternatively, N_a or N_b may be present or absent when there is a wing modification present.

[0181] The iRNA 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, 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 or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand 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 option, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In some options, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

[0182] In some options, the dsRNAi agent 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, or the 5'end of the antisense strand.

[0183] In some options, 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 dsRNAi 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.

[0184] In one option, the dsRNAi 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.

[0185] In certain options, the dsRNAi 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.

[0186] In certain options, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from 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.

[0187] In other options, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). For example, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

[0188] In certain options, the sense strand sequence may be represented by formula (I):

$$5'\ n_p\text{-}N_a\text{-}(XXX)_iN_b\text{-}YYY\text{-}N_b\text{-}(ZZZ)_j\text{-}N_a\text{-}n_q3' \quad (I)$$

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.

[0189] In some options, the N_a or N_b comprises modifications of alternating pattern.

[0190] In some options, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi 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 first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

[0191] In one option, 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:

$$5'\ n_p\text{-}N_a\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q\ 3' \quad (Ib);$$

$$5'\ n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_a\text{-}n_q3' \quad (Ic);$$

or

$$5'\ n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q3' \quad (Id).$$

[0192] 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.

[0193] 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.

[0194] 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.

[0195] Each of X, Y and Z may be the same or different from each other.

[0196] In other options, i is 0 and j is 0, and the sense strand may be represented by the formula:

$$5'\ n_p\text{-}N_a\text{-}YYY\text{-}\ N_a\text{-}n_q3' \quad (Ia).$$

[0197] 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.

[0198] In one option, the antisense strand sequence of the RNAi may be represented by formula (II):

$$5'\ n_{q'}\text{-}N_a'\text{-}(Z'Z'Z')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(X'X'X')_1\text{-}N'_a\text{-}n_p'3' \quad (II)$$

wherein:

k and 1 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.

[0199] In some options, the N_a' or N_b' comprises modifications of alternating pattern.

[0200] The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in 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 first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

[0201] In certain options, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

[0202] In certain options, k is 1 and 1 is 0, or k is 0 and 1 is 1, or both k and 1 are 1.

[0203] The antisense strand can therefore be represented by the following formulas:

5' $_{nq}$-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).

[0204] 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.

[0205] 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.

[0206] 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.

[0207] In other options, k is 0 and 1 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).

[0208] 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.

[0209] Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, 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.

[0210] In some options, the sense strand of the dsRNAi 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 first nucleotide from the 5'-end, or optionally, the count starting at the first 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.

[0211] In some options the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first 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.

[0212] 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.

[0213] Accordingly, the dsRNAi agents for use in the methods of the disclosure may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the iRNA 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 1 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.

[0214] In one option, 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 option, k is 0 and 1 is 0; or k is 1 and 1 is 0; k is 0 and 1 is 1; or both k and 1 are 0; or both k and 1 are 1.

[0215] Exemplary combinations of the sense strand and antisense strand forming an iRNA duplex include the formulas below:

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' (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'

3'n_p -N_a -X'X'X'-N_b -Y'Y'Y'-N_b -Z'Z'Z'-N_a-n_q 5' (IIId)

[0216] When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0217] When the dsRNAi agent is represented by formula (IIIb), each N_b independently 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.

[0218] When the dsRNAi 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 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0219] When the dsRNAi 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 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b, and Nb' independently comprises modifications of alternating pattern.

[0220] Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

[0221] When the dsRNAi 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.

[0222] When the dsRNAi 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.

[0223] When the dsRNAi 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.

[0224] In certain options, 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, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

[0225] In certain options, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In other options, 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 other options, 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 other options, 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.

[0226] In some options, when the dsRNAi agent is represented by formula (IIIa), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications , n_p' >0 and a_t 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.

[0227] In some options, the dsRNAi 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.

[0228] In some options, the dsRNAi 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.

[0229] In one option, two dsRNAi agents represented by at least one of formulas (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 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.

[0230] In certain options, an RNAi agent of the disclosure may contain a low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 10 or fewer nucleotides with 2'-fluoro modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific option, the RNAi agent of the disclosure contains 10 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific option, the RNAi agent of the disclosure contains 6 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

[0231] In other options, an RNAi agent of the disclosure may contain an ultra low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 of 0 nucleotides with a 2'-fluoro modification. In a specific option, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

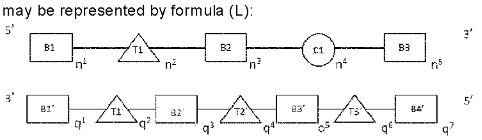
[0232] Various publications describe multimeric iRNAs that can be used in the methods of the disclosure. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520.

[0233] As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA 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.

[0234] 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 options 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, or 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 chemical entity, *e.g.*, a ligand to the constituent ring.

[0235] The iRNA 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, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin; preferably, the acyclic group is a serinol backbone or diethanolamine backbone.

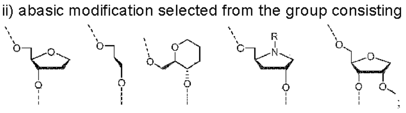
[0236] In another option of the disclosure, an iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



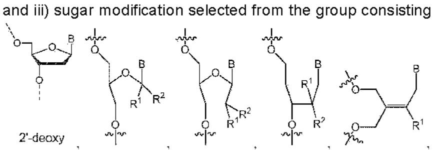
(L),

In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one option, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one option, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one option, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA) modification.

[0237] C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA). In one option, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:

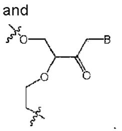


and iii) sugar modification selected from the group consisting of:

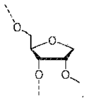


2'-deoxy

and



wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In one option, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally destabilizing modification in C1 is GNA or



T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one option, T1 is DNA. In one option, T 1' is DNA, RNA or LNA. In one option, T2' is DNA or RNA. In one option, T3' is DNA or RNA.

n^1, n^3, and q^1 are independently 4 to 15 nucleotides in length.

n^5, q^3, and q^7 are independently 1-6 nucleotide(s) in length.

n^4, q^2, and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

[0238] Alternatively, n^4 is 0-3 nucleotide(s) in length.

[0239] In one option, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0240] In one option, n^4, q^2, and q^6 are each 1.

[0241] In one option, n^2, n^4, q^2, q^4, and q^6 are each 1.

[0242] In one option, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In one option, C1 is at position 15 of the 5'-end of the sense strand

[0243] In one option, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

[0244] In one option, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

[0245] In an exemplary option, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

[0246] In one option, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

[0247] In one option, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

[0248] In one option, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

[0249] In one option, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary option, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

[0250] In one option, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

[0251] In an exemplary option, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

[0252] In one option, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

[0253] In one option, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is

1.

[0254] In one option, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0255] In one option, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0256] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0257] In one option, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. In one option, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0258] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0259] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

[0260] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

[0261] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

[0262] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

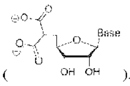
[0263] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

[0264] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

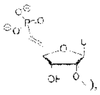
[0265] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

[0266] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

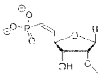
[0267] The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS_2), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-*C*-malonyl

().

When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either 5'-E-VP isomer (*i.e., trans*-vinylphosphate,

),

5'-Z-VP isomer (*i.e., cis*-vinylphosphate,

),

or mixtures thereof.

[0268] In one option, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In one option, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

[0269] In one option, the RNAi agent comprises a 5'-P. In one option, the RNAi agent comprises a 5'-P in the antisense strand.

[0270] In one option, the RNAi agent comprises a 5'-PS. In one option, the RNAi agent comprises a 5'-PS in the antisense strand.

[0271] In one option, the RNAi agent comprises a 5'-VP. In one option, the RNAi agent comprises a 5'-VP in the antisense strand. In one option, the RNAi agent comprises a 5'-E-VP in the antisense strand. In one option, the RNAi agent comprises a 5'-Z-VP in the antisense strand.

[0272] In one option, the RNAi agent comprises a 5'-PS_2. In one option, the RNAi agent comprises a 5'-PS_2 in the antisense strand.

[0273] In one option, the RNAi agent comprises a 5'-PS_2. In one option, the RNAi agent comprises a 5'-deoxy-5'-C-malonyl in the antisense strand.

[0274] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-PS.

[0275] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

[0276] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

[0277] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

[0278] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0279] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

[0280] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

[0281] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

[0282] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

[0283] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0284] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

[0285] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The dsRNA agent also comprises a 5'-PS.

[0286] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

[0287] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

[0288] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0289] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P.

[0290] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS.

[0291] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

[0292] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS_2.

[0293] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

[0294] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

[0295] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

[0296] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof. In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The dsRNAi RNA agent also comprises a 5'- PS_2.

[0297] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

[0298] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- P.

[0299] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS.

[0300] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

[0301] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

[0302] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0303] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

[0304] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

[0305] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-Z-VP, or combination thereof.

[0306] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

[0307] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0308] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- P.

[0309] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS.

[0310] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-Z-VP, or combination thereof.

[0311] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

[0312] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

[0313] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one option, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0314] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one option, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0315] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof), and a targeting ligand.

[0316] In one option, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0317] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one option, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0318] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one option, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0319] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P and a targeting ligand. In one option, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0320] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one option, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0321] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one option, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0322] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS_2 and a targeting ligand. In one option, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0323] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one option, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0324] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one option, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0325] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one option, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0326] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one option, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0327] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS_2 and a targeting ligand. In one option, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0328] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one option, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0329] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one option, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0330] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS and a targeting ligand. In one option, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0331] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP (*e.g.*, a 5'-*E*-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one option, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0332] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one option, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0333] In one option, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one option, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

[0334] In a particular option, an RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and
    3. (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22

(counting from the 5' end); and
3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0335] In another particular option, an RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end); and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

    and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

    wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0336] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

    and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

    wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0337] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

    and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

    wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0338] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

    and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

    wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0339] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

   wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0340] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 25 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (e.g. dT) at positions 24 and 25 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

   wherein the RNAi agents have a four nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0341] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

   wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0342] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 23 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and
    3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

   wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0343] In another particular option, a RNAi agent of the present disclosure comprises:

1. (a) a sense strand having:
    1. (i) a length of 19 nucleotides;
    2. (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
    3. (iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and
    4. (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

   and
2. (b) an antisense strand having:
    1. (i) a length of 21 nucleotides;
    2. (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5'

end); and

3. (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

[0344] In certain options, the iRNA for use in the methods of the disclosure is an agent selected from agents listed in Table 3, Table 5, or Table 6. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

[0345] Another modification of the RNA of an iRNA of the disclosure involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such 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). In other options, the ligand is 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 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 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).

[0346] In certain options, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In preferred options 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 do not take part in duplex pairing in a duplexed nucleic acid.

[0347] 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-acetylglucosamine, 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.

[0348] 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, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, 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 options, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

[0349] 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.

[0350] 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-glucosamine 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.

[0351] 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, or intermediate filaments. The drug can be, for example, taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

[0352] In some options, 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. 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 disclosure 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 options described herein.

[0353] Ligand-conjugated iRNAs of the disclosure 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.

[0354] The oligonucleotides used in the conjugates of the present disclosure 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 methods 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.

[0355] In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present disclosure, 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.

[0356] 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 options, the oligonucleotides or linked nucleosides of the present disclosure are synthesized by an automated synthesizer using phosphoramidites 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

[0357] In certain options, 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 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, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

[0358] A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate 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.

[0359] In certain options, the lipid based ligand binds HSA. Preferably, it binds 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.

[0360] In other options, 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 target to kidney cells can also be used in place of, or in addition to, the lipid based ligand.

[0361] 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

[0362] 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.

[0363] 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.

[0364] 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: 15). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:16) 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:17) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:18) 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.

[0365] An RGD peptide for use in the compositions and methods of the disclosure 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.

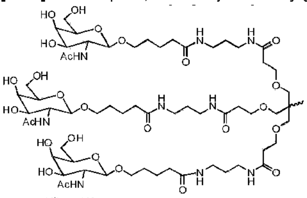
[0366] A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide 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

[0367] In some options of the compositions and methods of the disclosure, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is 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).

[0368] In certain options, a carbohydrate conjugate for use in the compositions and methods of the disclosure is a monosaccharide.

[0369] In one option, a carbohydrate conjugate for use in the compositions and methods of the disclosure is selected from the group consisting of: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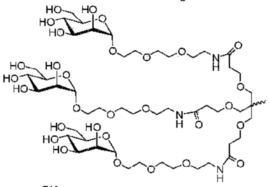
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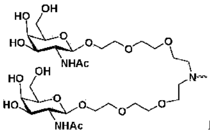
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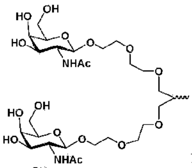
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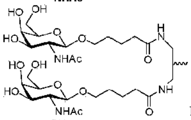
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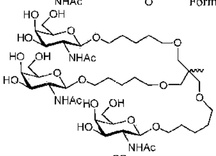
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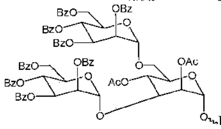
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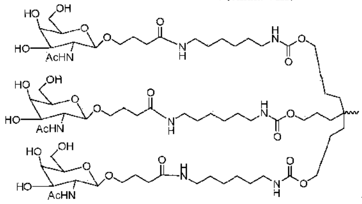
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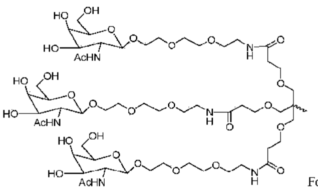
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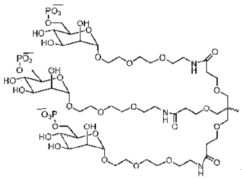
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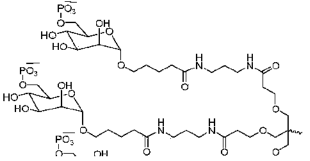

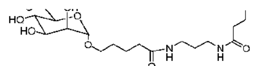
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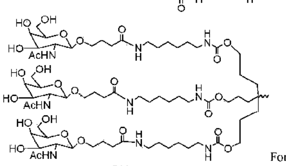
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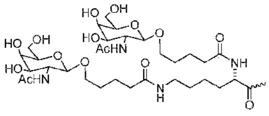
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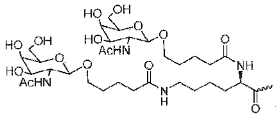
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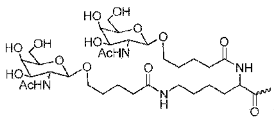
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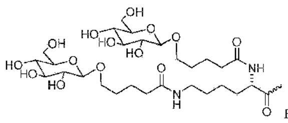
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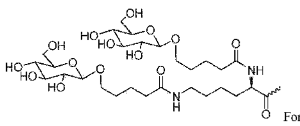
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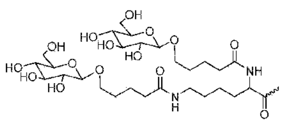
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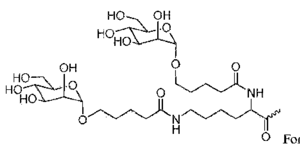
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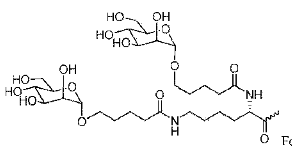
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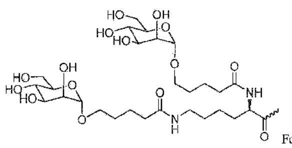
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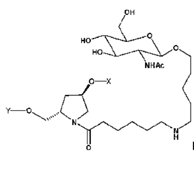
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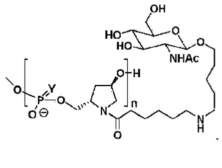
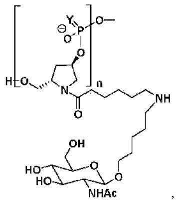
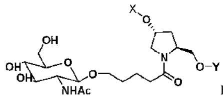

.

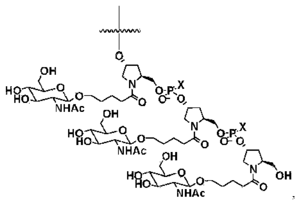
wherein Y is O or S and n is 3 -6 (Formula XXIV);



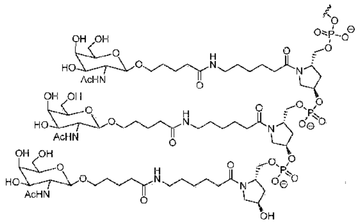
wherein Y is O or S and n is 3-6 (Formula XXV);

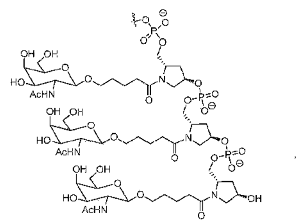


Formula XXVI;

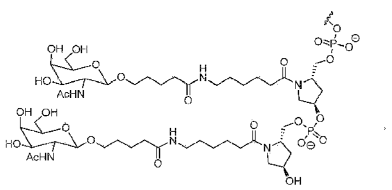


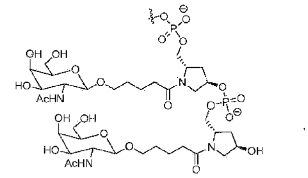
wherein X is O or S (Formula XXVII);





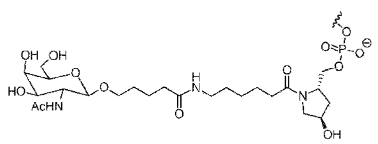
Formula XXVII; Formula XXIX;



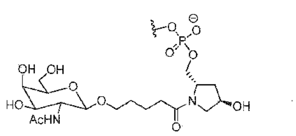


Formula XXX;

Formula XXXI;

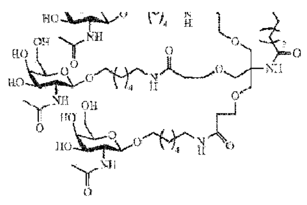


, and



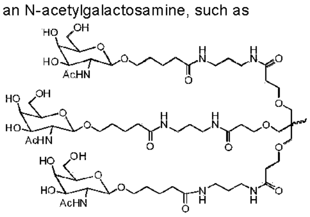
Formula XXXII;

Formula XXXIII.



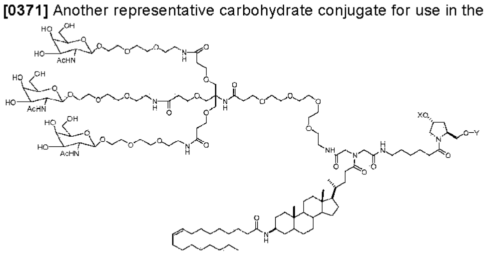
Formula XXXIV.

[0370] In another option, a carbohydrate conjugate for use in the compositions and methods of the disclosure is a monosaccharide. In one option, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

[0371] Another representative carbohydrate conjugate for use in the options described herein includes, but is not limited to,



(Formula XXXVI),

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

[0372] In certain options of the disclosure, the GalNAc or GalNAc derivative is attached to an iRNA agent of the disclosure *via* a monovalent linker. In some options, the GalNAc or GalNAc derivative is attached to an iRNA agent of the disclosure *via* a bivalent linker. In yet other options of the disclosure, the GalNAc or GalNAc derivative is attached to an iRNA agent of the disclosure *via* a trivalent linker.

[0373] In one option, the double stranded RNAi agents of the disclosure comprise one GalNAc or GalNAc derivative attached to the iRNA agent, *e.g.*, the 5'end of the sense strand of a dsRNA agent, or the 5' end of one or both sense strands of a dual targeting RNAi agent as described herein. In another option, the double stranded RNAi agents of the disclosure comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

[0374] In some options, for example, when the two strands of an iRNA agent of the disclosure 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 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.

[0375] In some options, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

[0376] Additional carbohydrate conjugates and linkers suitable for use in the present disclosure include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627.

D. Linkers

[0377] In some options, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

[0378] 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 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, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, 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), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic, or substituted aliphatic. In one option, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

[0379] A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred option, 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 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).

[0380] 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.

[0381] 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.

[0382] 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.

[0383] Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

[0384] 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 options, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 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

[0385] In certain options, 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 options, 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

[0386] In other options, 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-, -OP(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -OP(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 options are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -OP(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-, and -O-P(S)(H)-S-. A preferred option is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

[0387] In other options, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred options acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.5, 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 -C=NN-, C(O)O, or -OC(O). A preferred option 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.

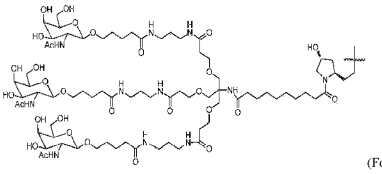
iv. Ester-based linking groups

[0388] In other options, 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 using methods analogous to those described above.

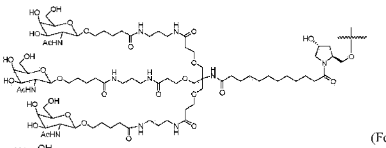
v. Peptide-based cleaving groups

[0389] In yet other options, 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 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 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.

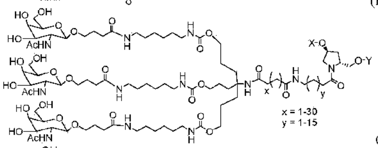
[0390] In some options, an iRNA of the disclosure is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the disclosure include, but are not limited to,



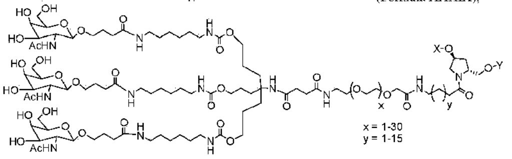
(Formula XXXVII),



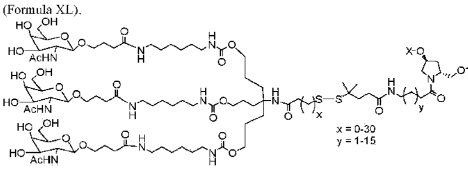
(Formula XXXVIII),



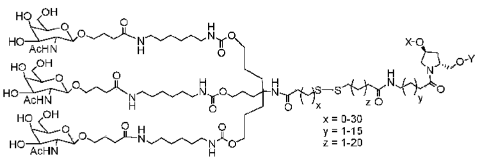
(Formula XXXIX),



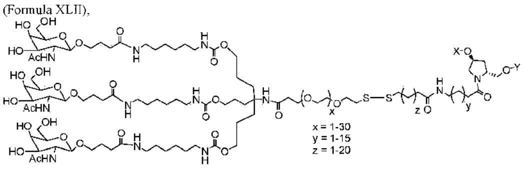
(Formula XL),



(Formula XLI),

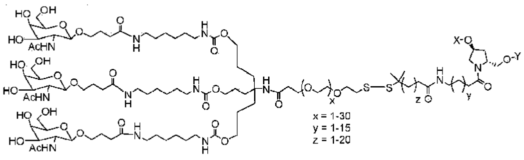


(Formula XLII),



(Formula XLIII),

and



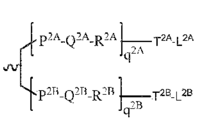
(Formula XLIV),

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

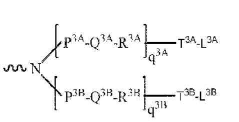
[0391] In certain options of the compositions and methods of the disclosure, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

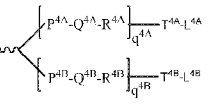
[0392] In one option, a dsRNA of the disclosure is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) - (XL VI):

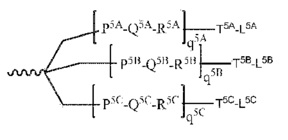
Formula XXXXV



Formula XLVI







Formula XLVII Formula XLVIII

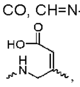
wherein:

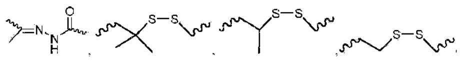
q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

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 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 wherein 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 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-O,

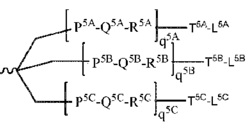
,



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, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^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 (XLIX):

Formula XLIX



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

[0393] 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.

[0394] Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent 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;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022.

[0395] 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 disclosure also includes iRNA compounds that are chimeric compounds.

[0396] "Chimeric" iRNA compounds or "chimeras," in the context of this disclosure, are iRNA compounds, preferably dsRNAi agents, that 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, 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.

[0397] 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 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 Disclosure

[0398] The delivery of an iRNA of the disclosure 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 susceptible to or diagnosed with an AGT associated disorder, *e.g.*, hypertension) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the disclosure 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.

[0399] 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 disclosure (see *e.g.*, Akhtar S. and Julian RL. (1992) Trends Cell. Biol. 2(5):139-144 and WO94/02595). 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. 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). Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA 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).

[0400] In an alternative option, 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 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) Journal of Controlled Release 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when 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). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN, *et al* (2003), *supra*), "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. 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 options, 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.

A. Vector encoded iRNAs of the Disclosure

[0401] iRNA targeting the AGT 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. WO 00/22114, and Conrad, U.S. Patent 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 extrachromosomal plasmid (Gassmann, et al., Proc. Natl. Acad. Sci. USA (1995) 92:1292).

[0402] 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, 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 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 target cells. Other aspects to consider for vectors and constructs are known in the art.

V. Pharmaceutical Compositions of the Disclosure

[0403] The present disclosure also includes pharmaceutical compositions and formulations which include the iRNAs of the disclosure. In one option, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for preventing or treating an AGT asociated disorder, *e.g.*, hypertension. 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. The pharmaceutical compositions of the disclosure may be administered in dosages sufficient to inhibit expression of an AGT gene.

[0404] The pharmaceutical compositions of the disclosure may be administered in dosages sufficient to inhibit expression of an AGT gene. In general, a suitable dose of an iRNA of the disclosure will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the disclosure will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once a year. In certain options, the iRNA is administered about once per month to about once per six months.

[0405] After an initial treatment regimen, the treatments can be administered on a less frequent basis. Duration of treatment can be determined based on the severity of disease.

[0406] In other options, a single dose of the pharmaceutical compositions can be long lasting, such that doses are administered at not more than 1, 2, 3, or 4 month intervals. In some options of the disclosure, a single dose of the pharmaceutical compositions of the disclosure is administered about once per month. In other options of the disclosure, a single dose of the pharmaceutical compositions of the disclosure is administered quarterly *(i.e.,* about every three months). In other options of the disclosure, a single dose of the pharmaceutical compositions of the disclosure is administered twice per year *(i.e.,* about once every six months).

[0407] 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 mutations present in the subject, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a prophylactically or therapeutically effective amount, as appropriate, of a composition can include a single treatment or a series of treatments.

[0408] The iRNA can be delivered in a manner to target a particular tissue (*e.g.,* hepatocytes).

[0409] Pharmaceutical compositions of the present disclosure 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. Formulations include those that target the liver.

[0410] The pharmaceutical formulations of the present disclosure, 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.

A. Additional Formulations

i. Emulsions

[0411] The compositions of the present disclosure 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 0.1 µm 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 either in 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.

[0412] 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. 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).

[0413] 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).

[0414] 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).

[0415] The application of emulsion formulations *via* dermatological, oral, and parenteral 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).

ii. Microemulsions

[0416] In one option of the present disclosure, 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.), 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., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

[0417] An iRNA of the disclosure 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

[0418] In one option, the present disclosure 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.

[0419] 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 and their use in manufacture of pharmaceutical compositions and delivery of pharmaceutical agents are well known in the art.

v. Excipients

[0420] 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 the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Such agent are well known in the art.

vi. Other Components

[0421] The compositions of the present disclosure 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 disclosure, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present disclosure. 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, or aromatic substances, and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0422] Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, or dextran. The suspension can also contain stabilizers.

[0423] In some options, pharmaceutical compositions featured in the disclosure include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating an AGT associated disorder, *e.g.*, hypertension.

[0424] Toxicity and prophylactic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose prophylactically 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.

[0425] The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the disclosure lies generally within a range of circulating concentrations that include the ED50, preferably an ED80 or ED90, 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 disclosure, the prophylactically effective dose can be estimated 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) or higher levels of inhibition 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.

[0426] In addition to their administration, as discussed above, the iRNAs featured in the disclosure can be administered in combination with other known agents used for the prevention or treatment of an AGT associated disorder, *e.g.*, hypertension. 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. Methods For Inhibiting AGT Expression

[0427] The present disclosure also provides methods of inhibiting expression of an AGT gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNA agent, in an amount effective to inhibit expression of AGT in the cell, thereby inhibiting expression of AGT in the cell.

[0428] Contacting of a cell with an iRNA, *e.g.*, a double stranded RNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished *via* a targeting ligand, including any ligand described herein or known in the art. In preferred options, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAcs ligand, or any other ligand that directs the RNAi agent to a site of interest.

[0429] The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

[0430] The phrase "inhibiting expression of an AGT" is intended to refer to inhibition of expression of any AGT gene (such as, *e.g.*, a mouse AGT gene, a rat AGT gene, a monkey AGT gene, or a human AGT gene) as well as variants or mutants of an AGTgene. Thus, the AGT gene may be a wild-type AGT gene, a mutant AGT gene, or a transgenic AGT gene in the context of a genetically manipulated cell, group of cells, or organism.

[0431] "Inhibiting expression of an AGT gene" includes any level of inhibition of an AGT gene, *e.g.*, at least partial suppression of the expression of an AGT gene. The expression of the AGT gene may be assessed based on the level, or the change in the level, of any variable associated with AGT gene expression, *e.g.*, AGT mRNA level or AGT protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject. It is understood that AGT is expressed predominantly in the liver, but also in the brain, gall bladder, heart, and kidney, and is present in circulation.

[0432] Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with AGT 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 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).

[0433] In some options of the methods of the disclosure, expression of an AGT gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In preferred options, expression of an AGT gene is inhibited by at least 70%.. It is further understood that inhibition of AGT expression in certain tissues, *e.g.*, in liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In preferred options, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

[0434] In certain options, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the human target gene (*i.e.*, AGT), *e.g.*, when administered a single dose at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

[0435] Inhibition of the expression of an AGT 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 an AGT gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an iRNA of the disclosure, or by administering an iRNA of the disclosure to a subject in which the cells are or were present) such that the expression of an AGT 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) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In preferred options, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

[0436] In other options, inhibition of the expression of an AGT gene may be assessed in terms of a reduction of a parameter that is functionally linked to AGT gene expression, *e.g.*, AGT protein level in blood or serum from a subject. AGT gene silencing may be determined in any cell expressing AGT, either endogenous or heterologous from an expression construct, and by any assay known in the art.

[0437] Inhibition of the expression of an AGT protein may be manifested by a reduction in the level of the AGT protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition 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, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

[0438] A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of an AGT gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the disclosure. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

[0439] The level of AGT 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 option, the level of expression of AGT in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the AGT 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, northern blotting, *in situ* hybridization, and microarray analysis.

[0440] In some options, the level of expression of AGT is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific AGT. 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.

[0441] Isolated mRNA can be used in hybridization or amplification assays that include, but 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 AGT mRNA. In one option, 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 from the gel to a membrane, such as nitrocellulose. In an alternative option, 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 AGT mRNA.

[0442] An alternative method for determining the level of expression of AGT in a sample involves the process of nucleic acid amplification 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. Patent 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. (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. Patent 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 in very low numbers. In particular aspects of the disclosure, the level of expression of AGT is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In preferred options, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

[0443] The expression levels of AGT 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 nucleic acids). See U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934. The determination of AGT expression level may also comprise using nucleic acid probes in solution.

[0444] In preferred options, 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. In preferred options, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

[0445] The level of AGT 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.

[0446] In some options, the efficacy of the methods of the disclosure are assessed by a decrease in AGT mRNA or protein level (*e.g.,* in a liver biopsy).

[0447] In some options of the methods of the disclosure, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of AGT may be assessed using measurements of the level or change in the level of AGT mRNA or agt protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.,* liver or blood).

[0448] As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

VII. Prohylactic and Treatment Methods of the Disclosure

[0449] The present disclosure also provides methods of using an iRNA of the disclosure or a composition containing an iRNA of the disclosure to inhibit expression of AGT, thereby preventing or treating a an AGT associated disorder, *e.g.*, high blood pressure, *e.g.*, hypertension.

[0450] In the methods of the disclosure the cell may be contacted with the siRNA *in vitro* or *in vivo, i.e.,* the cell may be within a subject.

[0451] A cell suitable for treatment using the methods of the disclosure may be any cell that expresses an AGT gene, *e.g.,* a liver cell, a brain cell, a gall bladder cell, a heart cell, or a kidney cell, but preferably a liver cell. A cell suitable for use in the methods of the disclosure may be a mammalian cell, *e.g.,* a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.,* a monkey cell or a chimpanzee cell), or a non-primate cell. In certain options, the cell is a human cell, *e.g.,* a human liver cell. In the methods of the disclosure, AGT expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

[0452] The *in vivo* methods of the disclosure may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the AGT gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain options, the compositions are administered by intravenous infusion or injection. In certain options, the compositions are administered by subcutaneous injection. In certain options, the compositions are administered by intramuscular injection.

[0453] In one aspect, the present disclosure also provides methods for inhibiting the expression of an AGTgene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an AGT gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the AGT gene, thereby inhibiting expression of the AGT gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.,* in Example 2. Reduction in protein production can be assessed by any methods known it the art, *e.g.* ELISA. In certain options, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the AGT gene or protein expression. In other options, a blood sample serves as the subject sample for monitoring the reduction in the agt protein expression.

[0454] The present disclosure further provides methods of treatment in a subject in need thereof, *e.g.,* a subject diagnosed with a hypertension.

[0455] The present disclosure further provides methods of prophylaxis in a subject in need thereof. The treatment methods of the disclosure include administering an iRNA of the disclosure to a subject, *e.g.,* a subject that would benefit from a reduction of AGT expression, in a prophylactically effective amount of an iRNA targeting an AGT gene or a pharmaceutical composition comprising an iRNA targeting an AGT gene.

[0456] An iRNA of the disclosure may be administered as a "free iRNA." A free 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 option, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

[0457] Alternatively, an iRNA of the disclosure may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

[0458] Subjects that would benefit from an inhibition of AGT gene expression are subjects susceptible to or diagnosed with hypertension.

[0459] In an option, the method includes administering a composition featured herein such that expression of the target AGT gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain options, the composition is administered once every 3-6 months.

[0460] Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target AGT gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

[0461] Administration of the iRNA according to the methods of the disclosure may result prevention or treatment of an AGT associated disorder disorder, *e.g.,* high blood pressure, *e.g.,* hypertension. Diagnostic criteria for various types of high blood pressure are provided below.

[0462] Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

[0463] The iRNA is preferably administered subcutaneously, *i.e.,* by subcutaneous injection. One or more injections may be used to deliver the desired dose of iRNA to a subject. The injections may be repeated over a period of time.

[0464] The administration may be repeated on a regular basis. In certain options, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as once per month to once a year. In certain options, the iRNA is administered about once per month to about once every three months, or about once every three months to about once every six months.

VIII. Diagnostic Criteria, Risk Factors, and Treatments for Hypertension

[0465] Recently practice guidelines for prevention and treatment of hypertension were revised. Extensive reports were published by Reboussin et al. (Systematic Review for the 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2017 Nov 7. pii: S0735-1097(17)41517-8. doi: 10.1016/j.jacc.2017.11.004.) and Whelton et al. (2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2017 Nov 7. pii: S0735-1097(17)41519-1. doi: 10.1016/j.jacc.2017.11.006.). Some highlights of the new Guidelines are provided below. However, the Guidelines should be understood as providing the knowledge of those of skill in the art regarding diagnostic and monitoring criteria and treatment for hypertension at the time of filing of this application.

A. Diagnostic Criteria

[0466] Although a continuous association exists between higher blood pressure and increased cardiovascular disease risk, it is useful to categorize blood pressure levels for clinical and public health decision making. Blood pressure can be categorized into 4 levels on the basis of average blood pressure measured in a healthcare setting (office pressures): normal, elevated, and stage 1 or 2 hypertension as shown in the table below (from Whelton *et al.*, 2017).

Blood Pressure Category	Systolic Blood Pressure		Diastolic Blood Pressure
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120-129 mm Hg	and	<80 mm Hg
Hypertension*			
Stage 1	130-139 mm Hg	or	80-89 mm Hg
Stage 2	≥140 mm Hg	or	≥ 90 mm Hg
*Individuals with systolic blood pressure and diastolic blood pressure in 2 categories should be designated to the higher blood pressure category.			

[0467] Blood pressure indicates blood pressure based on an average of ≥2 careful readings obtained on ≥2 occasions. Best practices for obtaining careful blood pressure readings are detailed in Whelton *et al.*, 2017 and are known in the art.

[0468] This categorization differs from that previously recommended in the JNC 7 report (Chobanian et al; the National High Blood Pressure Education Program Coordinating Committee. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42:1206-52) with stage 1 hypertension now defined as a systolic blood pressure (SBP) of 130-139 or a diastolic blood pressure (DBP) of 80-89 mm Hg, and with stage 2 hypertension in the present document corresponding to stages 1 and 2 in the JNC 7 report. The rationale for this categorization is based on observational data related to the association between SBP/DBP and cardiovascular disease risk, randomized clinical trials of lifestyle modification to lower blood pressure, and randomized clinical trials of treatment with antihypertensive medication to prevent cardiovascular disease.

[0469] The increased risk of cardiovascular disease among adults with stage 2 hypertension is well established. An increasing number of individual studies and meta-analyses of observational data have reported a gradient of progressively higher cardiovascular disease risk going from normal blood pressure to elevated blood pressure and stage 1 hypertension. In many of these meta-analyses, the hazard ratios for coronary heart disease and stroke were between 1.1 and 1.5 for the comparison of SBP/DBP of 120-129/80-84 mm Hg versus <120/80 mm Hg and between 1.5 and 2.0 for the comparison of SBP/DBP of 130-139/85-89 mm Hg versus <120/80 mm Hg. This risk gradient was consistent across subgroups defined by sex and race/ethnicity. The relative increase in cardiovascular disease risk associated with higher blood pressure was attenuated but still present among older adults. Lifestyle modification and pharmacological antihypertensive treatment are recommended for individuals with elevated blood pressure and stages 1 and 2 hypertension. Clinical benefit can be obtained by a reduction of the stage of elevated blood pressure, even if blood pressure is not normalized by a treatment.

B. Risk Factors

[0470] Hypertension is a complex disease that results from a combination of factors including, but not limited to, genetics, lifestyle, diet, and secondary risk factors. Hypertension can also be associated with pregnancy. It is understood that due to the complex nature of hypertension, it is understood that multiple interventions may be required for treatment of hypertension. Moreover, nonpharmacological interventions, including modification of diet and lifestyle, can be useful for the prevention and treatment of hypertension. Further, an intervention may provide a clinical benefit without fully normalizing blood pressure in an individual.

1. Genetic risk factors

[0471] Several monogenic forms of hypertension have been identified, such as glucocorticoid-remediable aldosteronism, Liddle's syndrome, Gordon's syndrome, and others in which single-gene mutations fully explain the pathophysiology of hypertension, these disorders are rare. The current tabulation of known genetic variants contributing to blood pressure and hypertension includes more than 25 rare mutations and 120 single nucleotide polymorphisms. However, although genetic factors may contribute to hypertension in some individuals, it is estimated that genetic variation accounts for only about 3.5% of blood pressure variability.

2. Diet and alcohol consumption

[0472] Common environmental and lifestyle risk factors leading to hypertension include poor diet, insufficient physical activity, and excess alcohol consumption. These factors can lead to a person to become overweight or obese, further increasing the likelihood of developing or exacerbating hypertension. Elevated blood pressure is even more strongly correlated with increased waist-to-hip ratio or other measures of central fat distribution. Obesity at a young age and ongoing obesity is strongly correlated with hypertension later in life. Achieving a normal weight can reduce the risk of developing high blood pressure to that of a person who has never been obese.

[0473] Intake of sodium, potassium, magnesium, and calcium can also have a significant effect on blood pressure. Sodium intake is positively correlated with blood pressure and accounts for much of the age-related increase in blood pressure. Certain groups are more sensitive to increased sodium consumption than others including black and older adults (≥ 65 years old), and those with a higher level of blood pressure or comorbidities such as chronic kidney disease, diabetes mellitus, or metabolic syndrome. In aggregate, these groups constitute more than half of all US adults. Salt sensitivity may be a marker for increased cardiovascular disease and all-cause mortality, independent of blood pressure. Currently, techniques for recognition of salt sensitivity are impractical in a clinical setting. Therefore, salt sensitivity is best considered as a group characteristic.

[0474] Potassium intake is inversely related to blood pressure and stroke, and a higher level of potassium seems to blunt the effect of sodium on blood pressure. A lower sodium-potassium ratio is associated with a lower blood pressure than that noted for corresponding levels of sodium or potassium on their own. A similar observation has been made for risk of cardiovascular disease.

[0475] Alcohol consumption has long been associated with high blood pressure. In the US, it has been estimated that alcohol consumption accounts for about 10% of the population burden of hypertension, with the burden being greater in men than women.

[0476] It is understood that changes in diet or alcohol consumption can be an aspect of prevention or treatment of hypertension.

3. Physical activity

[0477] There is a well-established inverse correlation between physical activity/ physical fitness and blood pressure levels. Even modest levels of physical activity have been demonstrated to be beneficial in decreasing hypertension.

[0478] It is understood that an increase in physical activity can be an aspect of prevention or treatment of hypertension.

4. Secondary risk factors

[0479] Secondary hypertension can underlie severe elevation of blood pressure, pharmacologically resistant hypertension, sudden onset of hypertension, increased blood pressure in patients with hypertension previously controlled on drug therapy, onset of diastolic hypertension in older adults, and target organ damage disproportionate to the duration or severity of the hypertension. Although secondary hypertension should be suspected in younger patients (<30 years of age) with elevated blood pressure, it is not uncommon for primary hypertension to manifest at a younger age, especially in blacks, and some forms of secondary hypertension, such as renovascular disease, are more common at older age (≥ 65 years of age). Many of the causes of secondary hypertension are strongly associated with clinical findings or groups of findings that suggest a specific disorder. In such cases, treatment of the underlying condition may resolve the findings of elevated blood pressure without administering agents typically used for the treatment of hypertension.

5. Pregnancy

[0480] Pregnancy is a risk factor for high blood pressure, and high blood pressure during pregnancy is a risk factor for cardiovascular disease and hypertension later in life. A Report on pregnancy associated hypertension was published in 2013 by the American College of Obstetrics and Gynecology (ACOG) (American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122:1122-31). Some highlights of the Report are provided below. However, the Report should be understood as providing the knowledge of those of skill in the art regarding diagnostic and monitoring criteria and treatment for hypertension in pregnancy at the time of filing of this application.'

[0481] The diagnostic criteria for preeclampsia are provided in the table below (from Table 1 of the ACOG report, 2013).

Blood Pressure	- ≥140 mm Hg diastolic or ≥ 90 mm Hg diastolic on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure
	- ≥ 160 mm Hg systolic or ≥ 110 mm Hg diastolic, hypertension can be confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy
and	
Proteinurea	- ≥ 300 mg per 24-hour urine collection (or this amount extrapolated for a timed collection)
	Or
	- Protein/ creatinine ratio ≥ 0.3 (each measured as mg/dL)
Or in the absence of proteinurea, new onset of hypertension with the new onset of an of the following:	
Thrombocytopenia	- Platelet count ≤ 100,000/microliter
Renal insufficiency	- Serum creatinine concentration ≥ 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease
Impaired liver function	- Elevated blood concentrations if liver transaminases to twice normal concentration
Pulmonary edema	
Cerebral or visual symptoms	

[0482] Blood Pressure management during pregnancy is complicated by the fact that many commonly used antihypertensive agents, including ACE inhibitors and ARBs, are contraindicated during pregnancy because of potential harm to the fetus. The goal of antihypertensive treatment during pregnancy includes prevention of severe hypertension and the possibility of prolonging gestation to allow the fetus more time to mature before delivery. A review of treatment for pregnancy-associated severe hypertension found insufficient evidence to recommend specific agents; rather, clinician experience was recommended in this setting (Duley L, Meher S, Jones L. Drugs for treatment of very high blood pressure during pregnancy. Cochrane Database Syst Rev. 2013;7:CD001449.).

C. Treatments

[0483] Treatment of high blood pressure is complex as it is frequently present with other comorbidities, often including reduced renal function, for which the subject may also be undergoing treatment. Clinicians managing adults with high blood pressure should focus on overall patient health, with a particular emphasis on reducing the risk of future adverse cardiovascular disease outcomes. All patient risk factors need to be managed in an integrated fashion with a comprehensive set of nonpharmacological and pharmacological strategies. As patient blood pressure and risk of future cardiovascular disease events increase, blood pressure management should be intensified.

[0484] Whereas treatment of high blood pressure with blood pressure-lowering medications on the basis of blood pressure level alone is considered cost effective, use of a combination of absolute cardiovascular disease risk and blood pressure level to guide such treatment is more efficient and cost effective at reducing risk of cardiovascular disease than is use of blood pressure level alone. Many patients started on a single agent will subsequently require ≥2 drugs from different pharmacological classes to reach their blood pressure goals. Knowledge of the pharmacological mechanisms of action of each agent is important. Drug regimens with complementary activity, where a second antihypertensive agent is used to block compensatory responses to the initial agent or affect a different pressor mechanism, can result in additive lowering of blood pressure. For example, thiazide diuretics may stimulate the renin-angiotensin-aldosterone system. By adding an ACE inhibitor or ARB to the thiazide, an additive blood pressure lowering effect may be obtained. Use of combination therapy may also improve adherence. Several 2- and 3-fixed-dose drug combinations of antihypertensive drug therapy are available, with complementary mechanisms of action among the components.
Table 18 from Whelton *et al.* 2017 listing oral antihypertensive drugs is provided below. Classes of therapeutic agents for the treatment of high blood pressure and drugs

that fall within those classes are provided. Dose ranges, frequencies, and comments are also provided.

Class	Drug	Usual Dose, (mg/d)*	Daily Frequency	Comments
Primary agents				
Thiazide or thiazide-type diuretics	Chlorthalidone	12.5-25	1	• Chlorthalidone is preferred on the basis of prolonged half-life and proven trial reduction of CVD.
	Hydrochlorothiazid e	25-50	1	
	Indapamide	1.25-2.5	1	• Monitor for hyponatremia and hypokalemia, uric acid and calcium levels.
	Metolazone	2.5-10	1	• Use with caution in patients with history of acute gout unless patient is on uric acid-lowering therapy.
ACE inhibitors	Benazepril	10-40	1 or 2	• Do not use in combination with ARBs or direct renin inhibitor
	Captopril	12.5-150	2 or 3	
	Enalapril	5-40	1 or 2	• There is an increased risk of hyperkalemia, especially in patents with CKD or in those on K^+ supplements or K^+ -sparing drugs.
	Fosinopril	10-40	1	
	Lisinopril	10-40	1	
	Moexipril	7.5-30	1 or 2	• There is a risk of acute renal failure in patients with severe bilateral renal artery stenosis.
	Perindopril	4-16	1	
	Quinapril	10-80	1 or 2	
	Ramipril	2.5-10	1 or 2	• Do no use if patient has history of angioedema with ACE inhibitors.
	Trandolapril	1-4	1	• Avoid in pregnancy.
ARBs	Azilsartan	40-80	1	• Do not use in combination with ACE inhibitors or direct renin inhibitors.
	Candesartan	8-32	1	
	Eprosartan	600-800	1 or 2	
	Irbesartan	150-300	1	• There is an increased risk of hyperkalemia in CKD or in those on K^+ supplements or K^+-sparing drugs.
	Losartan	50-100	1 or 2	
	Olmesartan	20-40	1	• There is a risk of acute renal failure in patients with severe bilateral renal artery stenosis.
	Telmisartan	20-80	1	
	Valsartan	80-320	1	• Do not use if patient has history of angioedema with ARBs. Patients with a history of angioedema with an ACE inhibitor can receive an ARB beginning 6 weeks after ACE inhibitor is discontinued.
				• Avoid in pregnancy.
CCB-dihydropyridines	Amlodipine	2.5-10	1	• Avoid use in patients with HF*r*EF; amlodipine or felodipine may be used if required
	Felodipine	5-10	1	
	Isradipine	5-10	2	
	Nicardipine SR	5-20	1	• They are associated with dose-related pedal edema, which is more common in women than men.
	Nifedipine LA	60-120	1	
	Nisoldipine	30-90	1	
CCB-nondihydropyridines	Diltiazem SR	180-360	2	• Avoid routine use with beta blockers because of increased risk of bradycardia and heart block.
	Diltiazem ER	120-480	1	
	Verapamil IR	40-80	3	
	Verapamil SR	120-480	1 or 2	• Do not use in patients with HF*r*EF.
	Verapamil-delayed onset ER (various forms)	100-480	1 (in the evening)	• There are drug interactions with diltiazem and verapamil (*CYP3A4* major substrate and moderate inhibitor).
Secondary agents				
Diuretics-loop	Bumetanide	0.5-4	2	• There are preferred diuretics in patients with symptomatic HF. They are preferred over thiazides in patients with moderate-to-severe CKD (e.g., GFR <30 mL/min).
	Furosemide	20-80	2	
	Torsemide	5-10	1	
Diuretics-potassium sparing	Amiloride	5-10	1 or 2	• These are monotherapy agents and minimally effective antihypertensive agents.
	Triamterene	50-100	1 or 2	• Combination therapy of potassium-sparing diuretic with a thiazide can be considered in patients with hypokalemia on thiazide monotherapy. • Avoid in patients with
				significate CKD (e.g. GFR <45 mL/min).
Diuretics-aldosterone antagonists	Eplerenone	50-100	12	• These are preferred agents in primary aldosteronism and resistant hypertension.
	Spironolactone	25-100	1	• Spironolactone is associated with greater risk of gynecomastia and impotence as compared with eplerenone.
				• This is common add-on therapy in resistant hypertension.
				• Avoid use with K^+ supplements, other K^+-sparing diuretics, or significant renal dysfunction.
				• Eplerenone often requires twice-daily dosing for adequate BP lowering.
Beta blockers-cardioselective	Atenolol	25-100	12	• Beta blockers are not recommended as first-line

Secondary agents				
	Betaxolol	5-20	1	agents unless the patient has IHD or HF.
	Bisoprolol	2.5-10	1	
	Metoprolol tartrate	100-400	2	• These are preferred patients with bronchospastic airway disease requiring a beta blocker.
	Metoprolol succinate	50-200	1	• Bisoprolol and metoprolol succinate are preferred in patients with HFrEF. • Avoid abrupt cessation.
Beta blockers-cardioselective and vasodilatory	Nebivolol	5-40	1	• Nebivolol induces nitric oxide-inducesd vasodilation. • Avoid abrupt cessation.
Beta blockers-noncardioselective	Nadolol	40-120	1	• Avoid in patients with reactive airways disease.
	Propranolol IR	160-480	2	• Avoid abrupt cessation.
	Propranolol LA	80-320	1	
Beta blockers-intrinsic sympathomimetic activity	Acebutolol	200-800	2	• Generally avoid, especially in patients with IHD or HF.
	Carteolol	2.5-10	1	
	Penbutolol	10-40	1	• Avoid abrupt
	Pindolol	10-60	2	cessation.
Beta blockers-combined alpha-and beta receptor	Carvedilol	12.5-50	2	• Carvedilol is preferred in patients with HFrEF.
	Carvedilol phosphate	20-80	1	• Avoid abrupt cessation.
	Labetalol	200-800	2	
Direct renin inhibitor	Aliskiren	150-300	1	• Do not use in combination with ACE inhibitors or ARBs. • Aliskiren is very long acting. • There is an increased risk of hyperkalemina in CKD or in those on K^+ supplements or K^+-sparing drugs. • Aliskiren may cause acute renal failure in patients with severe bilateral renal artery stenosis. • Avoid in pregnancy.
Alpha-1-blockers	Doxazosin	1-8	1	• These are associated with orthostatic hypotension, especially in older adults.
	Prazosin	2-20	2 or 3	
	Terazosin	1-20	1 or 2	• They may be considered as second-line agent in patients with concomitant BPH.
Central alpha$_1$-agonist and other centrally acting drugs	Clonidine oral	0.1-0.8	2	• These are generally reserved as last-line because of significant CNS adverse effects, especially in older adults.
	Clonidine patch	0.1-0.3	1 weekly	
	Methyldopa	250-1000	2	
	Guanfacine	0.5-2	1	• Avoid abrupt discontinuation of clonidine, which may induce hypertensive crisis; clonidine must be tapered to avoid rebound hypertension.
Direct vasodilators	Hydralazine	250-200	2 or 3	• These are associated with sodium and water retention and reflex tachycardia; use with a diuretic and beta blocker.
	Minoxidil	5-100	1-3	• Hydralazine is associated with drug-induced lupus-like syndrome at higher doses. • Minoxidil is associated with hirsutism and required a loop diurestic. Minoxidil can induce pericardial effusion.

*Dosages may vary from those listed in the FDA approved labeling (available at https://dailymed.nlm.nih.gov/dailymed/). ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BP, blood pressure; BPH, benign prostatic hyperplasia; CCB, calcium channel blocker; CKD, chronic kidney disease; CNS, central nervous system; CVD, cardiovascular disease; ER, extended release; GFR, glomerular filtration rate; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; IHD, ischemic heart disease; IR, immediate release; LA, long-acting; and SR, sustained release.
From, Chobanian et al. (2003) The JNC 7 Report. JAMA 289(19):2560.

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

[0485] Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA Design

[0486] A set of siRNAs targeting the human AGT gene (human: NCBI refseqID NM_000029.3; NCBI GeneID: 183) was designed using custom R and Python scripts. The human NM_000029 REFSEQ mRNA, version 3, has a length of 2587 bases.

[0487] A detailed list of the unmodified AGT sense and antisense strand nucleotide sequences is shown in Table 3. A detailed list of the modified AGT sense and antisense strand nucleotide sequences is shown in Table 5.

siRNA Synthesis

[0488] siRNAs were synthesized and annealed using routine methods known in the art.

Example 2. *In vitro* screening methods

Cell culture and 384-well transfections

[0489] Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization.

[0490] Transfection was performed by adding 4.9µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 20 minutes. Firty µl of complete growth media containing 5,000 Hep3b cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM final duplex concentration, and dose response experiments were performed using an eight-point six-fold serial dilution over the range of 10nM to 37.5fM.

[0491] Additional dsRNA agents targeting an AGT mRNA are described in PCT Publication No. WO 2015/179724.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

[0492] Cells were lysed in 75µl of Lysis/Binding Buffer containing 3uL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

[0493] A master mix of 1ul 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR:

[0494] Two µl of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human AGT (Hs00174854m1), 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

[0495] To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO:19) and antisense UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 20). Results from the screening are shown in Table 4.

Table 2. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
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

Abbreviation	Nucleotide(s)
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, modified or unmodified
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
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
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3)
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Ggn)	Guanosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphate
(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
(Tam)	2'-O-(N-methylacetamide)thymidine-3'-phosphate
(Tams)	2'-O-(N-methylacetamide)thymidine-3'-phosphorothioate
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
dUs	2'-deoxyuridine-3'-phosphorothioate
(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-methylcyridine-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-methyl-xylofuranosyluridine-2'-phosphate
(m5Cam)	2'-O-(N-methylacetamide)-5-methylcytidine-3'-phosphate

Abbreviation	Nucleotide(s)
(m5Cams)	2'-O-(N-methylacetamide)-5-methylcytidine-3'-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-3'-phosphorothioate
(pshe)	Hydroxyethylphosphorothioate

Table 3. Unmodified Sense and Antisense Strand Sequences of AGT dsRNA Agents

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
AD-84704	A-168477	CACAAUGAGAGUACCUGUGAA	21	644-664	A-168478	UUCACAGGUACUCUCAUUGUGGA	205	642-664
AD-84705	A-168479	GUCUCCCACCUUUUCUUCUAA	22	2076-2096	A-168480	UUAGAAGAAAAGGUGGGAGACUG	206	2074-2096
AD-84706	A-168481	ACUUUCCAGCAAAACUCCCUA	23	1586-1606	A-168482	UAGGGAGUUUUGCUGGAAAGUGA	207	1584-1606
AD-84707	A-168483	CCUCAACUGGAUGAAGAAACU	24	1603-1623	A-168484	AGUUUCUUCAUCCAGUUGAGGGA	208	1601-1623
AD-84708	A-168485	CUGUUUGCUGUGUAUGAUCAA	25	1889-1909	A-168486	UUGAUCAUACACAGCAAACAGGA	209	1887-1909
AD-84709	A-168487	UUUGCUGUGUAUGAUCAAAGA	26	1892-1912	A-168488	UCUUUGAUCAUACACAGCAAACA	210	1890-1912
AD-84710	A-168489	CCGACCAGCUUGUUUGUGAAA	27	2283-2303	A-168490	UUUCACAAACAAGCUGGUCGGUU	211	2281-2303
AD-84711	A-168491	UCCAACCGACCAGCUUGUUUA	28	2278-2298	A-168492	UAAACAAGCUGGUCGGUUGGAAU	212	2276-2298
AD-84712	A-168493	CCAUUCCUGUUUGCUGUGUAU	29	1883-1903	A-168494	AUACACAGCAAACAGGAAUGGGC	213	1881-1903
AD-84713	A-168495	CACCUUUUCUUCUAAUGAGUA	30	2082-2102	A-168496	UACUCAUUAGAAGAAAAGGUGGG	214	2080-2102
AD-84714	A-168497	GUUUGCUGUGUAUGAUCAAAA	31	1891-1911	A-168498	UUUUGAUCAUACACAGCAAACAG	215	1889-1911
AD-84715	A-168499	GCUGAGAAGAUUGACAGGUUA	32	1250-1270	A-168500	UAACCUGUCAAUCUUCUCAGCAG	216	1248-1270
AD-84716	A-168501	UUCCAGCAAAACUCCCUCAAA	33	1589-1609	A-168502	UUUGAGGGAGUUUUGCUGGAAAG	217	1587-1609
AD-84717	A-168503	UGCUGAGAAGAUUGACAGGUU	34	1249-1269	A-168504	AACCUGUCAAUCUUCUCAGCAGC	218	1247-1269
AD-84718	A-168505	UCUCACUUUCCAGCAAAACUA	35	1582-1602	A-168506	UAGUUUUGCUGGAAAGUGAGACC	219	1580-1602
AD-84719	A-168507	UCCACAAUGAGAGUACCUGUA	36	642-662	A-168508	UACAGGUACUCUCAUUGUGGAUG	220	640-662
AD-84720	A-168509	CCACCUCGUCAUCCACAAUGA	37	631-651	A-168510	UCAUUGUGGAUGACGAGGUGGAA	221	629-651
AD-84721	A-168511	UCACUUUCCAGCAAAACUCCA	38	1584-1604	A-168512	UGGAGUUUUGCUGGAAAGUGAGA	222	1582-1604
AD-84722	A-168513	UCCCUCAACUGGAUGAAGAAA	39	1601-1621	A-168514	UUUCUUCAUCCAGUUGAGGGAGU	223	1599-1621
AD-84723	A-168515	GAGAGUACCUGUGAGCAGCUA	40	650-670	A-168516	UAGCUGCUCACAGGUACUCUCAU	224	648-670
AD-84724	A-168517	AGAAUUCCAACCGACCAGCUU	41	2273-2293	A-168518	AAGCUGGUCGGUUGGAAUUCUUU	225	2271-2293
AD-84725	A-168519	CAUUCCUGUUUGCUGUGUAUA	42	1884-1904	A-168520	UAUACACAGCAAACAGGAAUGGG	226	1882-1904
AD-84726	A-168521	GAAUUCCAACCGACCAGCUUA	43	2274-2294	A-168522	UAAGCUGGUCGGUUGGAAUUCUU	227	2272-2294
AD-84727	A-168523	CAUCCACAAUGAGAGUACCUA	44	640-660	A-168524	UAGGUACUCUCAUUGUGGAUGAC	228	638-660
AD-84728	A-168525	CCCAUUCCUGUUUGCUGUGUA	45	1882-1902	A-168526	UACACAGCAAACAGGAAUGGGCG	229	1880-1902
AD-84729	A-168527	CUGGGUUUAUUUUAGAGAAUA	46	2202-2222	A-168528	UAUUCUCUAAAAUAAACCCAGCA	230	2200-2222
AD-84730	A-168529	GCUGGGUUUAUUUUAGAGAAU	47	2201-2221	A-168530	AUUCUCUAAAAUAAACCCAGCAA	231	2199-2221
AD-84731	A-168531	AUGGCAUGCACAGUGAGCUAU	48	861-881	A-168532	AUAGCUCACUGUGCAUGCCAUAU	232	859-881
AD-84732	A-168533	GAGAGAGCCCACAGAGUCUAA	49	1816-1836	A-168534	UUAGACUCUGUGGGCUCUCUCUC	233	1814-1836
AD-84733	A-168535	GCAAGAACCAGUGUUUAGCGA	50	2234-2254	A-168536	UCGCUAAACACUGGUUCUUGCCU	234	2232-2254
AD-84734	A-168537	CCAGCAAAACUCCCUCAACUA	51	1591-1611	A-168538	UAGUUGAGGGAGUUUUGCUGGAA	235	1589-1611
AD-	A-	CACCUCGUCAUCCACAAUGAA	52	632-652	A-168540	UUCAUUGUGGAUGACGAGGUGGA	236	630-652

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
84735	168539							
AD-84736	A-168541	CGUCAUCCACAAUGAGAGUAA	53	637-657	A-168542	UUACUCUCAUUGUGGAUGACGAG	237	635-657
AD-84737	A-168543	CUCCCACGCUCUCUGGACUUA	54	1211-1231	A-168544	UAAGUCCAGAGAGCGUGGGAGGA	238	1209-1231
AD-84738	A-168545	AACUCCCUCAACUGGAUGAAA	55	1598-1618	A-168546	UUUCAUCCAGUUGAGGGAGUUUU	239	1596-1618
AD-84739	A-168547	UGAGAAGAUUGACAGGUUCAU	56	1252-1272	A-168548	AUGAACCUGUCAAUCUUCUCAGC	240	1250-1272
AD-84740	A-168549	CCUCGUCAUCCACAAUGAGAA	57	634-654	A-168550	UUCUCAUUGUGGAUGACGAGGUG	241	632-654
AD-84741	A-168551	GAGAAGAUUGACAGGUUCAUA	58	1253-1273	A-168552	UAUGAACCUGUCAAUCUUCUCAG	242	1251-1273
AD-84742	A-168553	CUUCUUGGGCUUCCGUAUAUA	59	841-861	A-168554	UAUAUACGGAAGCCCAAGAAGUU	243	839-861
AD-84743	A-168555	UCCACCUCGUCAUCCACAAUA	60	630-650	A-168556	UAUUGUGGAUGACGAGGUGGAAG	244	628-650
AD-84744	A-168557	AGAUUGACAGGUUCAUGCAGA	61	1257-1277	A-168558	UCUGCAUGAACCUGUCAAUCUUC	245	1255-1277
AD-84745	A-168559	CUCCCUCAACUGGAUGAAGAA	62	1600-1620	A-168560	UUCUUCAUCCAGUUGAGGGAGUU	246	1598-1620
AD-84746	A-168561	AAUGAGAGUACCUGUGAGCAA	63	647-667	A-168562	UUGCUCACAGGUACUCUCAUUGU	247	645-667
AD-85431	A-168469	CCACCUUUUCUUCUAAUGAGU	64	2081-2101	A-170464	ACUCAUUAGAAGAAAAGGUGGGA	248	2079-2101
AD-85432	A-168471	CGACCAGCUUGUUUGUGAAAA	65	2284-2304	A-170465	UUUUCACAAACAAGCUGGUCGGU	249	2282-2304
AD-85433	A-168473	ACCUUUUCUUCUAAUGAGUCA	66	2083-2103	A-170466	UGACUCAUUAGAAGAAAAGGUGG	250	2081-2103
AD-85434	A-168475	GUCAUCCACAAUGAGAGUACA	67	638-658	A-170467	UGUACUCUCAUUGUGGAUGACGA	251	636-658
AD-85435	A-168477	CACAAUGAGAGUACCUGUGAA	68	644-664	A-170468	UUCACAGGUACUCUCAUUGUGGA	252	642-664
AD-85436	A-168479	GUCUCCCACCUUUUCUUCUAA	69	2076-2096	A-170469	UUAGAAGAAAAGGUGGGAGACUG	253	2074-2096
AD-85437	A-168481	ACUUUCCAGCAAAACUCCCUA	70	1586-1606	A-170470	UAGGGAGUUUUGCUGGAAAGUGA	254	1584-1606
AD-85438	A-168483	CCUCAACUGGAUGAAGAAACU	71	1603-1623	A-170471	AGUUUCUUCAUCCAGUUGAGGGA	255	1601-1623
AD-85439	A-168485	CUGUUUGCUGUGUAUGAUCAA	72	1889-1909	A-170472	UUGAUCAUACACAGCAAACAGGA	256	1887-1909
AD-85440	A-168487	UUUGCUGUGUAUGAUCAAAGA	73	1892-1912	A-170473	UCUUUGAUCAUACACAGCAAACA	257	1890-1912
AD-85441	A-168489	CCGACCAGCUUGUUUGUGAAA	74	2283-2303	A-170474	UUUCACAAACAAGCUGGUCGGUU	258	2281-2303
AD-85442	A-168491	UCCAACCGACCAGCUUGUUUA	75	2278-2298	A-170475	UAAACAAGCUGGUCGGUUGGAAU	259	2276-2298
AD-85443	A-168493	CCAUUCCUGUUUGCUGUGUAU	76	1883-1903	A-170476	AUACACAGCAAACAGGAAUGGGC	260	1881-1903
AD-85444	A-168495	CACCUUUUCUUCUAAUGAGUA	77	2082-2102	A-170477	UACUCAUUAGAAGAAAAGGUGGG	261	2080-2102
AD-85445	A-168497	GUUUGCUGUGUAUGAUCAAAA	78	1891-1911	A-170478	UUUUGAUCAUACACAGCAAACAG	262	1889-1911
AD-85446	A-168499	GCUGAGAAGAUUGACAGGUUA	79	1250-1270	A-170479	UAACCUGUCAAUCUUCUCAGCAG	263	1248-1270
AD-85447	A-168501	UUCCAGCAAAACUCCCUCAAA	80	1589-1609	A-170480	UUUGAGGGAGUUUUGCUGGAAAG	264	1587-1609
AD-85448	A-168503	UGCUGAGAAGAUUGACAGGUU	81	1249-1269	A-170481	AACCUGUCAAUCUUCUCAGCAGC	265	1247-1269
AD-85449	A-168505	UCUCACUUUCCAGCAAAACUA	82	1582-1602	A-170482	UAGUUUUGCUGGAAAGUGAGACC	266	1580-1602
AD-85450	A-168507	UCCACAAUGAGAGUACCUGUA	83	642-662	A-170483	UACAGGUACUCUCAUUGUGGAUG	267	640-662
AD-85451	A-168509	CCACCUCGUCAUCCACAAUGA	84	631-651	A-170484	UCAUUGUGGAUGACGAGGUGGAA	268	629-651
AD-85452	A-168511	UCACUUUCCAGCAAAACUCCA	85	1584-1604	A-170485	UGGAGUUUUGCUGGAAAGUGAGA	269	1582-1604
AD-85453	A-168513	UCCCUCAACUGGAUGAAGAAA	86	1601-1621	A-170486	UUUCUUCAUCCAGUUGAGGGAGU	270	1599-1621
AD-85454	A-168515	GAGAGUACCUGUGAGCAGCUA	87	650-670	A-170487	UAGCUGCUCACAGGUACUCUCAU	271	648-670
AD-	A-	AGAAUUCCAACCGACCAGCUU	88	2273-2293	A-170488	AAGCUGGUCGGUUGGAAUUCUUU	272	2271-2293

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
85455	168517							
AD-85456	A-168519	CAUUCCUGUUUGCUGUGUAUA	89	1884-1904	A-170489	UAUACACAGCAAACAGGAAUGGG	273	1882-1904
AD-85457	A-168521	GAAUUCCAACCGACCAGCUUA	90	2274-2294	A-170490	UAAGCUGGUCGGUUGGAAUUCUU	274	2272-2294
AD-85458	A-168523	CAUCCACAAUGAGAGUACCUA	91	640-660	A-170491	UAGGUACUCUCAUUGUGGAUGAC	275	638-660
AD-85459	A-168525	CCCAUUCCUGUUUGCUGUGUA	92	1882-1902	A-170492	UACACAGCAAACAGGAAUGGGCG	276	1880-1902
AD-85460	A-168527	CUGGGUUUAUUUUAGAGAAUA	93	2202-2222	A-170493	UAUUCUCUAAAAUAAACCCAGCA	277	2200-2222
AD-85461	A-168529	GCUGGGUUUAUUUUAGAGAAU	94	2201-2221	A-170494	AUUCUCUAAAAUAAACCCAGCAA	278	2199-2221
AD-85462	A-168531	AUGGCAUGCACAGUGAGCUAU	95	861-881	A-170495	AUAGCUCACUGUGCAUGCCAUAU	279	859-881
AD-85463	A-168533	GAGAGAGCCCACAGAGUCUAA	96	1816-1836	A-170496	UUAGACUCUGUGGGCUCUCUCUC	280	1814-1836
AD-85464	A-168535	GCAAGAACCAGUGUUUAGCGA	97	2234-2254	A-170497	UCGCUAAACACUGGUUCUUGCCU	281	2232-2254
AD-85465	A-168537	CCAGCAAAACUCCCUCAACUA	98	1591-1611	A-170498	UAGUUGAGGGAGUUUUGCUGGAA	282	1589-1611
AD-85466	A-168539	CACCUCGUCAUCCACAAUGAA	99	632-652	A-170499	UUCAUUGUGGAUGACGAGGUGGA	283	630-652
AD-85467	A-168541	CGUCAUCCACAAUGAGAGUAA	100	637-657	A-170500	UUACUCUCAUUGUGGAUGACGAG	284	635-657
AD-85468	A-168543	CUCCCACGCUCUCUGGACUUA	101	1211-1231	A-170501	UAAGUCCAGAGAGCGUGGGAGGA	285	1209-1231
AD-85469	A-168545	AACUCCCUCAACUGGAUGAAA	102	1598-1618	A-170502	UUUCAUCCAGUUGAGGGAGUUUU	286	1596-1618
AD-85470	A-168547	UGAGAAGAUUGACAGGUUCAU	103	1252-1272	A-170503	AUGAACCUGUCAAUCUUCUCAGC	287	1250-1272
AD-85471	A-168549	CCUCGUCAUCCACAAUGAGAA	104	634-654	A-170504	UUCUCAUUGUGGAUGACGAGGUG	288	632-654
AD-85472	A-168551	GAGAAGAUUGACAGGUUCAUA	105	1253-1273	A-170505	UAUGAACCUGUCAAUCUUCUCAG	289	1251-1273
AD-85473	A-168553	CUUCUUGGGCUUCCGUAUAUA	106	841-861	A-170506	UAUAUACGGAAGCCCAAGAAGUU	290	839-861
AD-85474	A-168555	UCCACCUCGUCAUCCACAAUA	107	630-650	A-170507	UAUUGUGGAUGACGAGGUGGAAG	291	628-650
AD-85475	A-168557	AGAUUGACAGGUUCAUGCAGA	108	1257-1277	A-170508	UCUGCAUGAACCUGUCAAUCUUC	292	1255-1277
AD-85476	A-168559	CUCCCUCAACUGGAUGAAGAA	109	1600-1620	A-170509	UUCUUCAUCCAGUUGAGGGAGUU	293	1598-1620
AD-85477	A-168561	AAUGAGAGUACCUGUGAGCAA	110	647-667	A-170510	UUGCUCACAGGUACUCUCAUUGU	294	645-667
AD-85478	A-168469	CCACCUUUUCUUCUAAUGAGU	111	2081-2101	A-170511	ACUCAUUAGAAGAAAAGGUGGGA	295	2079-2101
AD-85479	A-168471	CGACCAGCUUGUUUGUGAAAA	112	2284-2304	A-170512	UUUUCACAAACAAGCUGGUCGGU	296	2282-2304
AD-85480	A-168473	ACCUUUUCUUCUAAUGAGUCA	113	2083-2103	A-170513	UGACUCAUUAGAAGAAAAGGUGG	297	2081-2103
AD-85481	A-168475	GUCAUCCACAAUGAGAGUACA	114	638-658	A-170514	UGUACUCUCAUUGUGGAUGACGA	298	636-658
AD-85482	A-168477	CACAAUGAGAGUACCUGUGAA	115	644-664	A-170515	UUCACAGGUACUCUCAUUGUGGA	299	642-664
AD-85483	A-168479	GUCUCCCACCUUUUCUUCUAA	116	2076-2096	A-170516	UUAGAAGAAAAGGUGGGAGACUG	300	2074-2096
AD-85484	A-168481	ACUUUCCAGCAAAACUCCCUA	117	1586-1606	A-170517	UAGGGAGUUUUGCUGGAAAGUGA	301	1584-1606
AD-85485	A-168483	CCUCAACUGGAUGAAGAAACU	118	1603-1623	A-170518	AGUUUCUUCAUCCAGUUGAGGGA	302	1601-1623
AD-85486	A-168485	CUGUUUGCUGUGUAUGAUCAA	119	1889-1909	A-170519	UUGAUCAUACACAGCAAACAGGA	303	1887-1909
AD-85487	A-168487	UUUGCUGUGUAUGAUCAAAGA	120	1892-1912	A-170520	UCUUUGAUCAUACACAGCAAACA	304	1890-1912
AD-85488	A-168489	CCGACCAGCUUGUUUGUGAAA	121	2283-2303	A-170521	UUUCACAAACAAGCUGGUCGGUU	305	2281-2303
AD-85489	A-168491	UCCAACCGACCAGCUUGUUUA	122	2278-2298	A-170522	UAAACAAGCUGGUCGGUUGGAAU	306	2276-2298
AD-85490	A-168493	CCAUUCCUGUUUGCUGUGUAU	123	1883-1903	A-170523	AUACACAGCAAACAGGAAUGGGC	307	1881-1903
AD-	A-	CACCUUUUCUUCUAAUGAGUA	124	2082-2102	A-170524	UACUCAUUAGAAGAAAAGGUGGG	308	2080-2102

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
85491	168495							
AD-85492	A-168497	GUUUGCUGUGUAUGAUCAAAA	125	1891-1911	A-170525	UUUUGAUCAUACACAGCAAACAG	309	1889-1911
AD-85493	A-168499	GCUGAGAAGAUUGACAGGUUA	126	1250-1270	A-170526	UAACCUGUCAAUCUUCUCAGCAG	310	1248-1270
AD-85494	A-168501	UUCCAGCAAAACUCCCUCAAA	127	1589-1609	A-170527	UUUGAGGGAGUUUUGCUGGAAAG	311	1587-1609
AD-85495	A-168503	UGCUGAGAAGAUUGACAGGUU	128	1249-1269	A-170528	AACCUGUCAAUCUUCUCAGCAGC	312	1247-1269
AD-85496	A-168505	UCUCACUUUCCAGCAAAACUA	129	1582-1602	A-170529	UAGUUUUGCUGGAAAGUGAGACC	313	1580-1602
AD-85497	A-168507	UCCACAAUGAGAGUACCUGUA	130	642-662	A-170530	UACAGGUACUCUCAUUGUGGAUG	314	640-662
AD-85498	A-168509	CCACCUCGUCAUCCACAAUGA	131	631-651	A-170531	UCAUUGUGGAUGACGAGGUGGAA	315	629-651
AD-85499	A-168511	UCACUUUCCAGCAAAACUCCA	132	1584-1604	A-170532	UGGAGUUUUGCUGGAAAGUGAGA	316	1582-1604
AD-85500	A-168513	UCCCUCAACUGGAUGAAGAAA	133	1601-1621	A-170533	UUUCUUCAUCCAGUUGAGGGAGU	317	1599-1621
AD-85501	A-168515	GAGAGUACCUGUGAGCAGCUA	134	650-670	A-170534	UAGCUGCUCACAGGUACUCUCAU	318	648-670
AD-85502	A-168517	AGAAUUCCAACCGACCAGCUU	135	2273-2293	A-170535	AAGCUGGUCGGUUGGAAUUCUUU	319	2271-2293
AD-85503	A-168519	CAUUCCUGUUUGCUGUGUAUA	136	1884-1904	A-170536	UAUACACAGCAAACAGGAAUGGG	320	1882-1904
AD-85504	A-168521	GAAUUCCAACCGACCAGCUUA	137	2274-2294	A-170537	UAAGCUGGUCGGUUGGAAUUCUU	321	2272-2294
AD-85505	A-168523	CAUCCACAAUGAGAGUACCUA	138	640-660	A-170538	UAGGUACUCUCAUUGUGGAUGAC	322	638-660
AD-85506	A-168525	CCCAUUCCUGUUUGCUGUGUA	139	1882-1902	A-170539	UACACAGCAAACAGGAAUGGGCG	323	1880-1902
AD-85507	A-168527	CUGGGUUUAUUUUAGAGAAUA	140	2202-2222	A-170540	UAUUCUCUAAAAUAAACCCAGCA	324	2200-2222
AD-85508	A-168529	GCUGGGUUUAUUUUAGAGAAU	141	2201-2221	A-170541	AUUCUCUAAAAUAAACCCAGCAA	325	2199-2221
AD-85509	A-168531	AUGGCAUGCACAGUGAGCUAU	142	861-881	A-170542	AUAGCUCACUGUGCAUGCCAUAU	326	859-881
AD-85510	A-168533	GAGAGAGCCCACAGAGUCUAA	143	1816-1836	A-170543	UUAGACUCUGUGGGCUCUCUCUC	327	1814-1836
AD-85511	A-168535	GCAAGAACCAGUGUUUAGCGA	144	2234-2254	A-170544	UCGCUAAACACUGGUUCUUGCCU	328	2232-2254
AD-85512	A-168537	CCAGCAAAACUCCCUCAACUA	145	1591-1611	A-170545	UAGUUGAGGGAGUUUUGCUGGAA	329	1589-1611
AD-85513	A-168539	CACCUCGUCAUCCACAAUGAA	146	632-652	A-170546	UUCAUUGUGGAUGACGAGGUGGA	330	630-652
AD-85514	A-168541	CGUCAUCCACAAUGAGAGUAA	147	637-657	A-170547	UUACUCUCAUUGUGGAUGACGAG	331	635-657
AD-85515	A-168543	CUCCCACGCUCUCUGGACUUA	148	1211-1231	A-170548	UAAGUCCAGAGAGCGUGGGAGGA	332	1209-1231
AD-85516	A-168545	AACUCCCUCAACUGGAUGAAA	149	1598-1618	A-170549	UUUCAUCCAGUUGAGGGAGUUUU	333	1596-1618
AD-85517	A-168547	UGAGAAGAUUGACAGGUUCAU	150	1252-1272	A-170550	AUGAACCUGUCAAUCUUCUCAGC	334	1250-1272
AD-85518	A-168549	CCUCGUCAUCCACAAUGAGAA	151	634-654	A-170551	UUCUCAUUGUGGAUGACGAGGUG	335	632-654
AD-85519	A-168551	GAGAAGAUUGACAGGUUCAUA	152	1253-1273	A-170552	UAUGAACCUGUCAAUCUUCUCAG	336	1251-1273
AD-85520	A-168553	CUUCUUGGGCUUCCGUAUAUA	153	841-861	A-170553	UAUAUACGGAAGCCCAAGAAGUU	337	839-861
AD-85521	A-168555	UCCACCUCGUCAUCCACAAUA	154	630-650	A-170554	UAUUGUGGAUGACGAGGUGGAAG	338	628-650
AD-85522	A-168557	AGAUUGACAGGUUCAUGCAGA	155	1257-1277	A-170555	UCUGCAUGAACCUGUCAAUCUUC	339	1255-1277
AD-85523	A-168559	CUCCCUCAACUGGAUGAAGAA	156	1600-1620	A-170556	UUCUUCAUCCAGUUGAGGGAGUU	340	1598-1620
AD-85524	A-168561	AAUGAGAGUACCUGUGAGCAA	157	647-667	A-170557	UUGCUCACAGGUACUCUCAUUGU	341	645-667
AD-85619	A-168469	CCACCUUUUCUUCUAAUGAGU	158	2081-2101	A-170558	ACUCAUUAGAAGAAAAGGUGGGA	342	2079-2101
AD-85620	A-168471	CGACCAGCUUGUUUGUGAAAA	159	2284-2304	A-170559	UUUUCACAAACAAGCUGGUCGGU	343	2282-2304
AD-	A-	ACCUUUUCUUCUAAUGAGUCA	160	2083-2103	A-170560	UGACUCAUUAGAAGAAAAGGUGG	344	2081-2103

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
85621	168473							
AD-85622	A-168475	GUCAUCCACAAUGAGAGUACA	161	638-658	A-170561	UGUACUCUCAUUGUGGAUGACGA	345	636-658
AD-85623	A-168477	CACAAUGAGAGUACCUGUGAA	162	644-664	A-170562	UUCACAGGUACUCUCAUUGUGGA	346	642-664
AD-85624	A-168479	GUCUCCCACCUUUUCUUCUAA	163	2076-2096	A-170563	UUAGAAGAAAAGGUGGGAGACUG	347	2074-2096
AD-85625	A-168481	ACUUUCCAGCAAAACUCCCUA	164	1586-1606	A-170564	UAGGGAGUUUUGCUGGAAAGUGA	348	1584-1606
AD-85626	A-168483	CCUCAACUGGAUGAAGAAACU	165	1603-1623	A-170565	AGUUUCUUCAUCCAGUUGAGGGA	349	1601-1623
AD-85627	A-168485	CUGUUUGCUGUGUAUGAUCAA	166	1889-1909	A-170566	UUGAUCAUACACAGCAAACAGGA	350	1887-1909
AD-85628	A-168487	UUUGCUGUGUAUGAUCAAAGA	167	1892-1912	A-170567	UCUUUGAUCAUACACAGCAAACA	351	1890-1912
AD-85629	A-168489	CCGACCAGCUUGUUUGUGAAA	168	2283-2303	A-170568	UUUCACAAACAAGCUGGUCGGUU	352	2281-2303
AD-85630	A-168491	UCCAACCGACCAGCUUGUUUA	169	2278-2298	A-170569	UAAACAAGCUGGUCGGUUGGAAU	353	2276-2298
AD-85631	A-168493	CCAUUCCUGUUUGCUGUGUAU	170	1883-1903	A-170570	AUACACAGCAAACAGGAAUGGGC	354	1881-1903
AD-85632	A-168495	CACCUUUUCUUCUAAUGAGUA	171	2082-2102	A-170571	UACUCAUUAGAAGAAAAGGUGGG	355	2080-2102
AD-85633	A-168497	GUUUGCUGUGUAUGAUCAAAA	172	1891-1911	A-170572	UUUUGAUCAUACACAGCAAACAG	356	1889-1911
AD-85634	A-168499	GCUGAGAAGAUUGACAGGUUA	173	1250-1270	A-170573	UAACCUGUCAAUCUUCUCAGCAG	357	1248-1270
AD-85635	A-168501	UUCCAGCAAAACUCCCUCAAA	174	1589-1609	A-170574	UUUGAGGGAGUUUUGCUGGAAAG	358	1587-1609
AD-85636	A-168503	UGCUGAGAAGAUUGACAGGUU	175	1249-1269	A-170575	AACCUGUCAAUCUUCUCAGCAGC	359	1247-1269
AD-85637	A-168505	UCUCACUUUCCAGCAAAACUA	176	1582-1602	A-170576	UAGUUUUGCUGGAAAGUGAGACC	360	1580-1602
AD-85638	A-168507	UCCACAAUGAGAGUACCUGUA	177	642-662	A-170577	UACAGGUACUCUCAUUGUGGAUG	361	640-662
AD-85639	A-168509	CCACCUCGUCAUCCACAAUGA	178	631-651	A-170578	UCAUUGUGGAUGACGAGGUGGAA	362	629-651
AD-85640	A-168511	UCACUUUCCAGCAAAACUCCA	179	1584-1604	A-170579	UGGAGUUUUGCUGGAAAGUGAGA	363	1582-1604
AD-85641	A-168513	UCCCUCAACUGGAUGAAGAAA	180	1601-1621	A-170580	UUUCUUCAUCCAGUUGAGGGAGU	364	1599-1621
AD-85642	A-168515	GAGAGUACCUGUGAGCAGCUA	181	650-670	A-170581	UAGCUGCUCACAGGUACUCUCAU	365	648-670
AD-85643	A-168517	AGAAUUCCAACCGACCAGCUU	182	2273-2293	A-170582	AAGCUGGUCGGUUGGAAUUCUUU	366	2271-2293
AD-85644	A-168519	CAUUCCUGUUUGCUGUGUAUA	183	1884-1904	A-170583	UAUACACAGCAAACAGGAAUGGG	367	1882-1904
AD-85645	A-168521	GAAUUCCAACCGACCAGCUUA	184	2274-2294	A-170584	UAAGCUGGUCGGUUGGAAUUCUU	368	2272-2294
AD-85646	A-168523	CAUCCACAAUGAGAGUACCUA	185	640-660	A-170585	UAGGUACUCUCAUUGUGGAUGAC	369	638-660
AD-85647	A-168525	CCCAUUCCUGUUUGCUGUGUA	186	1882-1902	A-170586	UACACAGCAAACAGGAAUGGGCG	370	1880-1902
AD-85648	A-168527	CUGGGUUUAUUUUAGAGAAUA	187	2202-2222	A-170587	UAUUCUCUAAAAUAAACCCAGCA	371	2200-2222
AD-85649	A-168529	GCUGGGUUUAUUUUAGAGAAU	188	2201-2221	A-170588	AUUCUCUAAAAUAAACCCAGCAA	372	2199-2221
AD-85650	A-168531	AUGGCAUGCACAGUGAGCUAU	189	861-881	A-170589	AUAGCUCACUGUGCAUGCCAUAU	373	859-881
AD-85651	A-168533	GAGAGAGCCCACAGAGUCUAA	190	1816-1836	A-170590	UUAGACUCUGUGGGCUCUCUCUC	374	1814-1836
AD-85652	A-168535	GCAAGAACCAGUGUUUAGCGA	191	2234-2254	A-170591	UCGCUAAACACUGGUUCUUGCCU	375	2232-2254
AD-85653	A-168537	CCAGCAAAACUCCCUCAACUA	192	1591-1611	A-170592	UAGUUGAGGGAGUUUUGCUGGAA	376	1589-1611
AD-85654	A-168539	CACCUCGUCAUCCACAAUGAA	193	632-652	A-170593	UUCAUUGUGGAUGACGAGGUGGA	377	630-652
AD-85655	A-168541	CGUCAUCCACAAUGAGAGUAA	194	637-657	A-170594	UUACUCUCAUUGUGGAUGACGAG	378	635-657
AD-85656	A-168543	CUCCCACGCUCUCUGGACUUA	195	1211-1231	A-170595	UAAGUCCAGAGAGCGUGGGAGGA	379	1209-1231
AD-	A-	AACUCCCUCAACUGGAUGAAA	196	1598-1618	A-170596	UUUCAUCCAGUUGAGGGAGUUUU	380	1596-1618

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3_
85657	168545							
AD-85658	A-168547	UGAGAAGAUUGACAGGUUCAU	197	1252-1272	A-170597	AUGAACCUGUCAAUCUUCUCAGC	381	1250-1272
AD-85659	A-168549	CCUCGUCAUCCACAAUGAGAA	198	634-654	A-170598	UUCUCAUUGUGGAUGACGAGGUG	382	632-654
AD-85660	A-168551	GAGAAGAUUGACAGGUUCAUA	199	1253-1273	A-170599	UAUGAACCUGUCAAUCUUCUCAG	383	1251-1273
AD-85661	A-168553	CUUCUUGGGCUUCCGUAUAUA	200	841-861	A-170600	UAUAUACGGAAGCCCAAGAAGUU	384	839-861
AD-85662	A-168555	UCCACCUCGUCAUCCACAAUA	201	630-650	A-170601	UAUUGUGGAUGACGAGGUGGAAG	385	628-650
AD-85663	A-168557	AGAUUGACAGGUUCAUGCAGA	202	1257-1277	A-170602	UCUGCAUGAACCUGUCAAUCUUC	386	1255-1277
AD-85664	A-168559	CUCCCUCAACUGGAUGAAGAA	203	1600-1620	A-170603	UUCUUCAUCCAGUUGAGGGAGUU	387	1598-1620
AD-85665	A-168561	AAUGAGAGUACCUGUGAGCAA	204	647-667	A-170604	UUGCUCACAGGUACUCUCAUUGU	388	645-667

Table 4. AGT Single 10 nM and 0.1 nM Dose Screen in Hep3B cells

Duplex ID	10 nM Avg.	10 nM SD	0.1 nM Avg.	0.1 nM SD
AD-67327	6.2	1.5	30.1	5.9
AD-84700	14.5	5.7	127.9	53.6
AD-84701	8.7	6.5	59.7	5.5
AD-84702	11.4	4.2	95.1	14.6
AD-84703	6.7	4.2	36.7	2.4
AD-84704	7.2	4.3	54.9	5.5
AD-84705	7.8	2.3	52.7	10.3
AD-84706	6.3	4.3	65.0	13.0
AD-84707	10.2	9.2	52.2	2.6
AD-84708	10.1	4.1	98.7	24.6
AD-84709	19.0	4.3	102.7	18.5
AD-84710	11.3	7.9	65.9	19.0
AD-84711	10.4	6.5	66.5	13.8
AD-84712	7.5	6.4	66.4	13.9
AD-84713	9.7	5.9	66.4	5.6
AD-84714	13.7	2.0	89.0	28.9
AD-84715	7.7	5.3	59.0	2.7
AD-84716	7.4	2.4	45.9	15.0
AD-84717	14.6	4.2	102.9	15.8
AD-84718	6.8	0.7	66.9	8.3
AD-84719	21.1	4.0	92.5	19.6
AD-84720	11.3	0.4	96.0	22.1
AD-84721	14.7	5.2	88.4	12.7
AD-84722	35.9	14.8	106.6	21.2
AD-84723	13.4	6.5	83.7	25.9
AD-84724	20.8	6.7	108.0	17.6
AD-84725	17.4	1.2	100.5	22.3
AD-84726	9.7	0.6	73.1	9.0
AD-84727	16.5	5.0	102.4	20.5
AD-84728	17.6	2.6	103.6	12.8
AD-84729	11.6	3.4	78.9	16.1
AD-84730	11.2	1.5	77.6	8.7
AD-84731	11.4	2.9	80.4	15.9
AD-84732	26.9	2.6	88.2	19.5
AD-84733	21.4	2.2	101.5	13.9
AD-84734	20.4	1.9	92.8	20.5
AD-84735	11.4	1.9	85.0	6.0
AD-84736	8.4	2.5	68.0	20.7
AD-84737	22.9	4.0	80.0	13.1
AD-84738	16.0	1.8	98.3	29.5
AD-84739	6.6	2.1	40.5	6.8
AD-84740	10.2	1.8	78.9	32.7
AD-84741	6.3	1.0	37.0	9.7
AD-84742	14.1	2.0	86.9	7.4
AD-84743	80.9	9.9	97.6	16.5

Duplex ID	10 nM Avg.	10 nM SD	0.1 nM Avg.	0.1 nM SD
AD-84744	23.1	5.8	93.4	18.9
AD-84745	27.8	5.0	79.2	25.3
AD-84746	7.5	1.2	48.0	14.4
AD-85431	12.3	3.9	61.8	29.6
AD-85432	10.1	6.6	50.5	19.8
AD-85433	11.5	4.0	61.4	25.4
AD-85434	5.8	2.5	37.2	20.4
AD-85435	7.3	1.8	44.9	17.8
AD-85436	8.2	4.9	35.2	12.8
AD-85437	7.2	6.3	30.4	18.6
AD-85438	10.4	7.5	41.8	18.1
AD-85439	15.4	3.8	62.1	25.9
AD-85440	26.1	5.1	85.5	34.7
AD-85441	9.9	7.4	50.6	19.3
AD-85442	8.4	5.2	52.4	20.4
AD-85443	11.5	12.9	59.0	25.0
AD-85444	8.4	4.4	48.6	24.9
AD-85445	21.7	6.8	63.2	36.1
AD-85446	14.7	11.5	59.0	27.5
AD-85447	8.4	2.9	52.8	15.5
AD-85448	12.6	4.0	89.5	25.2
AD-85449	8.3	1.5	72.7	14.2
AD-85450	35.3	6.3	91.4	30.0
AD-85451	17.7	2.3	80.7	20.8
AD-85452	21.5	5.2	73.3	27.4
AD-85453	60.5	32.6	69.0	33.9
AD-85454	21.8	8.6	63.2	23.6
AD-85455	34.0	12.4	88.8	23.4
AD-85456	21.6	1.4	91.9	18.1
AD-85457	12.1	2.6	85.6	28.0
AD-85458	29.8	7.1	98.1	25.4
AD-85459	23.8	2.0	101.2	34.7
AD-85460	9.9	1.8	69.0	12.7
AD-85461	11.0	3.7	60.1	13.4
AD-85462	25.7	4.1	78.5	6.8
AD-85463	56.6	13.1	89.7	20.5
AD-85464	21.8	6.3	99.8	34.4
AD-85465	25.2	6.2	99.2	24.6
AD-85466	35.3	8.4	104.2	31.5
AD-85467	12.7	2.7	82.2	8.4
AD-85468	30.9	6.5	93.0	31.2
AD-85469	44.7	8.0	82.1	13.4
AD-85470	7.9	4.6	66.7	11.2
AD-85471	15.8	3.7	95.3	14.0
AD-85472	9.3	3.3	71.3	15.1
AD-85473	21.5	3.9	102.2	22.4
AD-85474	100.2	24.5	110.4	21.9
AD-85475	28.7	8.6	105.2	31.1
AD-85476	35.5	10.2	81.4	13.1
AD-85477	10.1	2.2	76.9	30.6
AD-85478	15.1	1.9	94.6	27.5
AD-85479	18.7	5.8	92.1	26.6
AD-85480	11.8	4.1	68.8	16.7
AD-85481	5.3	1.1	32.9	8.7
AD-85482	7.1	3.4	52.7	11.5
AD-85483	7.9	3.0	61.1	15.3
AD-85484	11.5	3.3	84.6	8.8
AD-85485	11.6	5.5	58.9	19.7
AD-85486	25.3	6.2	94.8	21.4
AD-85487	40.5	10.2	101.0	24.4
AD-85488	32.8	7.4	97.6	24.2
AD-85489	12.8	4.0	87.2	20.9
AD-85490	7.6	0.8	78.5	20.2

Duplex ID	10 nM Avg.	10 nM SD	0.1 nM Avg.	0.1 nM SD
AD-85491	12.5	4.5	92.3	19.0
AD-85492	89.7	17.7	112.3	20.2
AD-85493	6.3	2.6	51.0	14.5
AD-85494	11.0	2.4	79.8	24.0
AD-85495	10.4	2.8	70.1	23.2
AD-85496	6.8	2.2	55.0	20.8
AD-85497	72.4	18.6	108.5	36.5
AD-85498	7.3	2.3	85.3	26.5
AD-85499	94.7	6.8	104.2	24.5
AD-85500	63.1	21.9	98.4	21.4
AD-85501	38.3	12.3	129.0	47.3
AD-85502	27.2	8.3	92.0	21.9
AD-85503	36.2	11.5	108.3	25.4
AD-85504	9.2	3.7	56.1	9.4
AD-85505	50.3	12.2	111.6	15.5
AD-85506	40.8	8.7	109.4	31.6
AD-85507	7.7	2.2	64.3	22.6
AD-85508	82.0	21.9	100.9	29.0
AD-85509	20.9	5.5	101.5	23.0
AD-85510	104.0	28.6	90.1	42.0
AD-85511	14.1	2.6	84.2	9.3
AD-85512	50.6	14.7	98.5	30.7
AD-85513	35.0	9.0	78.5	24.4
AD-85514	13.0	0.9	80.3	27.9
AD-85515	69.1	5.7	72.5	35.1
AD-85516	54.0	16.1	88.4	38.9
AD-85517	7.4	1.3	47.9	7.0
AD-85518	12.0	4.6	79.4	42.4
AD-85519	8.9	2.5	54.6	6.2
AD-85520	10.9	3.0	81.3	19.1
AD-85521	79.1	15.6	108.4	35.4
AD-85522	51.3	10.1	114.7	27.9
AD-85523	23.6	2.9	91.3	26.6
AD-85524	9.9	2.8	46.9	10.8
AD-85619	20.7	1.0	114.4	53.5
AD-85620	20.0	5.8	96.8	25.7
AD-85621	8.9	6.9	80.4	20.3
AD-85622	6.4	3.7	50.2	12.9
AD-85623	7.6	6.0	38.0	4.6
AD-85624	9.2	3.7	81.0	15.9
AD-85625	6.0	2.4	58.9	8.7
AD-85626	7.6	6.3	43.8	3.9
AD-85627	25.5	8.3	103.0	20.0
AD-85628	39.0	7.3	114.5	19.9
AD-85629	23.6	18.5	86.1	14.8
AD-85630	17.2	15.9	83.0	22.5
AD-85631	10.1	6.2	79.0	18.2
AD-85632	10.2	4.3	67.5	10.8
AD-85633	42.1	19.9	95.1	4.5
AD-85634	7.5	2.9	54.6	11.7
AD-85635	8.2	2.6	53.5	24.0
AD-85636	11.4	2.2	96.0	23.7
AD-85637	9.9	5.9	58.2	17.0
AD-85638	53.1	6.2	112	31.1
AD-85639	13.7	2.1	96.9	19.6
AD-85640	72.8	11.7	106.9	7.2
AD-85641	47.2	19.8	89.6	9.5
AD-85642	15.3	3.5	86.7	24.5
AD-85643	35.4	12.9	494.9	779.7
AD-85644	79.0	7.2	110.6	23.5
AD-85645	7.8	1.2	63.6	13.1
AD-85646	33.6	8.6	105.6	28.1
AD-85647	19.4	3.1	102.3	9.7

Duplex ID	10 nM Avg.	10 nM SD	0.1 nM Avg.	0.1 nM SD
AD-85648	49.0	6.6	106.0	10.7
AD-85649	18.5	2.9	82.4	10.1
AD-85650	69.4	19.0	87.2	15.6
AD-85651	90.4	17.3	106.9	31.9
AD-85652	31.3	8.5	90.0	37.7
AD-85653	47.6	2.8	104.1	20.6
AD-85654	66.7	3.7	97.5	17.8
AD-85655	10.4	0.8	55.4	6.9
AD-85656	96.7	4.3	98.6	23.4
AD-85657	83.6	3.3	86.6	31.4
AD-85658	7.6	1.8	65.4	9.2
AD-85659	14.1	4.2	76.0	33.0
AD-85660	40.9	6.5	95.8	24.0
AD-85661	63.6	10.4	93.0	34.1
AD-85662	83.4	11.5	87.3	17.2
AD-85663	23.0	5.3	79.1	30.8
AD-85664	44.8	2.9	112.2	19.9
AD-85665	13.5	2.5	81.0	25.4

Table 5. Modified Sense and Antisense Strand Sequences of hAGT dsRNA Agents

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
AD-84704	csascaauGfaGfAfGfuaccugugaaL96	389	usUfscacAfgGfUfacucUfcAfuugugsgsa	573	UCCACAAUGAGAGUACCUGUGAG	757
AD-84705	gsuscuccCfaCfCfUfuuucuucuaaL96	390	usUfsagaAfgAfAfaaggUfgGfgagacsusg	574	CAGUCUCCCACCUUUUCUUCUAA	758
AD-84706	ascsuuucCfaGfCfAfaaacucccuaL96	391	usAfsgggAfgUfUfuugcUfgGfaaagusgsa	575	UCACUUUCCAGCAAAACUCCCUC	759
AD-84707	cscsucaaCfuGfGfAfugaagaaacuL96	392	asGfsuuuCfuUfCfauccAfgUfugaggsgsa	576	UCCCUCAACUGGAUGAAGAAACU	760
AD-84708	csusguuuGfcUfGfUfguaugaucaaL96	393	usUfsgauCfaUfAfcacaGfcAfaacagsgsa	577	UCCUGUUUGCUGUGUAUGAUCAA	761
AD-84709	ususugcuGfuGfUfAfugaucaaagaL96	394	usCfsuuuGfaUfCfauacAfcAfgcaaascsa	578	UGUUUGCUGUGUAUGAUCAAAGC	762
AD-84710	cscsgaccAfgCfUfUfguuugugaaaL96	395	usUfsucaCfaAfAfcaagCfuGfgucggsusu	579	AACCGACCAGCUUGUUUGUGAAA	763
AD-84711	uscscaacCfgAfCfCfagcuuguuuaL96	396	usAfsaacAfaGfCfugguCfgGfuuggasasu	580	AUUCCAACCGACCAGCUUGUUUG	764
AD-84712	cscsauucCfuGfUfUfugcuguguauL96	397	asUfsacaCfaGfCfaaacAfgGfaauggsgsc	581	GCCCAUUCCUGUUUGCUGUGUAU	765
AD-84713	csasccuuUfuCfUfUfcuaaugaguaL96	398	usAfscucAfuUfAfgaagAfaAfaggugsgsg	582	CCCACCUUUUCUUCUAAUGAGUC	766
AD-84714	gsusuugcUfgUfGfUfaugaucaaaaL96	399	usUfsuugAfuCfAfuacaCfaGfcaaacsasg	583	CUGUUUGCUGUGUAUGAUCAAAG	767
AD-84715	gscsugagAfaGfAfUfugacagguuaL96	400	usAfsaccUfgUfCfaaucUfuCfucagcsasg	584	CUGCUGAGAAGAUUGACAGGUUC	768
AD-84716	ususccagCfaAfAfAfcucccucaaaL96	401	usUfsugaGfgGfAfguuuUfgCfuggaasasg	585	CUUUCCAGCAAAACUCCCUCAAC	769
AD-84717	usgscugaGfaAfGfAfuugacagguuL96	402	asAfsccuGfuCfAfaucuUfcUfcagcasgsc	586	GCUGCUGAGAAGAUUGACAGGUU	770
AD-84718	uscsucacUfuUfCfCfagcaaaacuaL96	403	usAfsguuUfuGfCfuggaAfaGfugagascsc	587	GGUCUCACUUUCCAGCAAAACUC	771
AD-84719	uscscacaAfuGfAfGfaguaccuguaL96	404	usAfscagGfuAfCfucucAfuUfguggasusg	588	CAUCCACAAUGAGAGUACCUGUG	772
AD-84720	cscsaccuCfgUfCfAfuccacaaugaL96	405	usCfsauuGfuGfGfaugaCfgAfgguggsasa	589	UUCCACCUCGUCAUCCACAAUGA	773
AD-84721	uscsacuuUfcCfAfGfcaaaacuccaL96	406	usGfsgagUfuUfUfgcugGfaAfagugasgsa	590	UCUCACUUUCCAGCAAAACUCCC	774
AD-84722	uscsccucAfaCfUfGfgaugaagaaaL96	407	usUfsucuUfcAfUfccagUfuGfagggasgsu	591	ACUCCCUCAACUGGAUGAAGAAA	775
AD-84723	gsasgaguAfcCfUfGfugagcagcuaL96	408	usAfsgcuGfcUfCfacagGfuAfcucucsasu	592	AUGAGAGUACCUGUGAGCAGCUG	776
AD-84724	asgsaauuCfcAfAfCfcgaccagcuuL96	409	asAfsgcuGfgUfCfgguuGfgAfauucususu	593	AAAGAAUUCCAACCGACCAGCUU	777
AD-84725	csasuuccUfgUfUfUfgcuguguauaL96	410	usAfsuacAfcAfGfcaaaCfaGfgaaugsgsg	594	CCCAUUCCUGUUUGCUGUGUAUG	778
AD-84726	gsasauucCfaAfCfCfgaccagcuuaL96	411	usAfsagcUfgGfUfcgguUfgGfaauucsusu	595	AAGAAUUCCAACCGACCAGCUUG	779
AD-84727	csasuccaCfaAfUfGfagaguaccuaL96	412	usAfsgguAfcUfCfucauUfgUfggaugsasc	596	GUCAUCCACAAUGAGAGUACCUG	780
AD-	cscscauuCfcUfGfUfuugcuguguaL96	413	usAfscacAfgCfAfaacaGfgAfaugggscsg	597	CGCCCAUUCCUGUUUGCUGUGUA	781

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
84728						
AD-84729	csusggguUfuAfUfUfuuagagaauaL96	414	usAfsuucUfcUfAfaaauAfaAfcccagscsa	598	UGCUGGGUUUAUUUUAGAGAAUG	782
AD-84730	gscsugggUfuUfAfUfuuuagagaauL96	415	asUfsucuCfuAfAfaauaAfaCfccagcsasa	599	UUGCUGGGUUUAUUUUAGAGAAU	783
AD-84731	asusggcaUfgCfAfCfagugagcuauL96	416	asUfsagcUfcAfCfugugCfaUfgccausasu	600	AUAUGGCAUGCACAGUGAGCUAU	784
AD-84732	gsasgagaGfcCfCfAfcagagucuaaL96	417	usUfsagaCfuCfUfguggGfcUfcucucsusc	601	GAGAGAGAGCCCACAGAGUCUAC	785
AD-84733	gscsaagaAfcCfAfGfuguuuagcgaL96	418	usCfsgcuAfaAfCfacugGfuUfcuugcscsu	602	AGGCAAGAACCAGUGUUUAGCGC	786
AD-84734	cscsagcaAfaAfCfUfcccucaacuaL96	419	usAfsguuGfaGfGfgaguUfuUfgcuggsasa	603	UUCCAGCAAAACUCCCUCAACUG	787
AD-84735	csasccucGfuCfAfUfccacaaugaaL96	420	usUfscauUfgUfGfgaugAfcGfaggugsgsa	604	UCCACCUCGUCAUCCACAAUGAG	788
AD-84736	csgsucauCfcAfCfAfaugagaguaaL96	421	usUfsacuCfuCfAfuuguGfgAfugacgsasg	605	CUCGUCAUCCACAAUGAGAGUAC	789
AD-84737	csusccca CfgCfUfCfucuggacuuaL96	422	usAfsaguCfcAfGfagagCfgUfgggagsgsa	606	UCCUCCCACGCUCUCUGGACUUC	790
AD-84738	asascuccCfuCfAfAfcuggaugaaaL96	423	usUfsucaUfcCfAfguugAfgGfgaguususu	607	AAAACUCCCUCAACUGGAUGAAG	791
AD-84739	usgsagaaGfaUfUfGfacagguucauL96	424	asUfsgaaCfcUfGfucaaUfcUfucucasgsc	608	GCUGAGAAGAUUGACAGGUUCAU	792
AD-84740	cscsucguCfaUfCfCfacaaugagaaL96	425	usUfscucAfuUfGfuggaUfgAfcgaggsusg	609	CACCUCGUCAUCCACAAUGAGAG	793
AD-84741	gsasgaagAfuUfGfAfcagguucauaL96	426	usAfsugaAfcCfUfgucaAfuCfuucucsasg	610	CUGAGAAGAUUGACAGGUUCAUG	794
AD-84742	csusucuuGfgGfCfUfuccguauauaL96	427	usAfsuauAfcGfGfaagcCfcAfagaagsusu	611	AACUUCUUGGGCUUCCGUAUAUA	795
AD-84743	uscscaccUfcGfUfCfauccacaauaL96	428	usAfsuugUfgGfAfugacGfaGfguggasasg	612	CUUCCACCUCGUCAUCCACAAUG	796
AD-84744	asgsauugAfcAfGfGfuucaugcagaL96	429	usCfsugcAfuGfAfaccuGfuCfaaucususc	613	GAAGAUUGACAGGUUCAUGCAGG	797
AD-84745	csuscccuCfaAfCfUfggaugaagaaL96	430	usUfscuuCfaUfCfcaguUfgAfgggagsusu	614	AACUCCCUCAACUGGAUGAAGAA	798
AD-84746	asasugagAfgUfAfCfcugugagcaaL96	431	usUfsgcuCfaCfAfgguaCfuCfucauusgsu	615	ACAAUGAGAGUACCUGUGAGCAG	799
AD-85431	cscsaccuUfuUfCfUfucuaaugaguL96	432	asCfsucauuagaagaAfaAfggguggsgsa	616	UCCCACCUUUUCUUCUAAUGAGU	800
AD-85432	csgsaccaGfcUfUfGfuuugugaaaaL96	433	usUfsuucacaaacaaGfcUfggucgsgsu	617	ACCGACCAGCUUGUUUGUGAAAC	801
AD-85433	ascscuuuUfcUfUfCfuaaugagucaL96	434	usGfsacucauuagaaGfaAfaaggusgsg	618	CCACCUUUUCUUCUAAUGAGUCG	802
AD-85434	gsuscaucCfaCfAfAfugagaguacaL96	435	usGfsuacucucauugUfgGfaugacsgsa	619	UCGUCAUCCACAAUGAGAGUACC	803
AD-85435	csascaauGfaGfAfGfuaccugugaaL96	436	usUfscacagguacucUfcAfuugugsgsa	620	UCCACAAUGAGAGUACCUGUGAG	804
AD-85436	gsuscuccCfaCfCfUfuuucuucuaaL96	437	usUfsagaagaaaaggUfgGfgagacsusg	621	CAGUCUCCCACCUUUUCUUCUAA	805
AD-85437	ascsuuucCfaGfCfAfaaacucccuaL96	438	usAfsgggaguuuugcUfgGfaaagusgsa	622	UCACUUUCCAGCAAAACUCCCUC	806
AD-85438	cscsucaaCfuGfGfAfugaagaaacuL96	439	asGfsuuucuucauccAfgUfugaggsgsa	623	UCCCUCAACUGGAUGAAGAAACU	807
AD-85439	csusguuuGfcUfGfUfguaugaucaaL96	440	usUfsgaucauacacaGfcAfaacagsgsa	624	UCCUGUUUGCUGUGUAUGAUCAA	808
AD-85440	ususugcuGfuGfUfAfugaucaaagaL96	441	usCfsuuugaucauacAfcAfgcaaascsa	625	UGUUUGCUGUGUAUGAUCAAAGC	809
AD-85441	cscsgaccAfgCfUfUfguuugugaaaL96	442	usUfsucacaaacaagCfuGfgucggsusu	626	AACCGACCAGCUUGUUUGUGAAA	810
AD-85442	uscscaacCfgAfCfCfagcuuguuuaL96	443	usAfsaacaagcugguCfgGfuuggasasu	627	AUUCCAACCGACCAGCUUGUUUG	811
AD-85443	cscsauucCfuGfUfUfugcuguguauL96	444	asUfsacacagcaaacAfgGfaauggsgsc	628	GCCCAUUCCUGUUUGCUGUGUAU	812
AD-85444	csasccuuUfuCfUfUfcuaaugaguaL96	445	usAfscucauuagaagAfaAfaggugsgsg	629	CCCACCUUUUCUUCUAAUGAGUC	813
AD-85445	gsusuugcUfgUfGfUfaugaucaaaaL96	446	usUfsuugaucauacaCfaGfcaaacsasg	630	CUGUUUGCUGUGUAUGAUCAAAG	814
AD-85446	gscsugagAfaGfAfUfugacagguuaL96	447	usAfsaccugucaaucUfuCfucagcsasg	631	CUGCUGAGAAGAUUGACAGGUUC	815
AD-85447	ususccagCfaAfAfAfcucccucaaaL96	448	usUfsugagggaguuuUfgCfuggaasasg	632	CUUUCCAGCAAAACUCCCUCAAC	816
AD-	usgscugaGfaAfGfAfuugacagguuL96	449	asAfsccugucaaucuUfcUfcagcasgsc	633	GCUGCUGAGAAGAUUGACAGGUU	817

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
85448						
AD-85449	uscsucacUfuUfCfCfagcaaaacuaL96	450	usAfsguuuugcuggaAfaGfugagascsc	634	GGUCUCACUUUCCAGCAAAACUC	818
AD-85450	uscscacaAfuGfAfGfaguaccuguaL96	451	usAfscagguacucucAfuUfguggasusg	635	CAUCCACAAUGAGAGUACCUGUG	819
AD-85451	cscsaccuCfgUfCfAfuccacaaugaL96	452	usCfsauuguggaugaCfgAfggugsasa	636	UUCCACCUCGUCAUCCACAAUGA	820
AD-85452	uscsacuuUfcCfAfGfcaaaacuccaL96	453	usGfsgaguuuugcugGfaAfagugasgsa	637	UCUCACUUUCCAGCAAAACUCCC	821
AD-85453	uscsccucAfaCfUfGfgaugaagaaaL96	454	usUfsucuucauccagUfuGfagggasgsu	638	ACUCCCUCAACUGGAUGAAGAAA	822
AD-85454	gsasgaguAfcCfUfGfugagcagcuaL96	455	usAfsgcugcucacagGfuAfcucucsasu	639	AUGAGAGUACCUGUGAGCAGCUG	823
AD-85455	asgsaauuCfcAfAfCfcgaccagcuuL96	456	asAfsgcuggucgguuGfgAfauucususu	640	AAAGAAUUCCAACCGACCAGCUU	824
AD-85456	csasuuccUfgUfUfUfgcuguguauaL96	457	usAfsuacacagcaaaCfaGfgaaugsgsg	641	CCCAUUCCUGUUUGCUGUGUAUG	825
AD-85457	gsasauucCfaAfCfCfgaccagcuuaL96	458	usAfsagcuggucgguUfgGfaauucsusu	642	AAGAAUUCCAACCGACCAGCUUG	826
AD-85458	csasuccaCfaAfUfGfagaguaccuaL96	459	usAfsgguacucucauUfgUfggaugsasc	643	GUCAUCCACAAUGAGAGUACCUG	827
AD-85459	cscscauuCfcUfGfUfuugcuguguaL96	460	usAfscacagcaaacaGfgAfaugggscsg	644	CGCCCAUUCCUGUUUGCUGUGUA	828
AD-85460	csusggguUfuAfUfUfuuagagaauaL96	461	usAfsuucucuaaaauAfaAfcccagscsa	645	UGCUGGGUUUAUUUUAGAGAAUG	829
AD-85461	gscsugggUfuUfAfUfuuuagagaauL96	462	asUfsucucuaaaauaAfaCfccagcsasa	646	UUGCUGGGUUUAUUUUAGAGAAU	830
AD-85462	asusggcaUfgCfAfCfagugagcuauL96	463	asUfsagcucacugugCfaUfgccausasu	647	AUAUGGCAUGCACAGUGAGCUAU	831
AD-85463	gsasgagaGfcCfCfAfcagagucuaaL96	464	usUfsagacucuguggGfcUfcucucsusc	648	GAGAGAGAGCCCACAGAGUCUAC	832
AD-85464	gscsaagaAfcCfAfGfuguuuagcgaL96	465	usCfsgcuaaacacugGfuUfcuugcscsu	649	AGGCAAGAACCAGUGUUUAGCGC	833
AD-85465	cscsagcaAfaAfCfUfcccucaacuaL96	466	usAfsguugagggaguUfuUfgcuggsasa	650	UUCCAGCAAAACUCCCUCAACUG	834
AD-85466	csasccucGfuCfAfUfccacaaugaaL96	467	usUfscauuguggaugAfcGfaggugsgsa	651	UCCACCUCGUCAUCCACAAUGAG	835
AD-85467	csgsucauCfcAfCfAfaugagaguaaL96	468	usUfsacucucauuguGfgAfugacgsasg	652	CUCGUCAUCCACAAUGAGAGUAC	836
AD-85468	csuscccaCfgCfUfCfucuggacuuaL96	469	usAfsaguccagagagCfgUfgggagsgsa	653	UCCUCCCACGCUCUCUGGACUUC	837
AD-85469	asascuccCfuCfAfAfcuggaugaaaL96	470	usUfsucauccaguugAfgGfgaguususu	654	AAAACUCCCUCAACUGGAUGAAG	838
AD-85470	usgsagaaGfaUfUfGfacagguucauL96	471	asUfsgaaccugucaaUfcUfucucasgsc	655	GCUGAGAAGAUUGACAGGUUCAU	839
AD-85471	cscsucgucfaUfCfCfacaaugagaaL96	472	usUfscucauuguggaUfgAfcgaggsusg	656	CACCUCGUCAUCCACAAUGAGAG	840
AD-85472	gsasgaagAfuUfGfAfcagguucauaL96	473	usAfsugaaccugucaAfuCfuucucsasg	657	CUGAGAAGAUUGACAGGUUCAUG	841
AD-85473	csusucuuGfgGfCfUfuccguauauaL96	474	usAfsuauacggaagcCfcAfagaagsusu	658	AACUUCUUGGGCUUCCGUAUAUA	842
AD-85474	uscscaccUfcGfUfCfauccacaauaL96	475	usAfsuuguggaugacGfaGfguggasasg	659	CUUCCACCUCGUCAUCCACAAUG	843
AD-85475	asgsauugAfcAfGfGfuucaugcagaL96	476	usCfsugcaugaaccuGfuCfaaucususc	660	GAAGAUUGACAGGUUCAUGCAGG	844
AD-85476	csuscccuCfaAfCfUfggaugaagaaL96	477	usUfscuucauccaguUfgAfgggagsusu	661	AACUCCCUCAACUGGAUGAAGAA	845
AD-85477	asasugagAfgUfAfCfcugugagcaaL96	478	usUfsgcucacagguaCfuCfucauusgsu	662	ACAAUGAGAGUACCUGUGAGCAG	846
AD-85478	cscsaccuUfuUfCfUfucuaaugaguL96	479	asCfsuca(Tgn)uagaagaAfaAfgguggsgsa	663	UCCCACCUUUUCUUCUAAUGAGU	847
AD-85479	csgsaccaGfcUfUfGfuuugugaaaaL96	480	usUfsuuc(Agn)caaacaaGfcUfggucgsgsu	664	ACCGACCAGCUUGUUUGUGAAAC	848
AD-85480	ascsuuuUfcUfUfCfuaaugagucaL96	481	usGfsacu(Cgn)auuagaaGfaAfaaggusgsg	665	CCACCUUUUCUUCUAAUGAGUCG	849
AD-85481	gsuscaucCfaCfAfAfugagaguacaL96	482	usGfsuac(Tgn)cucauugUfgGfaugacsgsa	666	UCGUCAUCCACAAUGAGAGUACC	850
AD-85482	csascaauGfaGfAfGfuaccugugaaL96	483	usUfscac(Agn)gguacucUfcAfuugugsgsa	667	UCCACAAUGAGAGUACCUGUGAG	851
AD-85483	gsuscuccCfaCfCfUfuuucuucuaaL96	484	usUfsaga(Agn)gaaaaggUfgGfgagacsusg	668	CAGUCUCCCACCUUUUCUUCUAA	852
AD-	ascsuuucCfaGfCfAfaaacucccuaL96	485	usAfsggg(Agn)guuuugcUfgGfaaagusgsa	669	UCACUUUCCAGCAAAACUCCCUC	853

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
85484						
AD-85485	cscsucaaCfuGfGfAfugaagaaacuL96	486	asGfsuuu(Cgn)uucauccAfgUfugaggsgsa	670	UCCCUCAACUGGAUGAAGAAACU	854
AD-85486	csusguuuGfcUfGfUfguaugaucaaL96	487	usUfsgau(Cgn)auacacaGfcAfaacagsgsa	671	UCCUGUUUGCUGUGUAUGAUCAA	855
AD-85487	ususugcuGfuGfUfAfugaucaaagaL96	488	usCfsuuu(Ggn)aucauacAfcAfgcaaascsa	672	UGUUUGCUGUGUAUGAUCAAAGC	856
AD-85488	cscsgaccAfgCfUfUfguuugugaaaL96	489	usUfsuca(Cgn)aaacaagCfuGfgucggsusu	673	AACCGACCAGCUUGUUUGUGAAA	857
AD-85489	uscscaacCfgAfCfCfagcuuguuuaL96	490	usAfsaac(Agn)agcugguCfgGfuuggasasu	674	AUUCCAACCGACCAGCUUGUUUG	858
AD-85490	cscsauucCfuGfUfUfugcuguguauL96	491	asUfsaca(Cgn)agcaaacAfgGfaauggsgsc	675	GCCCAUUCCUGUUUGCUGUGUAU	859
AD-85491	csasccuuUfuCfUfUfcuaaugaguaL96	492	usAfscuc(Agn)uuagaagAfaAfaggugsgsg	676	CCCACCUUUUCUUCUAAUGAGUC	860
AD-85492	gsusuugcUfgUfGfUfaugaucaaaaL96	493	usUfsuug(Agn)ucauacaCfaGfcaaacsasg	677	CUGUUUGCUGUGUAUGAUCAAAG	861
AD-85493	gscsugagAfaGfAfUfugacagguuaL96	494	usAfsacc(Tgn)gucaaucUfuCfucagcsasg	678	CUGCUGAGAAGAUUGACAGGUUC	862
AD-85494	ususccagCfaAfAfAfcucccucaaaL96	495	usUfsuga(Ggn)ggaguuuUfgCfuggaasasg	679	CUUUCCAGCAAAACUCCCUCAAC	863
AD-85495	usgscugaGfaAfGfAfuugacagguuL96	496	asAfsccu(Ggn)ucaaucuUfcUfcagcasgsc	680	GCUGCUGAGAAGAUUGACAGGUU	864
AD-85496	uscsucacUfuUfCfCfagcaaaacuaL96	497	usAfsguu(Tgn)ugcuggaAfaGfugagascsc	681	GGUCUCACUUUCCAGCAAAACUC	865
AD-85497	uscscacaAfuGfAfGfaguaccuguaL96	498	usAfscag(Ggn)uacucucAfuUfguggasusg	682	CAUCCACAAUGAGAGUACCUGUG	866
AD-85498	cscsaccuCfgUfCfAfuccacaaugaL96	499	usCfsauu(Ggn)uggaugaCfgAfgguggsasa	683	UUCCACCUCGUCAUCCACAAUGA	867
AD-85499	uscsacuuUfcCfAfGfcaaaacuccaL96	500	usGfsgag(Tgn)uuugcugGfaAfagugasgsa	684	UCUCACUUUCCAGCAAAACUCCC	868
AD-85500	uscsccucAfaCfUfGfgaugaagaaaL96	501	usUfsucu(Tgn)cauccagUfuGfagggasgsu	685	ACUCCCUCAACUGGAUGAAGAAA	869
AD-85501	gsasgaguAfcCfUfGfugagcagcuaL96	502	usAfsgcu(Ggn)cucacagGfuAfcucucsasu	686	AUGAGAGUACCUGUGAGCAGCUG	870
AD-85502	asgsaauuCfcAfAfCfcgaccagcuuL96	503	asAfsgcu(Ggn)gucgguuGfgAfauucususu	687	AAAGAAUUCCAACCGACCAGCUU	871
AD-85503	csasuuccUfgUfUfUfgcuguguauaL96	504	usAfsuac(Agn)cagcaaaCfaGfgaaugsgsg	688	CCCAUUCCUGUUUGCUGUGUAUG	872
AD-85504	gsasauucCfaAfCfCfgaccagcuuaL96	505	usAfsagc(Tgn)ggucgguUfgGfaauucsusu	689	AAGAAUUCCAACCGACCAGCUUG	873
AD-85505	csasuccaCfaAfUfGfagaguaccuaL96	506	usAfsggu(Agn)cucucauUfgUfggaugsasc	690	GUCAUCCACAAUGAGAGUACCUG	874
AD-85506	cscscauuCfcUfGfUfuugcuguguaL96	507	usAfscac(Agn)gcaaacaGfgAfaugggscsg	691	CGCCCAUUCCUGUUUGCUGUGUA	875
AD-85507	csusggguUfuAfUfUfuuagagaauaL96	508	usAfsuuc(Tgn)cuaaaauAfaAfcccagscsa	692	UGCUGGGUUUAUUUUAGAGAAUG	876
AD-85508	gscsugggUfuUfAfUfuuuagagaauL96	509	asUfsucu(Cgn)uaaaauaAfaCfccagcsasa	693	UUGCUGGGUUUAUUUUAGAGAAU	877
AD-85509	asusggcaUfgCfAfCfagugagcuauL96	510	asUfsagc(Tgn)cacugugCfaUfgccausasu	694	AUAUGGCAUGCACAGUGAGCUAU	878
AD-85510	gsasgagaGfcCfCfAfcagagucuaaL96	511	usUfsaga(Cgn)ucugugqGfcUfcucucsusc	695	GAGAGAGAGCCCACAGAGUCUAC	879
AD-85511	gscsaagaAfcCfAfGfuguuuagcgaL96	512	usCfsgcu(Agn)aacacugGfuUfcuugcscsu	696	AGGCAAGAACCAGUGUUUAGCGC	880
AD-85512	cscsagcaAfaAfCfUfcccucaacuaL96	513	usAfsguu(Ggn)agggaguUfuUfgcuggsasa	697	UUCCAGCAAAACUCCCUCAACUG	881
AD-85513	csasccucGfuCfAfUfccacaaugaaL96	514	usUfscau(Tgn)guggaugAfcGfaggugsgsa	698	UCCACCUCGUCAUCCACAAUGAG	882
AD-85514	csgsucauCfcAfCfAfaugagaguaaL96	515	usUfsacu(Cgn)ucauuguGfgAfugacgsasg	699	CUCGUCAUCCACAAUGAGAGUAC	883
AD-85515	csuscccaCfgCfUfCfucuggacuuaL96	516	usAfsagu(Cgn)cagagagCfgUfgggagsgsa	700	UCCUCCCACGCUCUCUGGACUUC	884
AD-85516	asascuccCfuCfAfAfcuggaugaaaL96	517	usUfsuca(Tgn)ccaguugAfgGfgaguususu	701	AAAACUCCCUCAACUGGAUGAAG	885
AD-85517	usgsagaaGfaUfUfGfacagguucauL96	518	asUfsgaa(Cgn)cugucaaUfcUfucucasgsc	702	GCUGAGAAGAUUGACAGGUUCAU	886
AD-85518	cscsucguCfaUfCfCfacaaugagaaL96	519	usUfscuc(Agn)uuguggaUfgAfcgaggsusg	703	CACCUCGUCAUCCACAAUGAGAG	887
AD-85519	gsasgaagAfuUfGfAfcagguucauaL96	520	usAfsuga(Agn)ccugucaAfuCfuucucsasg	704	CUGAGAAGAUUGACAGGUUCAUG	888
AD-	csusucuuGfgGfCfUfuccguauauaL96	521	usAfsuau(Agn)cggaagcCfcAfagaagsusu	705	AACUUCUUGGGCUUCCGUAUAUA	889

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
85520						
AD-85521	uscscaccUfcGfUfCfauccacaauaL96	522	usAfsuug(Tgn)ggaugacGfaGfguggasasg	706	CUUCCACCUCGUCAUCCACAAUG	890
AD-85522	asgsauugAfcAfGfGfuucaugcagaL96	523	usCfsugc(Agn)ugaaccuGfuCfaaucususc	707	GAAGAUUGACAGGUUCAUGCAGG	891
AD-85523	csuscccuCfaAfCfUfggaugaagaaL96	524	usUfscuu(Cgn)auccaguUfgAfgggagsusu	708	AACUCCCUCAACUGGAUGAAGAA	892
AD-85524	asasugagAfgUfAfCfcugugagcaaL96	525	usUfsgcu(Cgn)acagguaCfuCfucauusgsu	709	ACAAUGAGAGUACCUGUGAGCAG	893
AD-85619	cscsaccuUfuUfCfUfucuaaugaguL96	526	asCfsucau(Tgn)agaagaAfaAfgguggsgsa	710	UCCCACCUUUUCUUCUAAUGAGU	894
AD-85620	csgsaccaGfcUfUfGfuuugugaaaaL96	527	usUfsuuca(Cgn)aaacaaGfcUfggucgsgsu	711	ACCGACCAGCUUGUUUGUGAAAC	895
AD-85621	ascscuuuUfcUfUfCfuaaugagucaL96	528	usGfsacuc(Agn)uuagaaGfaAfaaggusgsg	712	CCACCUUUUCUUCUAAUGAGUCG	896
AD-85622	gsuscaucCfaCfAfAfugagaguacaL96	529	usGfsuacu(Cgn)ucauugUfgGfaugacsgsa	713	UCGUCAUCCACAAUGAGAGUACC	897
AD-85623	csascaauGfaGfAfGfuaccugugaaL96	530	usUfscaca(Ggn)guacucUfcAfuugugsgsa	714	UCCACAAUGAGAGUACCUGUGAG	898
AD-85624	gsuscuccCfaCfCfUfuuucuucuaaL96	531	usUfsagaa(Ggn)aaaaggUfgGfgagacsusg	715	CAGUCUCCCACCUUUUCUUCUAA	899
AD-85625	ascsuuucCfaGfCfAfaaacucccuaL96	532	usAfsggga(Ggn)uuuugcUfgGfaaagusgsa	716	UCACUUUCCAGCAAAACUCCCUC	900
AD-85626	cscsucaaCfuGfGfAfugaagaaacuL96	533	asGfsuuuc(Tgn)ucauccAfgUfugaggsgsa	717	UCCCUCAACUGGAUGAAGAAACU	901
AD-85627	csusguuuGfcUfGfUfguaugaucaaL96	534	usUfsgauc(Agn)uacacaGfcAfaacagsgsa	718	UCCUGUUUGCUGUGUAUGAUCAA	902
AD-85628	ususugcuGfuGfUfAfugaucaaagaL96	535	usCfsuuug(Agn)ucauacAfcAfgcaaascsa	719	UGUUUGCUGUGUAUGAUCAAAGC	903
AD-85629	cscsgaccAfgCfUfUfguuugugaaaL96	536	usUfsucac(Agn)aacaagCfuGfgucggsusu	720	AACCGACCAGCUUGUUUGUGAAA	904
AD-85630	uscscaacCfgAfCfCfagcuuguuuaL96	537	usAfsaaca(Agn)gcugguCfgGfuuggasasu	721	AUUCCAACCGACCAGCUUGUUUG	905
AD-85631	cscsauucCfuGfUfUfugcuguguauL96	538	asUfsacac(Agn)gcaaacAfgGfaauggsgsc	722	GCCCAUUCCUGUUUGCUGUGUAU	906
AD-85632	csasccuuUfuCfUfUfcuaaugaguaL96	539	usAfscuca(Tgn)uagaagAfaAfaggugsgsg	723	CCCACCUUUUCUUCUAAUGAGUC	907
AD-85633	gsusuugcUfgUfGfUfaugaucaaaaL96	540	usUfsuuga(Tgn)cauacaCfaGfcaaacsasg	724	CUGUUUGCUGUGUAUGAUCAAAG	908
AD-85634	gscsugagAfaGfAfUfugacagguuaL96	541	usAfsaccu(Ggn)ucaaucUfuCfucagcsasg	725	CUGCUGAGAAGAUUGACAGGUUC	909
AD-85635	ususccagCfaAfAfAfcucccucaaaL96	542	usUfsugag(Ggn)gaguuuUfgCfuggaasasg	726	CUUUCCAGCAAAACUCCCUCAAC	910
AD-85636	usgscugaGfaAfGfAfuugacagguuL96	543	asAfsccug(Tgn)caaucuUfcUfcagcasgsc	727	GCUGCUGAGAAGAUUGACAGGUU	911
AD-85637	uscsucacUfuUfCfCfagcaaaacuaL96	544	usAfsguuu(Tgn)gcuggaAfaGfugagascsc	728	GGUCUCACUUUCCAGCAAAACUC	912
AD-85638	uscscacaAfuGfAfGfaguaccuguaL96	545	usAfscagg(Tgn)acucucAfuUfguggasusg	729	CAUCCACAAUGAGAGUACCUGUG	913
AD-85639	cscsaccuCfgUfCfAfuccacaaugaL96	546	usCfsauug(Tgn)ggaugaCfgAfgguggsasa	730	UUCCACCUCGUCAUCCACAAUGA	914
AD-85640	uscsacuuUfcCfAfGfcaaaacuccaL96	547	usGfsgagu(Tgn)uugcugGfaAfagugasgsa	731	UCUCACUUUCCAGCAAAACUCCC	915
AD-85641	uscsccucAfaCfUfGfgaugaagaaaL96	548	usUfsucuu(Cgn)auccagUfuGfagggasgsu	732	ACUCCCUCAACUGGAUGAAGAAA	916
AD-85642	gsasgaguAfcCfUfGfugagcagcuaL96	549	usAfsgcug(Cgn)ucacagGfuAfcucucsasu	733	AUGAGAGUACCUGUGAGCAGCUG	917
AD-85643	asgsaauuCfcAfAfCfcgaccagcuuL96	550	asAfsgcug(Ggn)ucgguuGfgAfauucususu	734	AAAGAAUUCCAACCGACCAGCUU	918
AD-85644	csasuuccUfgUfUfUfgcuguguauaL96	551	usAfsuaca(Cgn)agcaaaCfaGfgaaugsgsg	735	CCCAUUCCUGUUUGCUGUGUAUG	919
AD-85645	gsasauucCfaAfCfCfgaccagcuuaL96	552	usAfsagcu(Ggn)gucgguUfgGfaauucsusu	736	AAGAAUUCCAACCGACCAGCUUG	920
AD-85646	csasuccaCfaAfUfGfagaguaccuaL96	553	usAfsggua(Cgn)ucucauUfgUfggaugsasc	737	GUCAUCCACAAUGAGAGUACCUG	921
AD-85647	cscscauuCfcUfGfUfuugcuguguaL96	554	usAfscaca(Ggn)caaacaGfgAfaugggscsg	738	CGCCCAUUCCUGUUUGCUGUGUA	922
AD-85648	csusggguUfuAfUfUfuuagagaauaL96	555	usAfsuucu(Cgn)uaaaauAfaAfcccagscsa	739	UGCUGGGUUUAUUUUAGAGAAUG	923
AD-85649	gscsugggUfuUfAfUfuuuagagaauL96	556	asUfsucuc(Tgn)aaaauaAfaCfccagcsasa	740	UUGCUGGGUUUAUUUUAGAGAAU	924
AD-	asusggcaUfgCfAfCfagugagcuauL96	557	asUfsagcu(Cgn)acugugCfaUfgccausasu	741	AUAUGGCAUGCACAGUGAGCUAU	925

Duplex Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Modified Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence 5' to 3'	SEQ ID NO:
85650						
AD-85651	gsasgagaGfcCfCfAfcagagucuaaL96	558	usUfsagac(Tgn)cuguggGfcUfcucucsusc	742	GAGAGAGAGCCCACAGAGUCUAC	926
AD-85652	gscsaagaAfcCfAfGfuguuuagcgaL96	559	usCfsgcua(Agn)acacugGfuUfcuugcscsu	743	AGGCAAGAACCAGUGUUUAGCGC	927
AD-85653	cscsagcaAfaAfCfUfcccucaacuaL96	560	usAfsguug(Agn)gggaguUfuUfgcuggsasa	744	UUCCAGCAAAACUCCCUCAACUG	928
AD-85654	csasccucGfuCfAfUfccacaaugaaL96	561	usUfscauu(Ggn)uggaugAfcGfaggugsgsa	745	UCCACCUCGUCAUCCACAAUGAG	929
AD-85655	csgsucauCfcAfCfAfaugagaguaaL96	562	usUfsacuc(Tgn)cauuguGfgAfugacgsasg	746	CUCGUCAUCCACAAUGAGAGUAC	930
AD-85656	csuscccaCfgCfUfCfucuggacuuaL96	563	usAfsaguc(Cgn)agagagCfgUfgggagsgsa	747	UCCUCCCACGCUCUCUGGACUUC	931
AD-85657	asascuccCfuCfAfAfcuggaugaaaL96	564	usUfsucau(Cgn)caguugAfgGfgaguusasu	748	AAAACUCCCUCAACUGGAUGAAG	932
AD-85658	usgsagaaGfaUfUfGfacagguucauL96	565	asUfsgaac(Cgn)ugucaaUfcUfucucasgsc	749	GCUGAGAAGAUUGACAGGUUCAU	933
AD-85659	cscsucguCfaUfCfCfacaaugagaaL96	566	usUfscuca(Tgn)uguggaUfgAfcgaggsusg	750	CACCUCGUCAUCCACAAUGAGAG	934
AD-85660	gsasgaagAfuUfGfAfcagguucauaL96	567	usAfsugaa(Cgn)cugucaAfuCfuucucsasg	751	CUGAGAAGAUUGACAGGUUCAUG	935
AD-85661	csusucuuGfgGfCfUfuccguauauaL96	568	usAfsuaua(Cgn)ggaagcCfcAfagaagsusu	752	AACUUCUUGGGCUUCCGUAUAUA	936
AD-85662	uscscaccUfcGfUfCfauccacaauaL96	569	usAfsuugu(Ggn)gaugacGfaGfguggasasg	753	CUUCCACCUCGUCAUCCACAAUG	937
AD-85663	asgsauugAfcAfGfGfuucaugcagaL96	570	usCfsugca(Tgn)gaaccuGfuCfaaucsusc	754	GAAGAUUGACAGGUUCAUGCAGG	938
AD-85664	csuscccuCfaAfCfUfggaugaagaaL96	571	usUfscuuc(Agn)uccaguUfgAfgggagsusu	755	AACUCCCUCAACUGGAUGAAGAA	939
AD-85665	asasugagAfgUfAfCfcugugagcaaL96	572	usUfsgcuc(Agn)cagguaCfuCfucauusgsu	756	ACAAUGAGAGUACCUGUGAGCAG	940

Table 6. Additional Modified Sense and Antisense Strand Sequences of hAGT dsRNA Agents

Duplex Name	Sense Oligo Name	Modified Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000029.3	Antisense Oligo Name	Modified Antisense Sequence	SEQ ID NO:	Range in NM_000029.3
AD-126306	A-168475	gsuscaucCfaCfAfAfugagaguacaL96	941	638-658	A-250785	usGfsua(Cgn)ucucauugUfgGfaugacsgsa	951	636-658
AD-126307	A-168477	csascaauGfaGfAfGfuaccugugaaL96	942	644-664	A-250786	usUfsca(Cgn)agguacucUfcAfuugugsgsa	952	642-664
AD-126308	A-168479	gsuscuccCfaCfCfUfuuucuucuaaL96	943	2076-2096	A-250787	usUfsag(Agn)agaaaaggUfgGfgagacsusg	953	2074-2096
AD-126310	A-168483	cscsucaaCfuGfGfAfugaagaaacuL96	944	1603-1623	A-250789	asGfsuu(Tgn)cuucauccAfgUfugaggsgsa	954	1601-1623
AD-126343	A-168549	cscsucguCfaUfCfCfacaaugagaaL96	945	634-654	A-250822	usUfscu(Cgn)auuguggaUfgAfcgaggsusg	955	632-654
AD-133360	A-168475	gsuscaucCfaCfAfAfugagaguacaL96	946	638-658	A-264752	usGfs(Tgn)acucucauugUfgGfaugacsgsa	956	636-658
AD-133361	A-168475	gsuscaucCfaCfAfAfugagaguacaL96	947	638-658	A-264753	usGfsu(Agn)cucucauugUfgGfaugacsgsa	957	636-658
AD-133362	A-168475	gsuscaucCfaCfAfAfugagaguacaL96	948	638-658	A-264754	usGfsuacuc(Tgn)cauugUfgGfaugacsgsa	958	636-658
AD-133374	A-168479	gsuscuccCfaCfCfUfuuucuucuaaL96	949	2076-2096	A-264766	usUfsagaag(Agn)aaggUfgGfgagacsusg	959	2074-2096
AD-133385	A-168477	csascaauGfaGfAfGfuaccugugaaL96	950	644-664	A-264777	usUfsc(Agn)cagguacucUfcAfuugugsgsa	960	642-664

Example 3. *In vivo* screening of dsRNA Duplexes in Mice Trandsuced with AAV expressing human AGT

[0496] To express human angiotensinogen, C57/BL6 mice were first transduced with an AAV (adeno-associated virus) vector exressing the human AGT transcript. After at least two weeks from AAV introduction, blood was obtained from mice for baseline circulating human AGT levels, and animals then received a single 3 mg/kg subcutaneous dose of one of a subset of the dsRNA agents provided in Tables 5 and 6 (N=3 per group). Blood was obtained from animals again at fourteen days post-dose of dsRNA agent. Human AGT levels were quantified using an ELISA specific for human angiotensinogen, according to manufacturer's protocol (IBL America #27412). Data were expressed as percent of baseline value, and presented as mean plus standard deviation. Certain dsRNA duplexes were selected for further analysis.

Table 7. AGT Single 3 mg/kg Dose Screen in AAV-human AGT transduced mouse

DuplexID	Avg	SD
AD-67327	13.2	4.5
AD-85110	97.0	3.6
AD-85117	86.7	10.8
AD-85118	107.2	14.2
AD-85434	18.4	0.6

DuplexID	Avg	SD
AD-85435	25.7	1.7
AD-85438	12.6	2.7
AD-85446	8.4	1.0
AD-85481	27.0	11.5
AD-85482	64.2	13.0
AD-85482	48.5	5.9
AD-85483	38.9	4.6
AD-85485	63.6	3.0
AD-85493	35.7	20.5
AD-85496	78.5	10.3
AD-85517	93.8	4.6
AD-85524	90.4	14.7
AD-85622	38.1	10.6
AD-85623	28.0	8.4
AD-85625	87.6	13.7
AD-85626	29.9	14.0
AD-85634	27.7	4.5
AD-85635	87.1	13.5
AD-126306	12.4	1.4
AD-126307	21.6	9.0
AD-126308	15.7	1.8
AD-126310	38.3	1.1
AD-126343	65.5	13.4
AD-133360	50.6	7.5
AD-133361	23.2	12.4
AD-133362	12.2	4.1
AD-133374	26.2	3.3
AD-133385	33.8	8.7

Example 4. *In vivo* screening of dsRNA Duplexes in Cynomolgus Monkeys

[0497] Duplexes of interest, identified from the above mouse studies, were evaluated in cynomolgus monkey. Animals (N=3 per group) received a single 3 mg/kg subcutaneous dose of a dsRNA agent (AD-85481, AD-126306, AD-126307, AD-126308, or AD-133362) on day 1. Blood was obtained on days -6, 1, 4, 8, 15, 22, 29, 32, 35, 43, 57, 71, 85, and 99 post-dose. Circulating AGT levels were quantified using an ELISA specific for human angiotensinogen (and cross-reactive with cynomolgus), according to manufacturer's protocol (IBL America #27412). Data were expressed as percent of baseline value, and presented as mean plus/minus standard deviation. The results are showin in Figure 1A. The apparent differences between AD-85481, AD-126306, and AD-133362 are within the range of typical study-to-study variability in non-human primates.

[0498] Dose-response studies were performed to assess the activity of AD-67327 and AD-85481. Although the experiments were performed separately, the methods used were essentially the same and the results are presented together in Figure 1B. Cynomolgus monkeys received a single 0.3 mg/kg, 1 mg/kg, or 3 mg/kg subcutaneous dose of dsRNA agent on day 1. Blood was obtained on days -6, 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71 and 78 post-dose for AD-67327 and on days -6, 1, 4, 8, 15, 22, 29, 32, 35, 43, 57, 71, 85, and 99 post-dose for AD-85481. Circulating AGT levels were quantified using an ELISA specific for human angiotensinogen (and cross-reactive with cynomolgus), according to manufacturer's protocol (IBL America #27412). Data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results are shown in Figure 1B. These data demonstrate a roughly 3-fold improvement in efficacy and duration for AD-85481 over AD-67327.

[0499] Multidose studies were performed to determine the potency and durability of AD-67327 and AD-85481. Although the experiments were performed separately, the methods used were essentially the same and the results are presented together in Figure 1C. Cynomolgus monkeys were subcutaneously administered a 1 mg/kg dose of AD-67327 or AD-85481 once every four weeks for three weeks (q4w dosing) (days 1, 29, and 57 post first dose). Blood was obtained for evaluation of circulating AGT at days -6, 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71 and 78 post first dose of AD-67327 and at days -8, 1, 4, 8, 15, 22, 29, 36, 43, 57, 64, 71, 85, 99 post first dose of AD-85481. These data demonstrate an increase in potency and durability of target silencing by AD-85481 compared to AD-67327.

Example 5. Treatement of Hypertension with an AGT dsRNA in a Spontaneous Hypertensive Rat Model

[0500] A rat specific dsRNA was designed to test the effect of AGT knockdown in a spontaneously hypertensive rat model. Spontaneously hypertensive rats (N=9 per group) were subcutaneously administered a 10 mg/kg dose of the rat-specific AGT dsRNA once every 2 weeks (10 mg/kg q2w), or daily oral doses of the ARB valsartan (31 mg/kg/day), or daily oral doses of the ACE inhibitor captopril (100 mg/kg/day). Select combinations (the rat specific dsRNA agent plus valsartan or captopril plus valsartan) were also evaluated, dosed as noted above. Mean arterial pressure was measured by telemetry over a 4-week period. After four weeks of treatment, animals were anaesthetized by i.p. pentobarbital injection, and blood collected from the hepatic portal vein for the measurement of plasma AGT, plasma Renin, plasma Angiotensin II, plasma aldosterone, plasma K^+, and plasma Renin activity. Heart weights and tibia length (for normalization of heart weights) were also obtained.

[0501] Treatment with the rat-specific dsRNA agent knocked down plasma AGT levels by over 98% when administered alone or in combination with valsartan relative to pre-treatment levels (Figure 2A). Treatment with the combination of valsartan and captopril was also demonstrated to significantly decrease serum AGT levels. All treatments increased the level of Renin, with the greatest increase occurring in animals treated with a combination of valsartan and the dsRNA agent, followed by a substantial increase in the dsRNA agent alone-treated group. Only combination treatment with valsartan and the dsRNA agent was found to lower circulating Angiotensin II levels. A trend towards reduced urinary AGT was observed after treatment with the dsRNA agent, suggesting that levels of AGT protein in the kidney are not significantly inhibited by treatment with the dsRNA agent (Figure 3). Only the combination of valsartan and the dsRNA agent was found to significantly lower urinary AGT. No treatments altered aldosterone levels. Plasma K^+ tended to increase in all groups, with significance only being reached in the combination valsartan plus siRNA treatment group.

[0502] Figure 2B shows mean arterial pressure levels measured by telemetry throughout the experiment and graphed relative to starting blood pressure levels. Each of the treatments caused a statistically significant decrease in blood pressure as compared to untreated animals. Statistical comparisons ($p<0.05$) are noted relative to baseline (#) or valsartan plus captopril ($). Treatment with valsaratan plus the rat-specific dsRNA agent was significantly better than treatment with captopril plus valsartan in lowering mean arterial pressure.

[0503] Figure 2C shows heart weights normalized to tibial lengths to provide a measure of cardiac hypertrophy. Treatment with both valsartan plus captopril and valsartan plus the dsRNA agent were effective at reducing cardiac hypertrophy relative to control ($p<0.05$), with valsartan plus the dsRNA agent also reducing cardiac hypertrophy relative to valsartan plus captopril ($p<0.05$). Figure 2E depicts the same data as a scatterplot of heart weight to tibial length versus MAP. A linear relationship between cardiac hypertrophy and MAP is observed, with valsartan plus the dsRNA agent providing the greatest reduction in cardiac hypertrophy. Cardiomyocyte size was reduced relative to vehicle by all groups except valsartan (Figure 2F), while NT-proBNP was reduced in the captopril plus valsartan group and a trend to reduction was observed in the valsartan plus dsRNA group (Figure 2G).

[0504] Figure 2D shows relative Renin activity at level at 4 weeks relative to baseline. Plasma Renin activity (PRA), which reflects reduced angiotensin signaling, indicates a clear increase in PRA with the dsRNA agent treatment ($p<0.05$ relative to both baseline and control group, for both the dsRNA agent alone and valsartan plus siRNA). The PRA assay measures Renin activity by quantifying the amount of Angiotensin I produced by Renin in a blood sample, in the presence of excess angiotensinogen. These data demonstrate a reduction in Angiotensin II signaling following that treatment with AGT-dsRNA agent, and that the effect is enhanced by co-treatment with valsartan. Due to upregulation, circulating angiotensin II remains intact even when AGT levels are almost completely knocked down. These data demonstrate that AGT-dsRNA agent causes a similar antihypertensive effect as valsartan and captopril. Without being bound by theory, it is proposed that only when combining dsRNA agent plus valsartan do angiotensin II levels collapse, resulting in a synergistic decrease in blood pressure.

[0505] The effect of various treatments on blood and renal AngI and AngII levels was investigated after four weeks of treatment. Figures 4A-4C show that treatment with valsartan and captopril, either alone or in combination, significantly increased blood levels of AngI as compared to vehicle control (Figure 4A). The dsRNA agent alone did not significantly alter blood levels of AngI, but the combination of valsartan with the dsRNA agent significantly decreased blood AngI as compared to vehicle control and treatment with the dsRNA agent alone. Valsartan alone was found to significantly increase blood AngII as compared to vehicle control (Figure 4B). Combination of valsartan with the dsRNA agent was found to significantly decrease blood AngII as compared to vehicle control. These changes resulted in a significant decrease in the ratio of AngII/AngI in the captopril and captopril + valsartan treated animals. The data for captopril and valsartan and consistent with their mechanisms of action, while the data for the dsRNA alone indicates little effect on AngII/I in the blood.

[0506] Figures 5A-5C show that the dsRNA agent reduced renal AngI without apparent effect on renal AngII, resulting in an upregulated renal AngII/I ratio. Figure 5A shows that each valsartan and captopril significantly increased renal AngI, while the combination of the agents did not have a significant effect on the level of AngI. Renal AngI was significantly decreased by the dsRNA agent and the combination of the dsRNA agent with valsartan. Moreover, the combination treatment significantly reduced renal AngI level as compared to treatment with the dsRNA agent alone. Figure 5B shows no significant change in renal AngII after treatment with any of the monotherapies except captoril, *i.e.,* valsartan or the dsRNA agent alone. However, the combination of captopril and valsartan and the combination of valsartan and the dsRNA agent were demonstrated to significantly reduce renal Ang II. The data for captopril and valsartan are consistent with their mechanisms of action, while the data for the dsRNA alone indicates little apparent effect on AngII in the kidneys. The renal AngII/AngI ratio increased by 4-fold after administration of the AGT dsRNA agent alone (Figure 5C). Conversely, a decrease of over 70% in the Ang II/Ang I ratio was seen after treatment with valsartan, captopril, and the combination of valsartan + captopril. No significant change in the ratio of AngII/AngI was observed after treatment with the valsartan + AGT dsRNA agent. The increase in renal AngII was demonstrated to not be a result of alterations in renal angiotensin receptor levels or ACE mRNA expression in renal cortex or medulla. Figures 6A-6C show no significant changes in AT1a receptor, AT1b receptor, or ACE mRNA level in kidney under any treatment conditions except one. Treatment with captopril caused a significant increase in AT1b receptor level in kidney medulla. The effect of the treatment regimens on kidney function was also assessed. No changes in glomerular filtration rate (GFR), natriuresis, and albuminuria were observed. This indicates that these treatments did not impair kidney function.

[0507] Urinary volume and urinary sodium were monitored during the experiment. Treatment with valsartan + dsRNA agent, valsartan + captopril, and captopril alone caused a significant increase in urinary volume within groups between baseline and 4 weeks (Figure 7). Comparison of urinary volume across groups at 4 weeks showed a significant increase in urinary output in captopril treated animals as compared to all other groups. Treatement with valsartan + dsRNA agent resulted in a significant increase in urinary output at 4 weeks as compared to treatment with valsartan alone or vehicle. No significant changes in urinary sodium were observed across or within groups during the experiment.

[0508] These data demonstrate that the reduced renal Ang II/I ratio during both ACEi and ARB confirms that renal ACE generates Ang II, and that tissue Ang II represents AT1R-internalized Ang II (van Esch et al., Cardiovasc Res 2010 86(3):401-409). Further, the lowering of renal Ang I after liver-targeted AGT siRNA treatment demonstrates that renal Ang generation depends on hepatic AGT. Although, urinary AGT is partly kidney-derived, this renal AGT does not contribute to renal Ang generation, as has been suggested before (Matsusaka et al., JASN 2012 23: 1181-1189). The increased renal Ang II/I ratio after AGT dsRNA treatment, allowing renal Ang II levels to remain intact, is suggestive for enhanced Ang II internalization, albeit in the absence of AT1b receptor upregulation. In agreement with this concept, additive ARB exposure virtually abolished renal Ang II. Treatment with the liver-specific AGT dsRNA agent synergistically lowers arterial pressure when combined with existing RAS blockers and lowers renal Ang production, without apparent negative effects on renal function.

Example 6. Treatement of Hypertension with an AGT dsRNA in a High Salt Rat Model

[0509] The deoxycorticosterone acetate (DOCA)-salt rat model is a well established model for hypertension in the context of high salt levels, and is considered a model of neurogenic hypertension due to the effect on central and peripheral nervous systems (Basting T & Lazartigues E, Cur Hypertension Rep 2017).

[0510] Upon arrival, Sprague-Dawley rats are allowed to acclimatize for 7 days. Subsequently, telemetry transmitters are implanted intra-abdominally, in the abdominal aorta, in the rats under isoflurane anaesthesia. The rats are allowed to recover from this procedure for 10 days. From then onwards, blood pressure, heart rate, and other indicators of hypertension are measured by telemetry over a 7-week time period. During the first 4 weeks, animals are subcutaneoulsy implanted with a 200 mg DOCA pellet and receive 0.9% salt in the drinking water (ad libidum) on a chronic basis to induce hypertension. After this period during which hypertension begins, a 3-week treatment period is initiated. Rats are treated with

1. 1) vehicle;
2. 2) valsartan, 31 mg/kg/day added to drinking water;
3. 3) an AGT dsRNA agent, 10 mg/kg once every two weeks, subcutaneously;
4. 4) spironolactone, 50 mg/kg/day, subcutaenously; a combination of an AGT dsRNA agent, 10 mg/kg once every two weeks, subcutaneously, and valsartan, 31 mg/kg/day added to drinking water;
5. 5) an AGT dsRNA agent, 10 mg/kg once every two weeks, subcutaneously, and spironolactone, 50 mg/kg/day, subcutaneously;
6. 6) valsartan, 31 mg/kg/day added to drinking water, and spironolactone, 50 mg/kg/day, subcutaneously; or
7. 7) an AGT dsRNA agent, 30 mg/kg once every two weeks, subcutaneously. In addition, one group of rats that do not receive DOCA and salt in the drinking water serves as controls to evaluate the effect of the treatments to normalize blood pressure.

Example 7. Treatment of Obesity with an AGT dsRNA Agent in a High Fat Fed Mouse Model of Diet Induced Obesity (DIO)

[0511] Sixteen-week old high fat fed (HFF) obese mice (diet-induced obesity (DIO)) and normal-weight control animals were purchased and kept on their respective high fat diet (60% of calories as fat) or normal chow. After acclimatization, animals were divided into four groups: Normal weight + PBS; Normal weight + AGT dsRNA; DIO + PBS; and DIO + AGT dsRNA (n=5/group). Animals received 10 mg/kg mouse-specific dsRNA or PBS every other week for 12 weeks starting at week 0. Animals were weighed and blood obtained biweekly. Serum AGT levels were determined by ELISA. A fasting glucose tolerance test was performed predose, at 6 weeks post-first dose, and at twelve weeks post-first dose. Organ weights were determined at study end.

[0512] Administration of the AGT dsRNA agent was effective at silencing AGT in both the high fat and normal chow animals with sustained knockdown of about 93% across AGT dsRNA agent treatment groups starting at the first time point, two weeks after the first administration of the dsRNA agent.

[0513] Treatment with the AGT dsRNA agent was effective at significantly decreasing weight gain as compared to the PBS treated DIO mice, as determined by two-way repeated measures ANOVA in the DIO mice, starting at two weeks post first dose and maintained throughout the study (Figure 8A). In an analysis comparing starting weights, the DIO+AGT dsRNA group did not gain weight relative to starting weight until the last time-point. Prior to the final time point, mice either lost weight relative to start weight (weeks 2, 4, 6), or there was no difference in weight (weeks 8, 10). No significant difference in weight was observed between the PBS and AGT dsRNA agent chow fed mice until weeks 10 and 12 of the study.

[0514] Organ weights were determined at the end of the study to assess the effect of treatment with the AGT dsRNA agent on the location of fat deposition. Liver weights of the AGT dsRNA agent treated DIO mice were significantly lower than the PBS treated DIO mice (Figure 8B). No significant difference in liver weight was observed between the AGT dsRNA agent and PBS treated normal chow fed mice. Adipose tissue (epidydymal) weight was statistically higher in the DIO+AGT siRNA group than the DIO+PBS, while the opposite was true for the normal-weight animals. There was no significant difference in calf muscle weights across all four groups.

[0515] Glucose tolerance tests were performed at week 0 (predose), week 6, and week 12 using a standard protocol. Blood glucose was measured at predose, 30, 60, 90, and 120 minutes post bolus intraperitoneal glucose dose administration using an AlphaTRAK®2 glucometer (Abbott Animal Health). The results are shown in Figures 9A-9C. At week 0, DIO mice had decreased glucose tolerance as compared to the chow fed controls. By six weeks, a significance difference was observed in multiple comparisons post-test between the AGT dsRNA agent treated DIO mice and the PBS treated DIO mice. At twelve weeks, and excluding one DIO+PBS animal whose values were above the glucometer limit, there continued to be a significant difference between AGT dsRNA agent treated DIO mice and the PBS treated DIO mice. Additionally, the data from DIO+AGT siRNA group were not different from either control group (AGT dsRNA agent treated or PBS treated chow fed mice) at six or twelve weeks.

Example 8. Treatment of NASH with an AGT dsRNA Agent in a High Fat High Fructose Mouse Model

[0516] A high fat-high fructose (HF HFr) fed mouse model of NASH (Softic et al. J Clin Invest 127 (11):4059-4074, 2017) was used to demonstrate the efficacy of AGT siRNA to treat NASH and signs of metabolic disorder.

[0517] Six to eight weeks-old C57BL/6 male mice obtained from Jackson Laboratories were fed a high fat diet containing 60% of calories as fat plus 30% fructose in water (Hf Hfr diet) for 12 weeks prior to treatment with an AGT dsRNA agent or PBS (control) in order to induce NASH or fed a standard chow and water diet. Food and water were provided *ad libitum*. Starting at week 12, HF HFr fed mice were subcutaneously administered a 10 mg/kg dose of a dsRNA agent targeted to AGT every other week for a total of four doses. Two weeks after the final dose (at week 20), livers were harvested, RNA was isolated, and AGT knockdown in liver was determined by RT-qPCR using the method described above. A 93% decrease in liver AGT mRNA was observed in the AGT dsRNA agent treated Hf Hfr fed mice as compared to the PBS treated HF HFr fed mice.

[0518] As expected, body weight (Figure 10A), cumulative weight gain (Figure 10B), and terminal liver weight were significantly higher in the HF HFr fed control mice as compared to chow fed mice at all time points. Treatment with the AGT dsRNA agent in the HF HFr mice lost weight from week 12 till week 20 which resulted in a significant decrease in terminal body weight as compared to the control treated mice ($p = 0.0023$). No significant difference in terminal liver weights was observed.

[0519] Serum and liver lipids and glucose, and serum insulin were assessed to determine the effect of the dsRNA agent in the HF HFr model. At week 20, serum triglycerides and glucose levels were substantially the same across all three groups (normal chow, HF HFr dsRNA, HF HFr control). Serum cholesterol and insulin levels were about the same in both HF Hfr groups, and significantly higher than in the chow fed group, as expected. Treatment with the AGT dsRNA agent was demonstrated to significantly decrease serum non-esterified fatty acids (NEFA) ($p = 0.01$). In liver, cholesterol was elevated in both HF HFr groups as compared to normal chow control, but again, no significant decrease was observed after treatment with the AGT dsRNA agent. A significant decrease in liver triglycerides ($p = 0.017$) and free fatty acids ($p = 0.001$) was observed in the AGT dsRNA agent treated group as compared to the control treated group. A possible trend towards decreased thiobarbituric acid (TBA), an indicator of lipid oxidation, was seen in the AGT dsRNA treated group.

[0520] Liver injury was indicated by a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH) levels in the control treated Hf Hfr mice as compared to chow fed mice. Treatment with the AGT dsRNA agent resulted in a significant decrease in ALT ($p = 0.01$) (Figure 11A) with a trend towards decreased AST (Figure 11B) and GLDH (Figure 11C) as compared to control treated HF HFr mice.

[0521] Liver injury was also assessed by histopathology and NAS scores. As expected, the HF HFr diet induced significant steatosis, balloon degeneration, and lobular inflammation resulting in an increase in the overall NAS score as compared to chow fed mice. Treatment with the AGT dsRNA agent resulted in a significant decrease in balloon degeneration ($p = 0.04$) with a trend towards decreased lobular inflammation resulting in a significant decrease in the overall NAS score ($p = 0.01$) in the AGT dsRNA agent treated HF HFr fed animals as compared to the control treated HF HFr fed animals.

[0522] These data demonstrate that treatment with the AGT dsRNA agent is effective in ameliorating some of the signs of NASH. Notably, treatment with the AGT dsRNA agent was effective in reducing weight and liver injury enzymes, with a significant reduction in ALT and a slight reduction in AST and GLDH. Reductions in lobular inflammation and ballooning scores were also observed, bringing down the overall NAS score.

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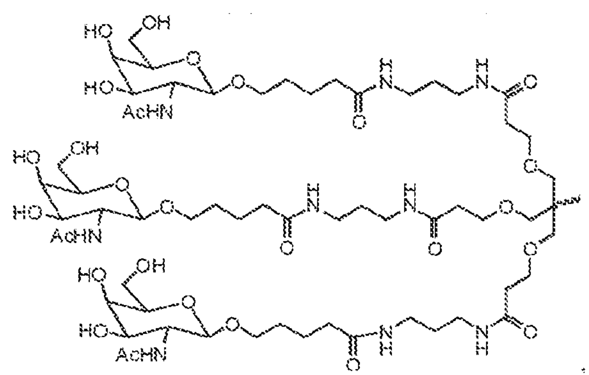
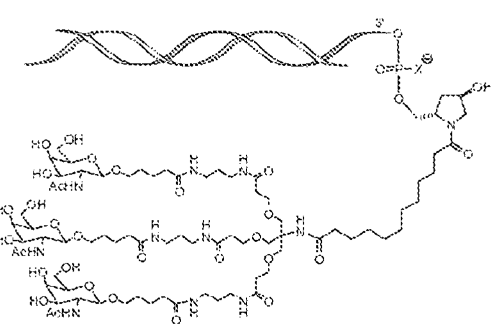
Titel; Angiotensinogen (AGT) iRNA sammensætninger og anvendelsesmetoder deraf

1. Et dobbeltstrenget ribonukleinsyre (dsRNA)-middel eller salt deraf til inhibering af ekspression af angiotensinogen (AGT), hvori dsRNA-midlet eller saltet deraf omfatter en sense-streng og en antisense-streng som danner en dobbeltstrenget region, hvor sense-strengen omfatter nukleotidsekvensen 5'-gsuscaucCfaCfAfAfuga-gaguaca-3' (SEQ ID NO:482), og antisense-strengen omfatter nukleotidsekvensen 5'-usGfsuac(Tgn)cu-cauugUfgGfaugacsgsa-3' (SEQ ID NO:666),
hvori a, g, c og u er henholdsvis 2'-O-methyl (2'-OMe) A, G, C og U; Af, Gf, Cf og Uf er henholdsvis 2'-fluor A, G, C og U; s er en phosphorthioatbinding; og (Tgn) er en thymidin-glycol-nukleinsyre (GNA) S-isomer, og
som yderligere omfatter en ligand, som er et N-acetylgalactosamin (GalNAc)-derivat.

2. DsRNA-midlet eller saltet deraf ifølge krav 1, hvor liganden er:
(i) konjugeret til 3'-enden af sense-strengen af dsRNA-midlet eller saltet deraf;
og/eller
(ii) et N-acetylgalactosamin (GalNAc) derivat som er



hvori yderligere dsRNA-midlet eller saltet deraf er eventuelt konjugeret til liganden som vist skematisk i det følgende



og hvor X er O eller S; eller
(iv) et eller flere GalNAc-derivater bundet gennem en monovalent, bivalent eller trivalent forgrenet linker.

3. En isoleret celle indeholdende dsRNA-midlet eller saltet deraf ifølge krav 1 eller 2.

4. En farmaceutisk sammensætning til inhibering af ekspression af et gen, der koder for AGT, omfattende dsRNA-midlet, eller salt deraf ifølge krav 1 eller 2.

5. En farmaceutisk sammensætning omfattende dsRNA-midlet eller saltet deraf ifølge krav 1 eller 2 og en lipidformulering.

6. En *in vitro* fremgangsmåde til inhibering af ekspression af et AGT gen i en celle, hvor fremgangsmåde omfatter at bringe cellen i kontakt med dsRNA-midlet eller saltet deraf ifølge krav 1 eller 2 eller den farmaceutiske sammensætning ifølge krav 4 eller 5, hvorved ekspression af AGT genet i cellen inhiberes.

7. DsRNA-midlet eller saltet deraf ifølge krav 1 eller 2 eller den farmaceutiske sammensætning ifølge krav 4 eller 5 til anvendelse ved behandling af et individ med en AGT-associeret lidelse.

8. DsRNA-midlet, eller saltet deraf, eller den farmaceutiske sammensætning til anvendelse ifølge krav 7, hvor individet har været diagnosticeret med en AGT-associeret lidelse, eventuelt hvor den AGT-associerede lidelse er valgt fra gruppen bestående af højt blodtryk, forhøjet blodtryk, borderline forhøjet blodtryk, primær forhøjet blodtryk, sekundær forhøjet blodtryk isoleret systolisk eller diastolisk forhøjet blodtryk, graviditetsassocieret forhøjet blodtryk, diabetisk forhøjet blodtryk, resistent forhøjet blodtryk, refraktær forhøjet blodtryk, paroxysmal forhøjet blodtryk, renovaskulær forhøjet blodtryk, Goldblatt forhøjet blodtryk, forhøjet blodtryk forbundet med lav plasmareninaktivitet eller plasmareninkoncentration, okulær forhøjet blodtryk, glaukom, pulmonal forhøjet blodtryk, portal forhøjet blodtryk, systemisk venøs forhøjet blodtryk, systolisk forhøjet blodtryk, labil forhøjet blodtryk; hypertensivt hjerte sygdom, hypertensiv nefropati, åreforkalkning, arteriosklerose, vaskulopati, diabetisk nefropati, diabetisk retinopati, kronisk hjertesvigt, kardiomyopati, diabetisk hjertemyopati, glomerulosklerose, koarktation af aorta, aortaaneurisme, ventrikulær fibrose, hjertesvigt, myokardieinfarkt, angina, slagtilfælde, nyresygdom, nyresvigt, systemisk sklerose, intrauterin vækstrestriktion (IUGR), føtal vækstrestriktion, fedme, leversteatose/ fedtlever, ikke-alkoholisk Steatohepatitis (NASH), ikke-alkoholisk fedtleversygdom (NAFLD); glukoseintolerance, type 2-diabetes (ikke-insulinafhængig diabetes) og metabolisk syndrom.

9. DsRNA-middelet, eller saltet deraf, eller den farmaceutiske sammensætning til anvendelse ifølge krav 7 eller 8, hvor kontakt med cellen med dsRNA-midlet, eller salt et deraf, eller den farmaceutiske sammensætning hæmmer ekspressionen af AGT med mindst 50 %, 60 %, 70 %, 80 %, 90 %, 95 %.

10. DsRNA-midlet eller saltet deraf eller den farmaceutiske sammensætning til anvendelse ifølge et hvilket som helst af kravene 7-9, hvor individet:
(i) har et systolisk blodtryk på mindst 130 mm Hg eller et diastolisk blodtryk på mindst 80 mm Hg;
(ii) har et systolisk blodtryk på mindst 140 mm Hg og et diastolisk blodtryk på mindst 80 mm Hg;
(iii) er et menneske; og/eller

(iv) er en del af en gruppe, der er modtagelig for saltfølsomhed, er overvægtig, er svær overvægtig eller er gravid.

11. DsRNA-midlet eller saltet deraf eller den farmaceutiske sammensætning til anvendelse ifølge et hvilket som helst af kravene 7-10, hvor dsRNA-midlet indgives til individet i en dosis på ca. 0,01 mg/kg til ca. 50 mg/kg, og/eller hvor dsRNA- midlet eller saltet deraf eller den farmaceutiske sammensætning indgives subkutant; og/eller hvor et yderligere terapeutisk middel til behandling af forhøjet blodtryk eventuelt indgives til individet hvor det yderligere terapeutiske middel:
(a) er valgt fra gruppen bestående af et diuretikum, en angiotensin-konverterende enzym (ACE)-hæmmer, en angiotensin II-receptorantagonist, en betablokker, en vasodialator, en calciumkanalblokker, en aldosteron antagonist, en alfa2-agonist, en reninhæmmer, en alfablokker, et perifert virkende adrenergen middel, en selektiv D1 receptor partiel agonist, en ikke-selektiv alfa-adrenergen antagonist, en syntetisk, et steroid antimineralocorticoid middel, en angiotensin-receptor-neprilysin-hæmmer (ARNi), Entresto®, sacubitril/valsartan; eller en endotelin receptor antagonist (ERA), sitaxentan, ambrisentan, atrasentan, BQ-123, zibotentan, bosentan, macitentan og tezosentan; en kombination af et hvilket som helst af de foregående; og et hypertensionsterapeutisk middel formuleret som en kombination af midler; eller
(b) omfatter en angiotensin II-receptor antagonist, yderligere eventuelt hvor angiotensin II-receptorantagonisten er valgt fra gruppen bestående af losartan, valsartan, olmesartan, eprosartan og azilsartan.

12. Et kit omfattende dsRNA-midlet eller saltet deraf ifølge krav 1 eller 2 eller den farmaceutiske sammensætning ifølge krav 4 eller 5.

DRAWINGS

FIG. 1A

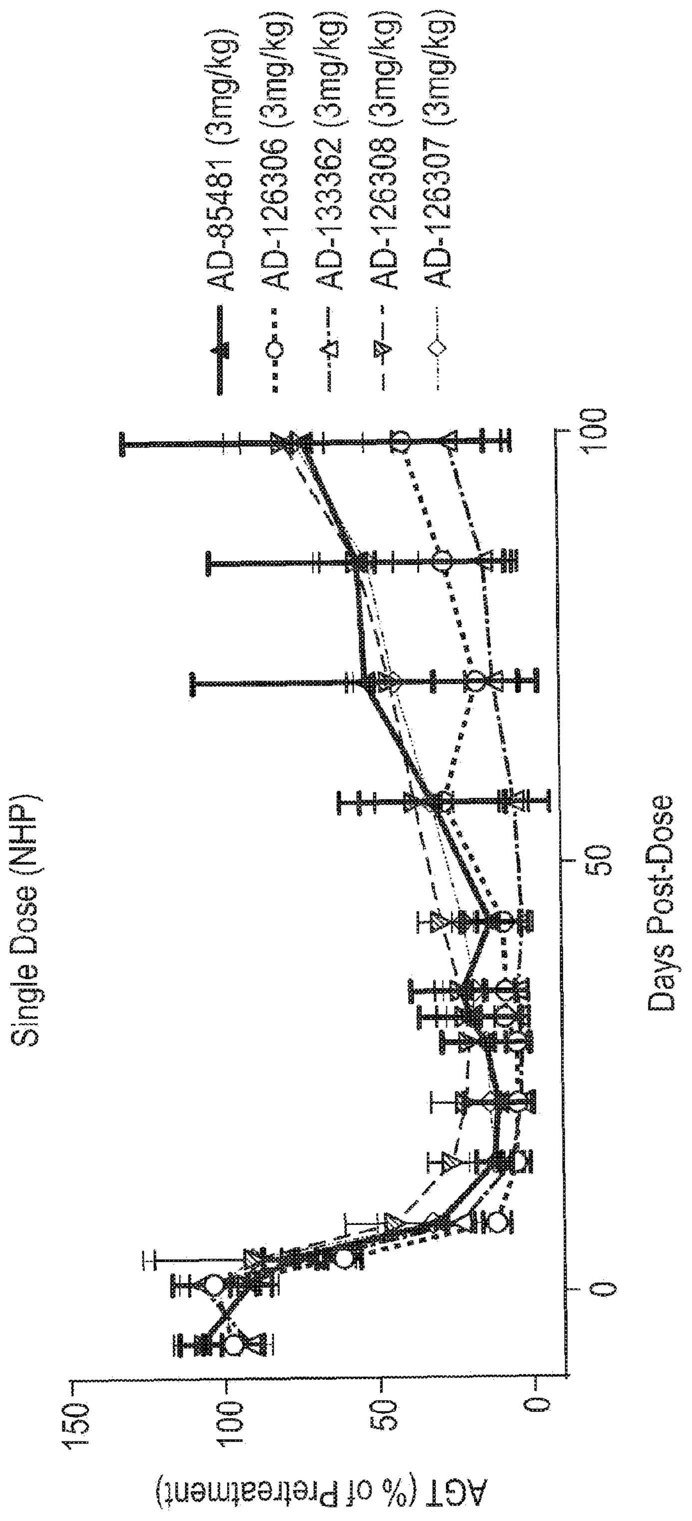


FIG. 1B

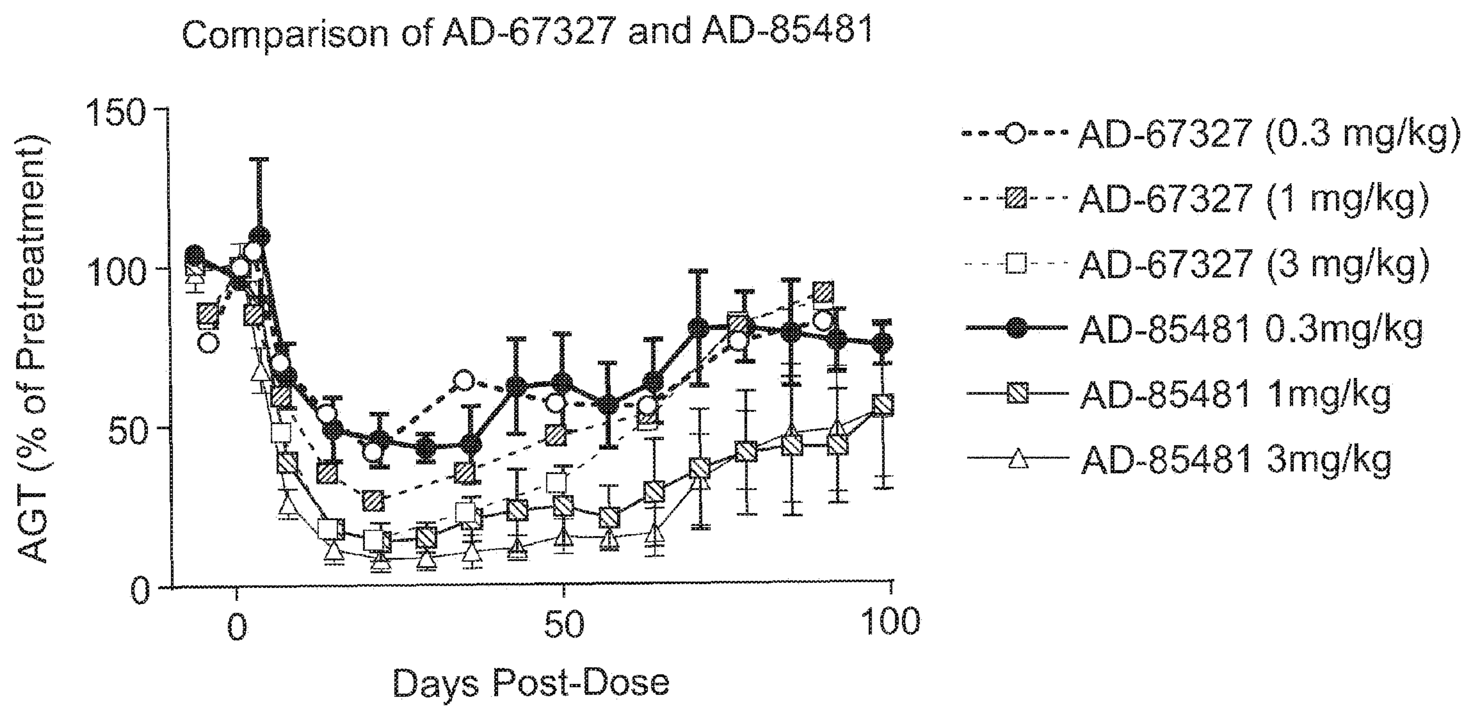

Comparison of AD-67327 and AD-85481
150
100
50
0
AGT (% of Pretreatment)
0
50
100
Days Post-Dose
AD-67327 (0.3 mg/kg)
AD-67327 (1 mg/kg)
AD-67327 (3 mg/kg)
AD-85481 0.3mg/kg
AD-85481 1mg/kg
AD-85481 3mg/kg

FIG. 1C

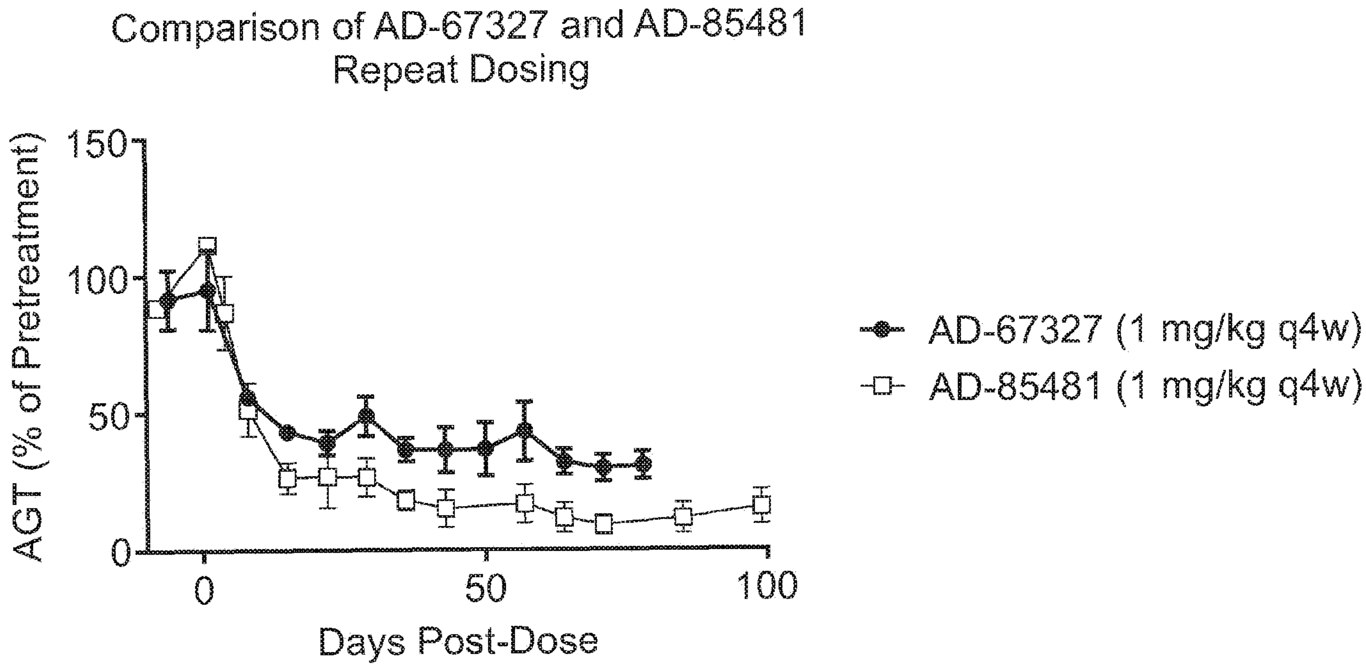

Comparison of AD-67327 and AD-85481
Repeat Dosing
AGT (% of Pretreatment)
150
100
50
0
0
50
100
Days Post-Dose
AD-67327 (1 mg/kg q4w)
AD-85481 (1 mg/kg q4w)

FIG. 2A

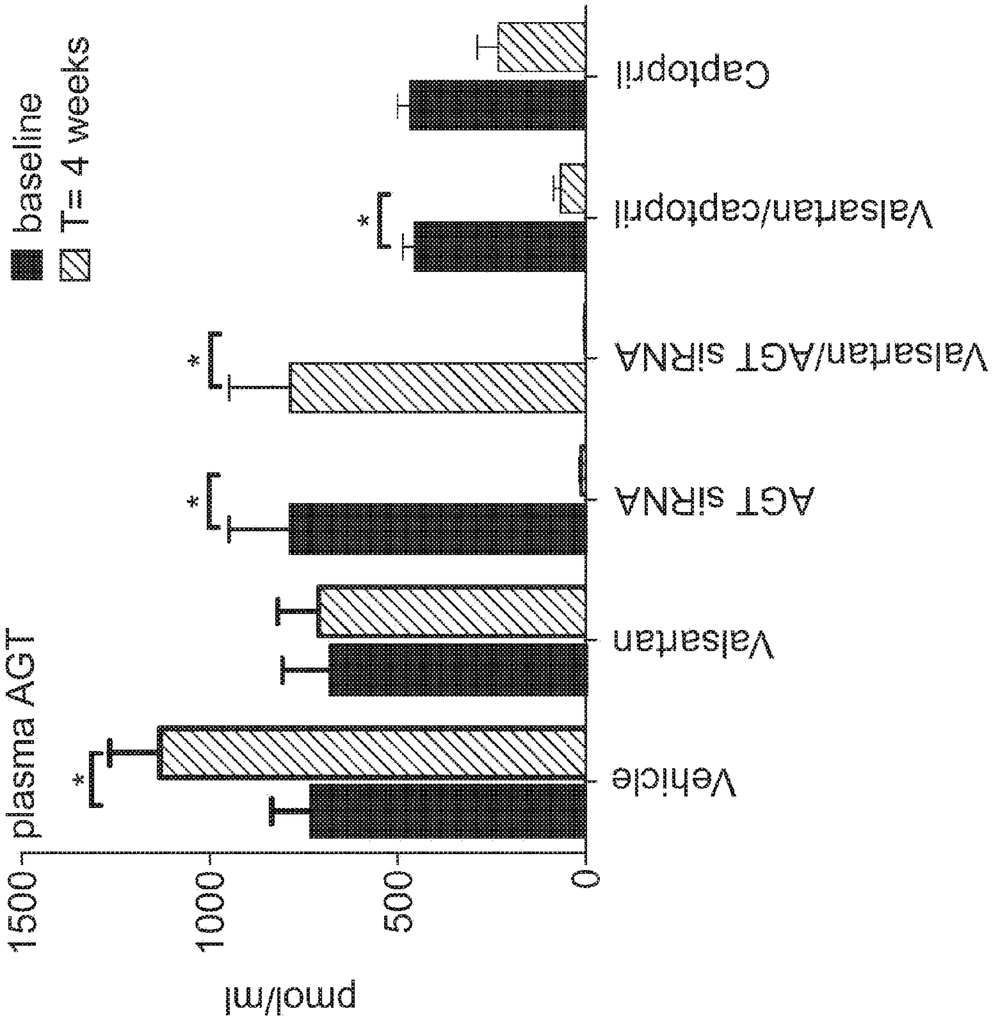

plasma AGT
baseline
T= 4 weeks
pmol/ml
1500
1000
500
0
*
*
*
*
Vehicle
Valsartan
AGT siRNA
Valsartan/AGT siRNA
Valsartan/captopril
Captopril

FIG. 2B

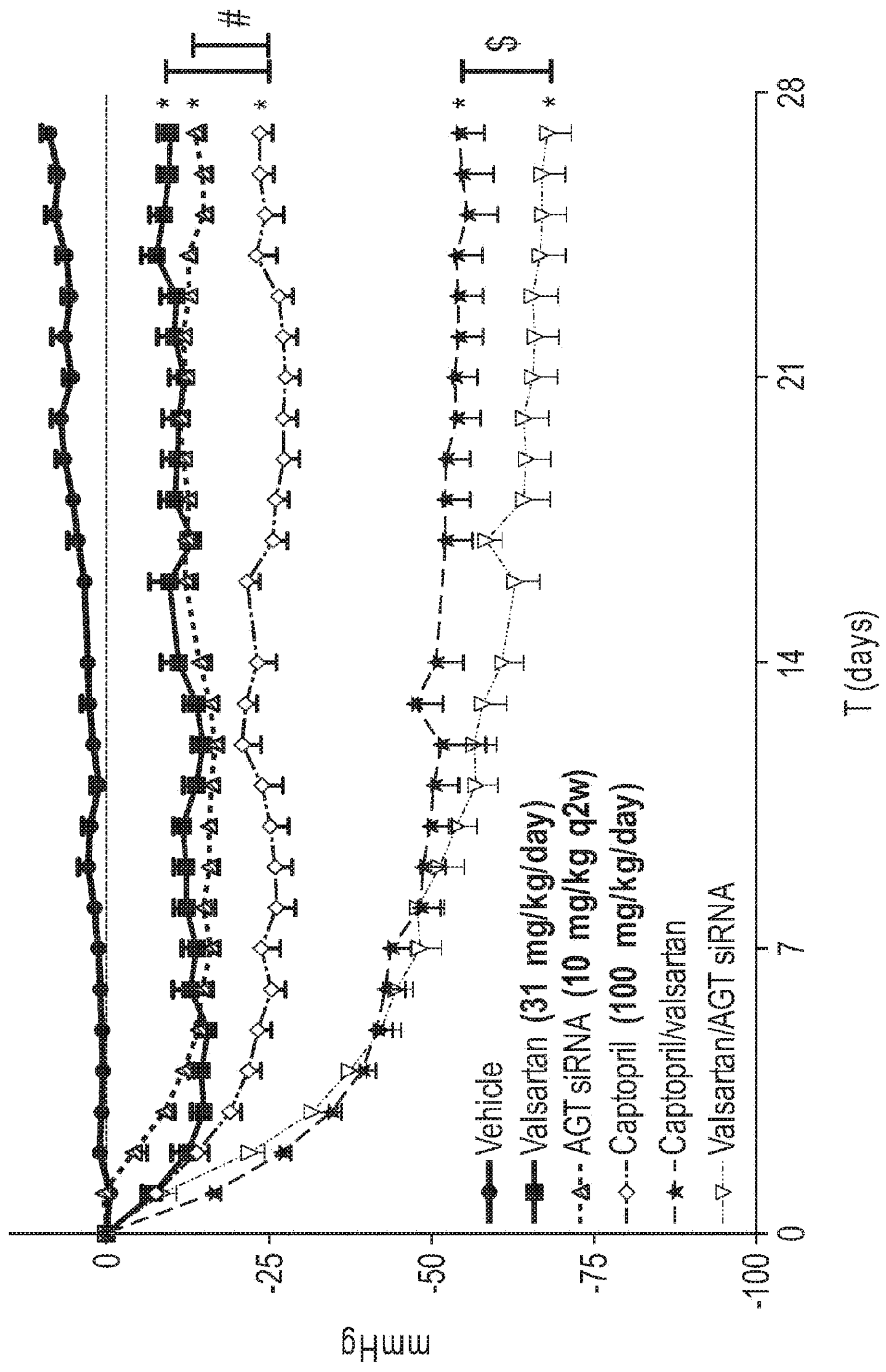

Vehicle
Valsartan (31 mg/kg/day)
AGT siRNA (10 mg/kg q2w)
Captopril (100 mg/kg/day)
Captopril/valsartan
Valsartan/AGT siRNA
mmHg
0
-25
-50
-75
-100
T (days)
0
7
14
21
28
#
$
*

FIG. 2C

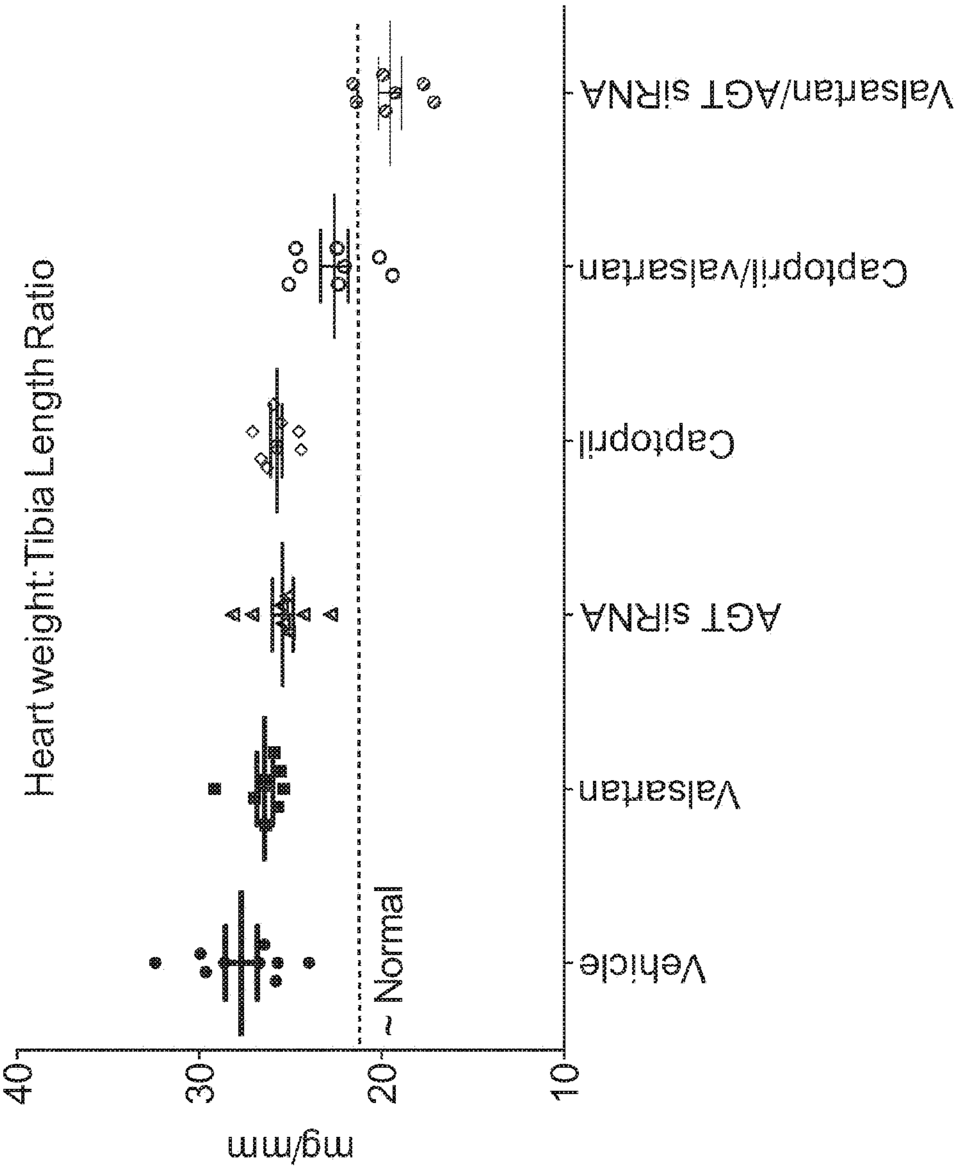

Heart weight: Tibia Length Ratio
mg/mm
40
30
20
10
~ Normal
Vehicle
Valsartan
AGT siRNA
Captopril
Captopril/valsartan
Valsartan/AGT siRNA

FIG. 2D

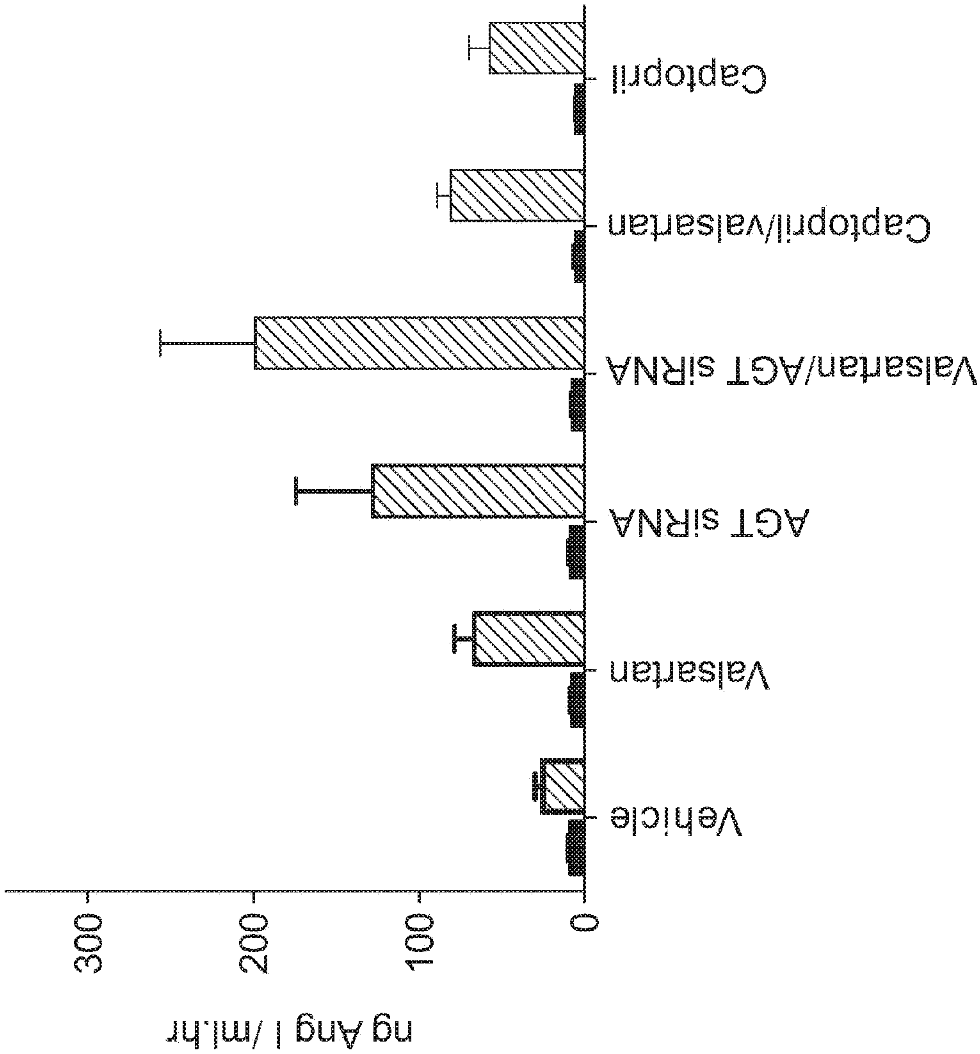

ng Ang I /ml.hr
0
100
200
300
Vehicle
Valsartan
AGT siRNA
Valsartan/AGT siRNA
Captopril/valsartan
Captopril

FIG. 2E

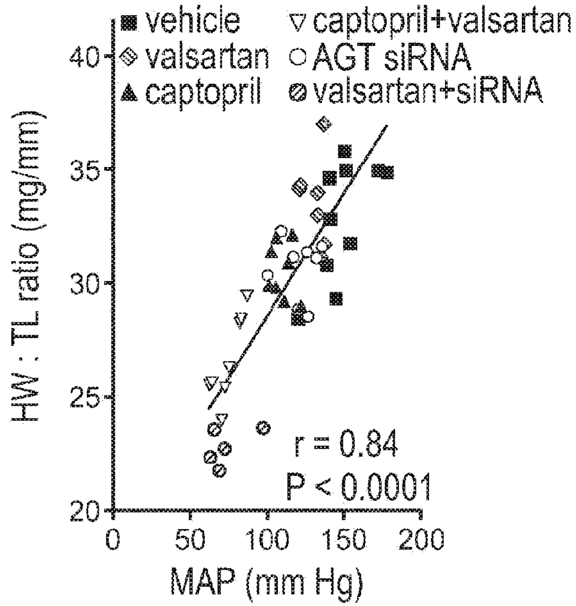


FIG. 2F

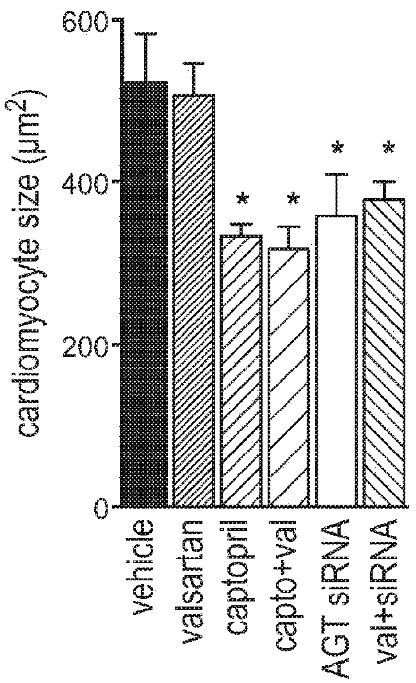


FIG. 2G

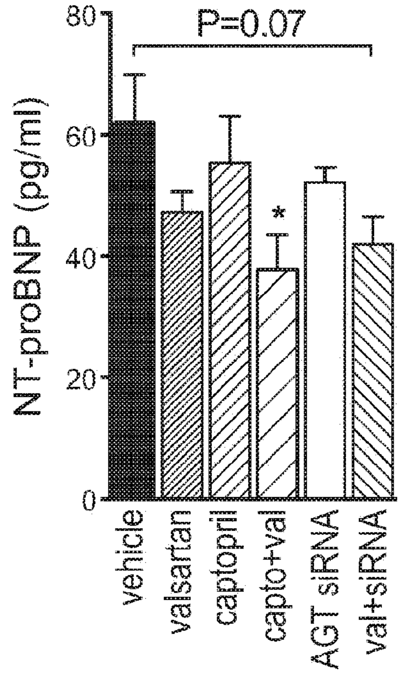


FIG. 3

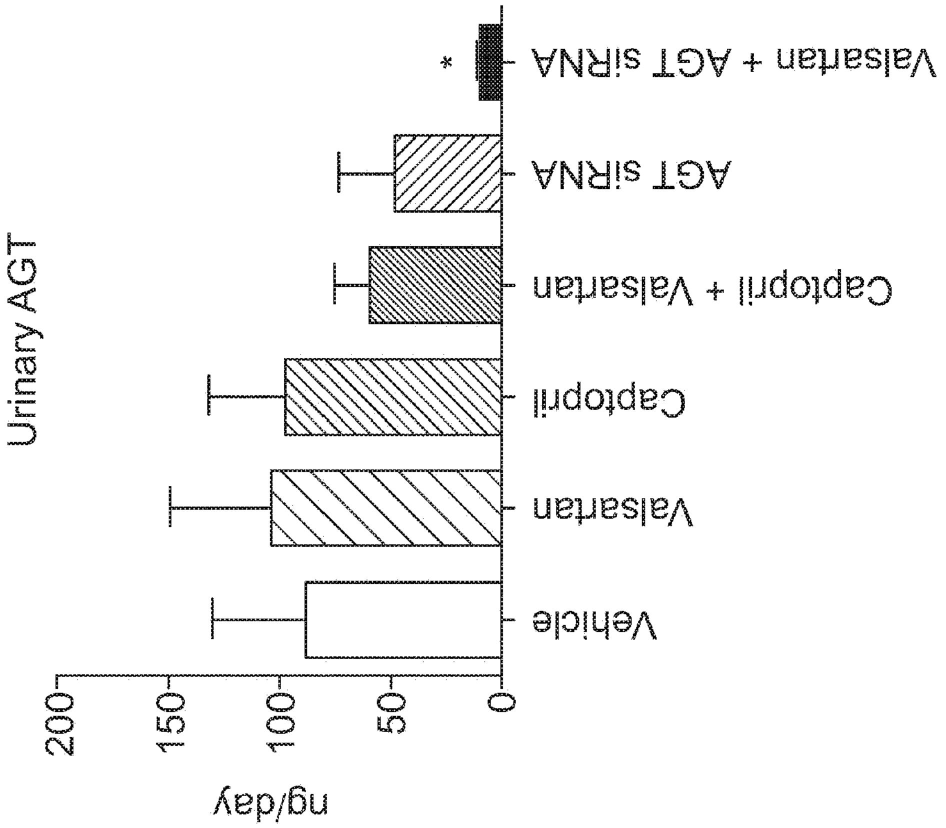

Urinary AGT
ng/day
0
50
100
150
200
Vehicle
Valsartan
Captopril
Captopril + Valsartan
AGT siRNA
Valsartan + AGT siRNA
*

FIG. 4C

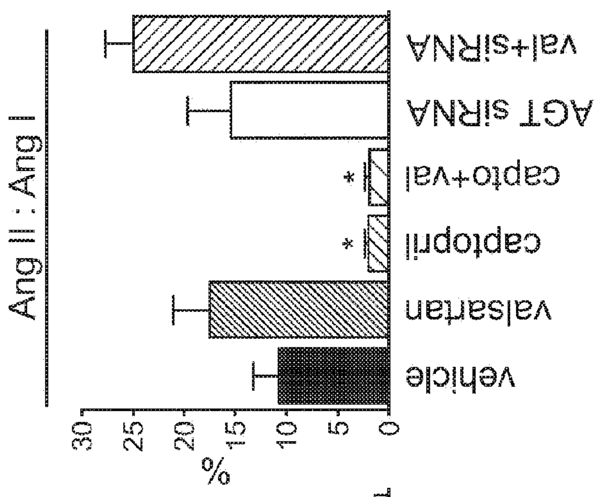


FIG. 4B

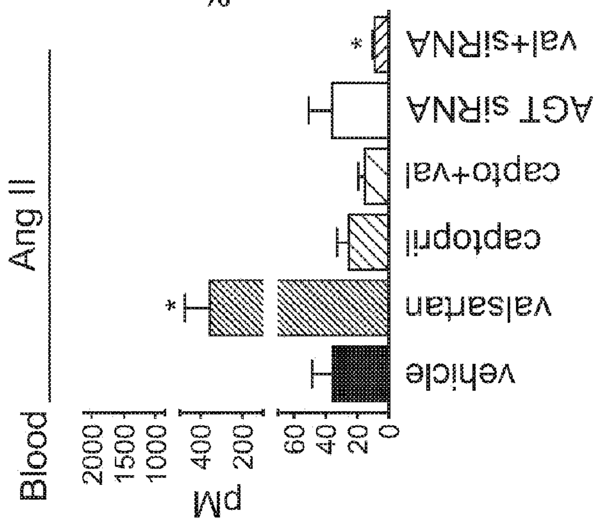


FIG. 4A

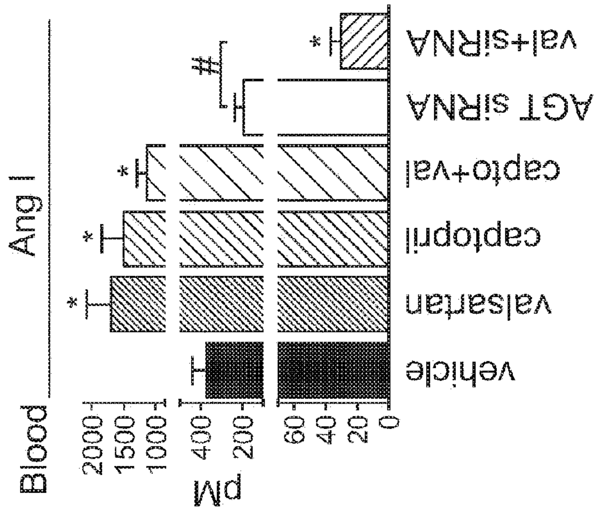


FIG. 5A

FIG. 5B

FIG. 5C

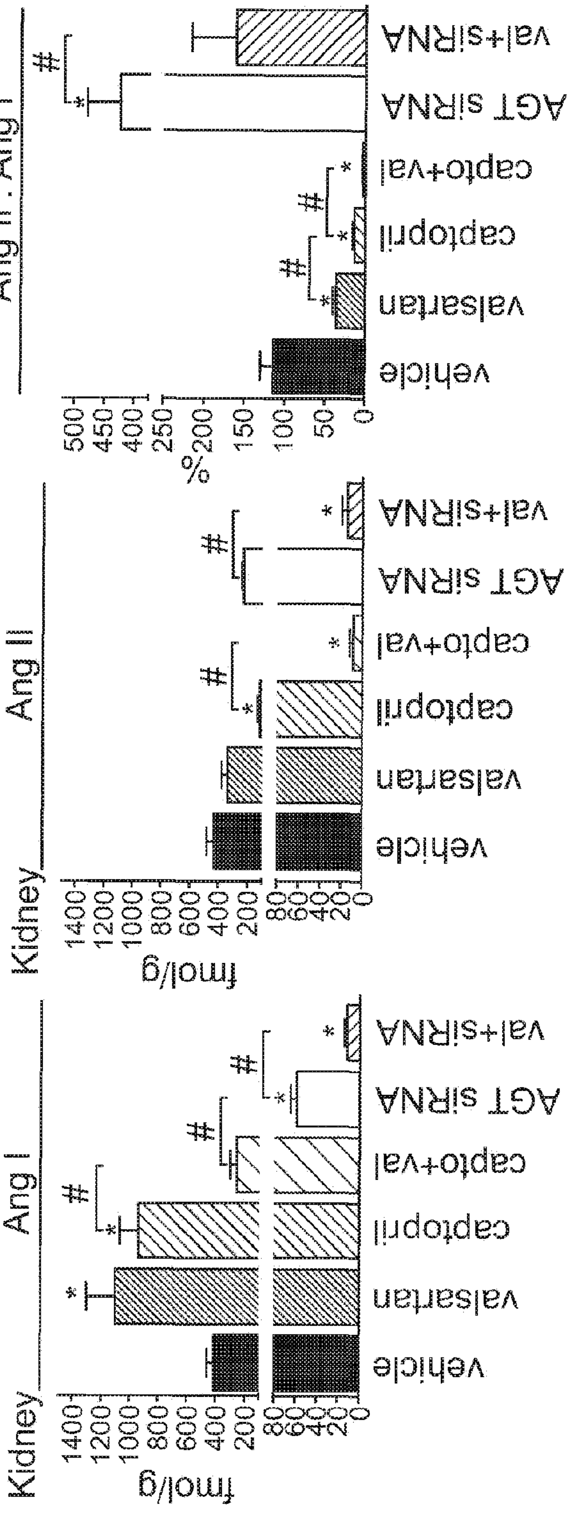


FIG. 6A

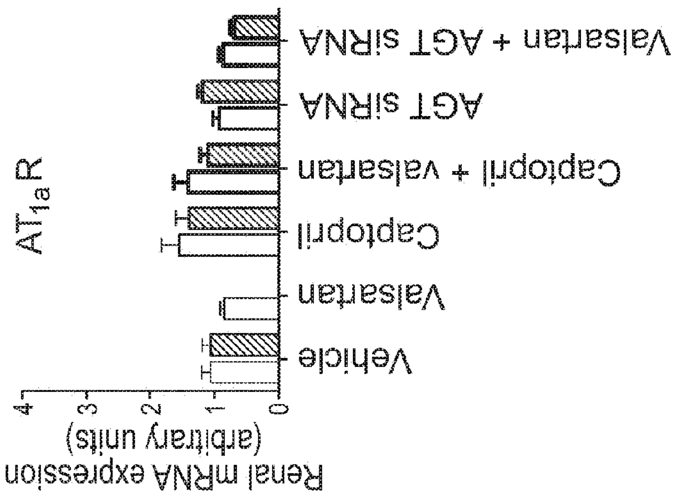


FIG. 6B

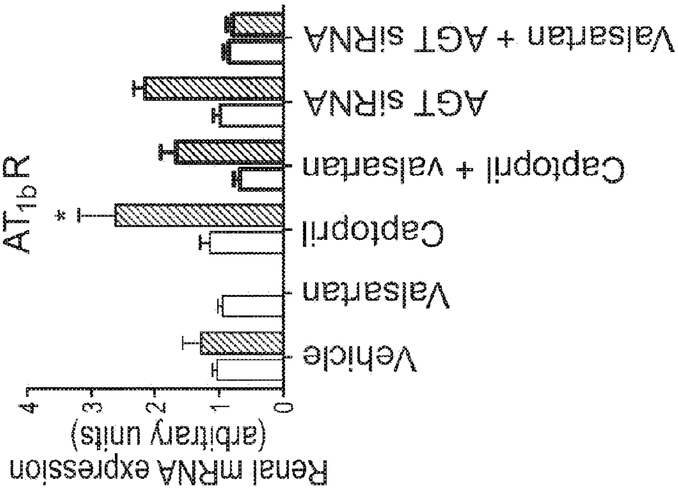


FIG. 6C

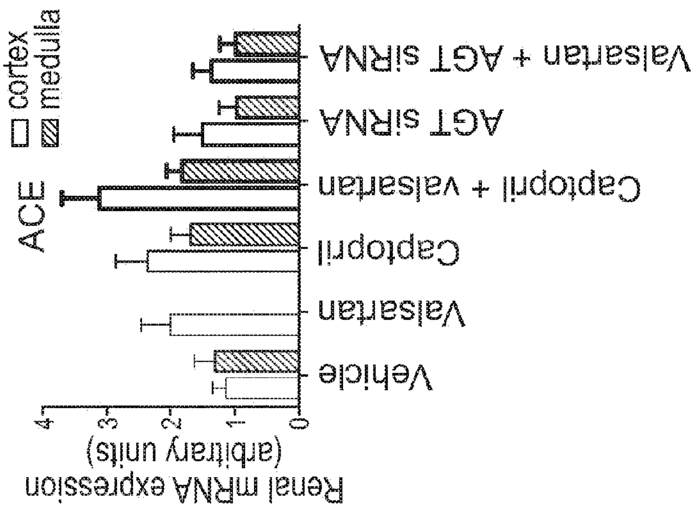
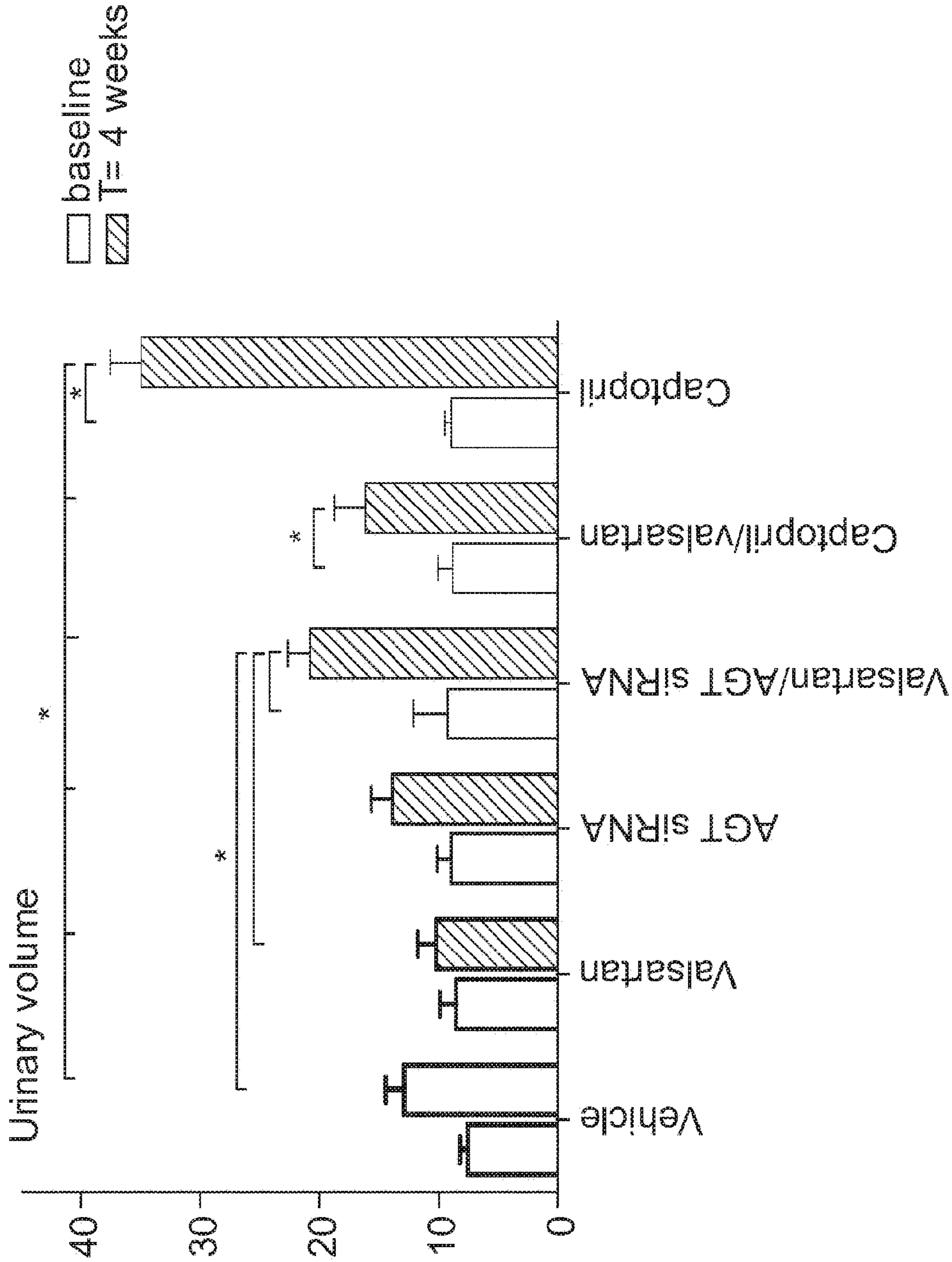


FIG. 7


Urinary volume
mL/day
0
10
20
30
40
Vehicle
Valsartan
AGT siRNA
Valsartan/AGT siRNA
Captopril/valsartan
Captopril
baseline
T= 4 weeks
*
*
*
*

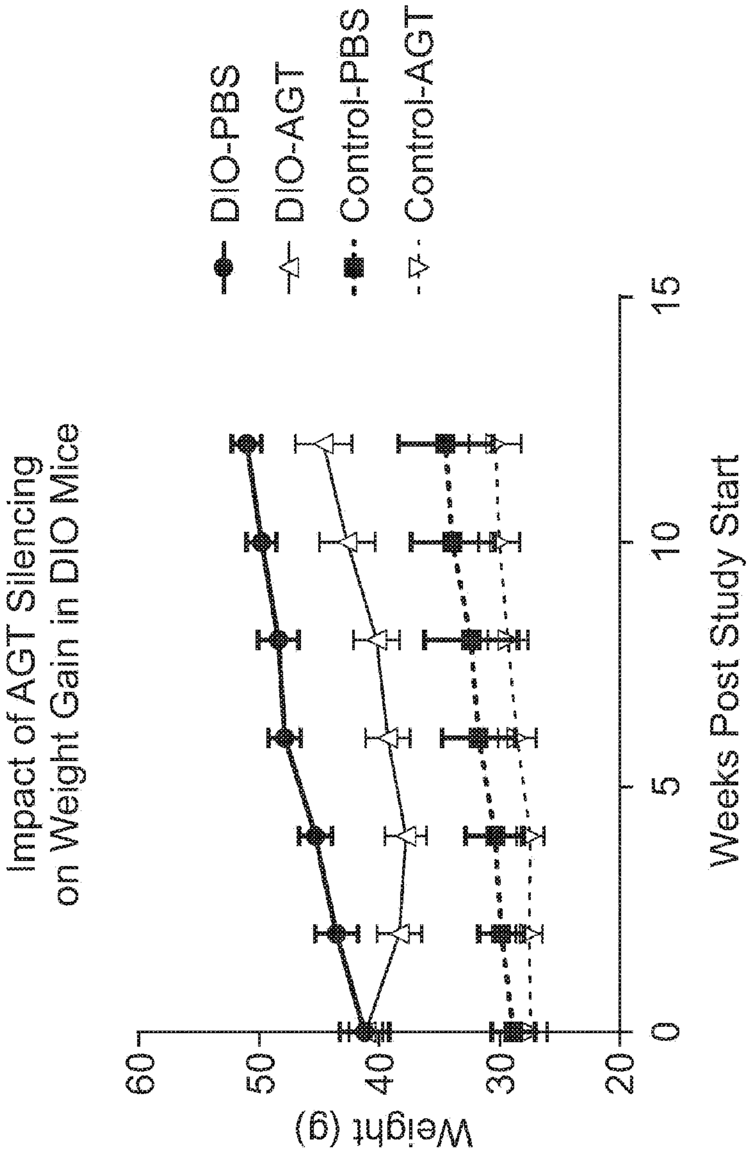

Impact of AGT Silencing
on Weight Gain in DIO Mice
Weight (g)
60
50
40
30
20
0
5
10
15
Weeks Post Study Start
DIO-PBS
DIO-AGT
Control-PBS
Control-AGT

FIG. 8A

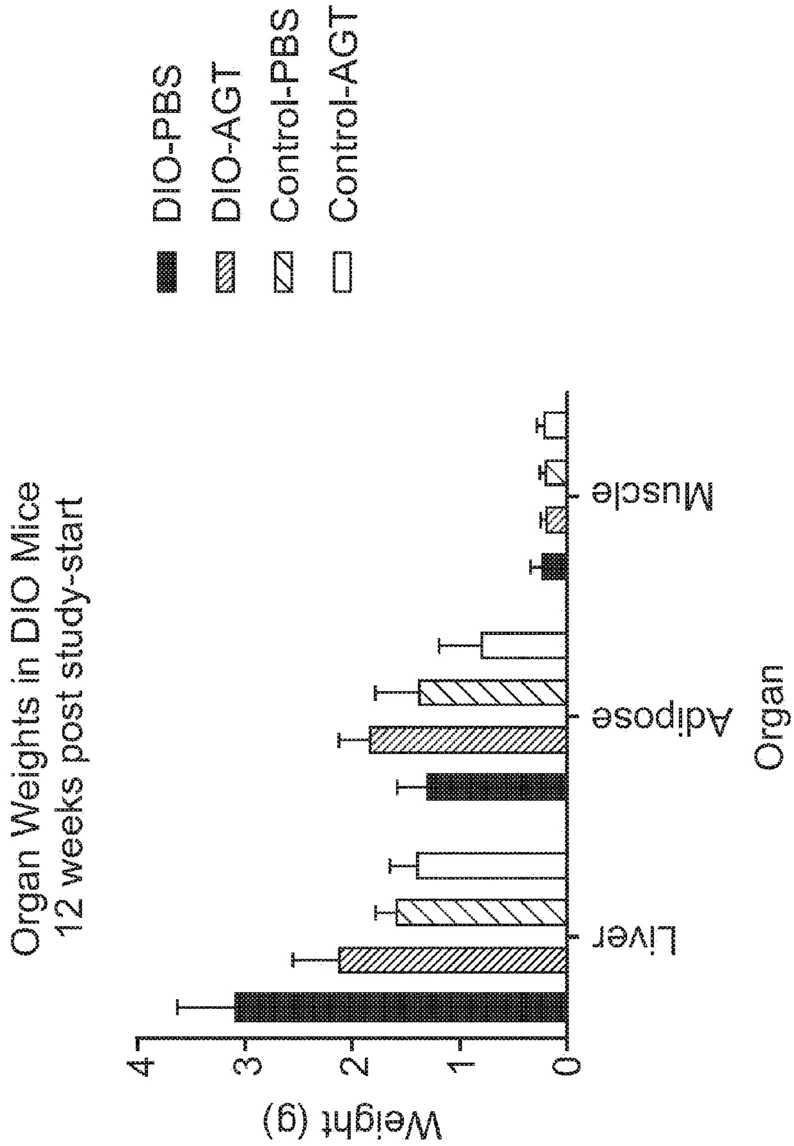

Organ Weights in DIO Mice
12 weeks post study-start
DIO-PBS
DIO-AGT
Control-PBS
Control-AGT
Weight (g)
4
3
2
1
0
Liver
Adipose
Muscle
Organ

FIG. 8B

FIG. 9A

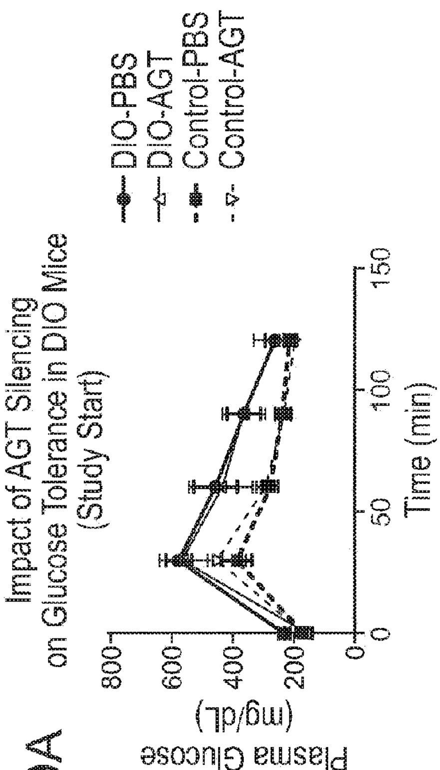


FIG. 9B

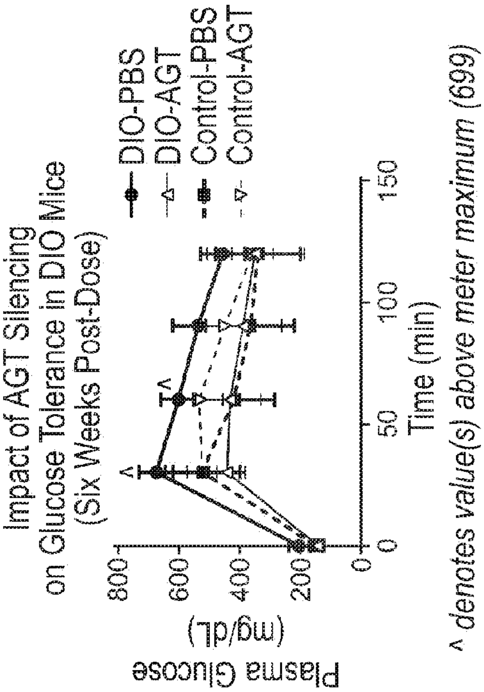


FIG. 9C

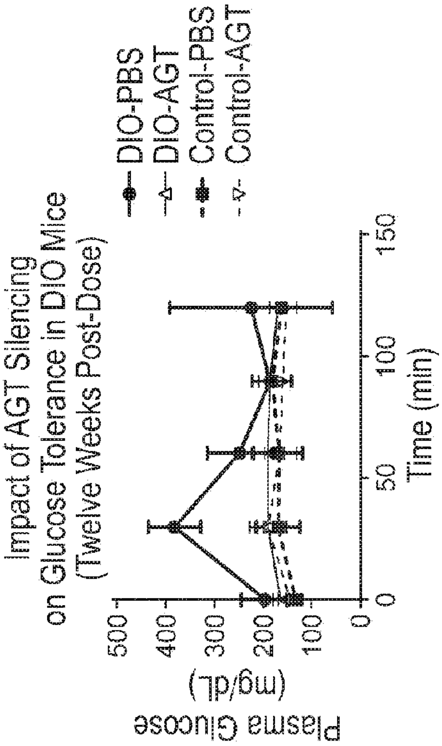


FIG. 10A

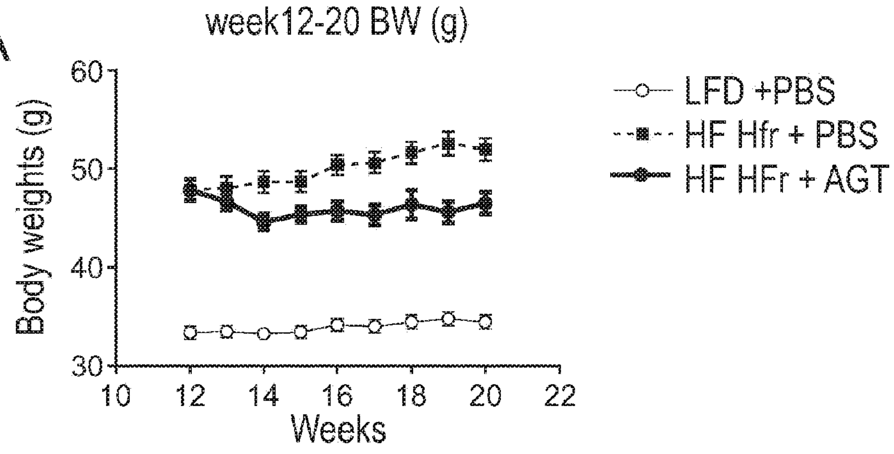


FIG. 10B

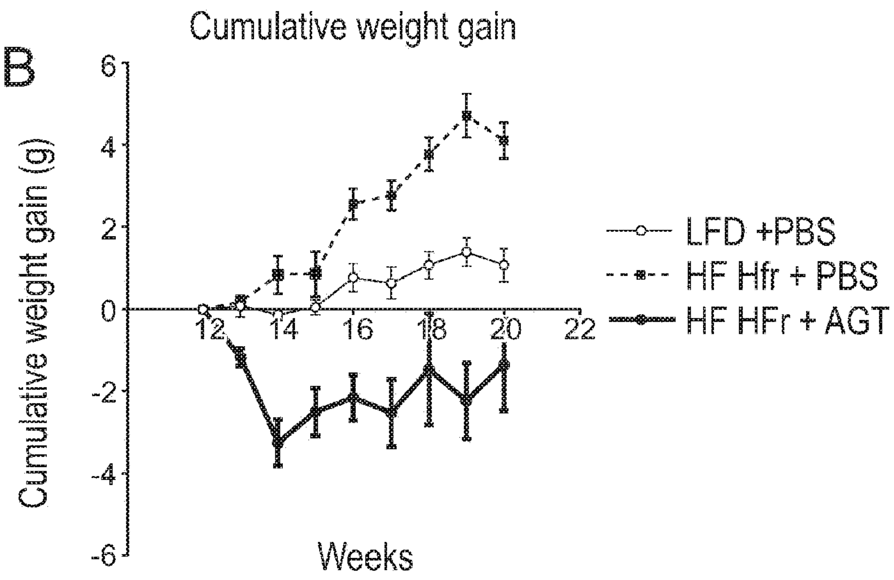


FIG. 11A

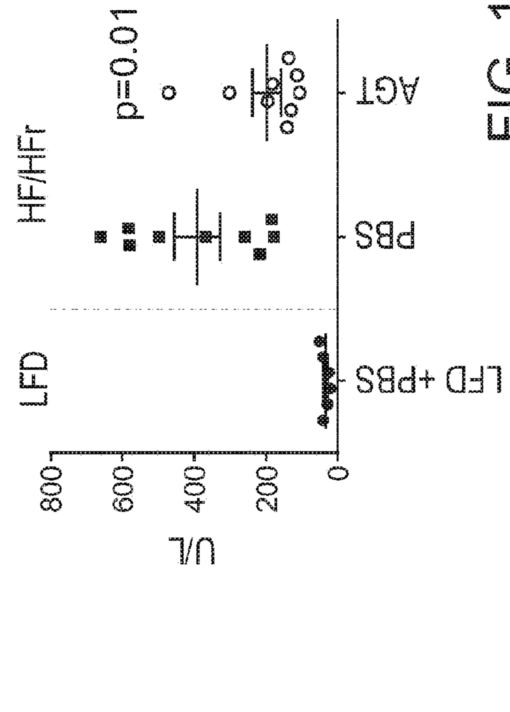


FIG. 11B

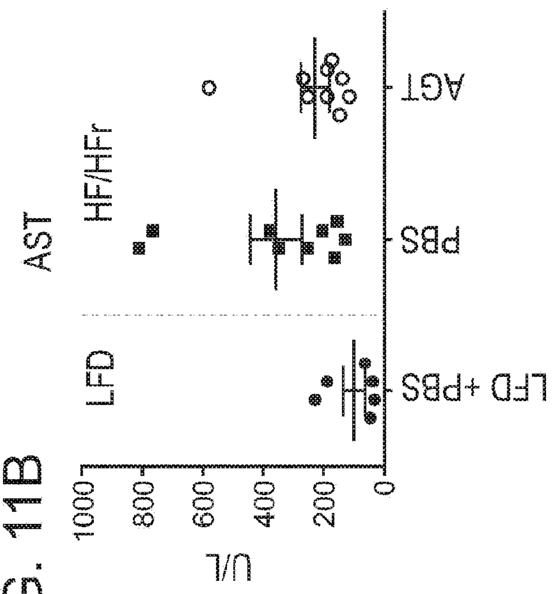
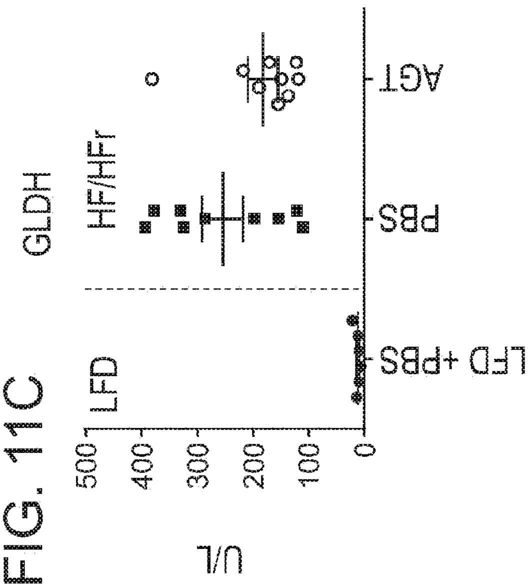


FIG. 11C



SEKVENSLISTE

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