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(54) **Title**: POLYNUCLEOTIDE AGENTS TARGETING ANGIOTENSINOGEN (AGT) AND METHODS OF USE THEREOF

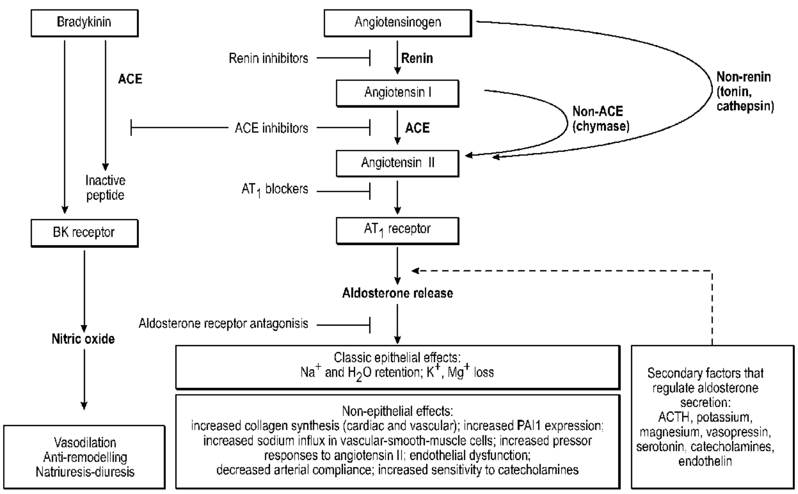

FIG. 1

(57) **Abstract**: The invention relates to polynucleotide agents, *e.g.*, antisense polynucleotide agents, targeting an angiotensinogen (AGT) gene, and methods of using such polynucleotide agents to inhibit expression of AGT and to treat subjects having an AGT-associated disease, *e.g.*, hypertension.

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POLYNUCLEOTIDE AGENTS TARGETING ANGIOTENSINOGEN (AGT) AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/169,055, filed on June 1, 2015, the entire contents of which are hereby incorporated herein by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 13, 2016, is named 121301-03520_SL.txt and is 88,565 bytes in size.

Background of the Invention

The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the regulation of blood pressure. The RAAS cascade begins with the release of 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, and/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_1R), 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_1R stimulation leads to aldosterone release which, in turn, promotes Na+ and K+ excretion in the renal distal convoluted tubule.

Dysregulation of the RAAS leading to, for example, excessive angiotensin II production and/or AT_1R 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.

Hypertension is the most prevalent, controllable disease in developed countries, affecting 20–50% of adult populations. It 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 and/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.

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 compliance and side-effects increase with increasing medication.

Accordingly, there is a need in the art for alternative therapies and combination therapies for subjects having an angiotensinogen-associated disease.

Summary of the Invention

The present invention provides polynucleotide agents and compositions comprising such agents which which target nucleic acids encoding angiotensinogen (AGT) and interfere with the normal function of the targeted nucleic acid. The AGT nucleic acid may be within a cell, *e.g.,* a cell within a subject, such as a human. The present invention also provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a AGT mRNA, *e.g.,* an angiotensinogen-associated disease, such as hypertension, using the polynucleotide agents and compositions of the invention.

Accordingly, in one aspect, the present invention provides antisense polynucleotide agents for inhibiting expression of an angiotensinogen (AGT) gene. The agents include about 4 to about 50 contiguous nucleotides, wherein at least one of the contiguous nucleotides is a modified nucleotide, and wherein the nucleotide sequence of the agent is about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%,or about 100% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1-4.

In one embodiment, the equivalent region is selected from the group consisting of nucleotides 1223-1296, 1596-1626, 1727-1813, 1727-1770, 1772-1813, 1838-1868, 2091-2120, 2200-2243, 2321-2340, 474-493, 981-1000, 1003-1022, 1167-1186, 1223-1242, 1234-1253, 1245-1264, 1254-1273, 1277-1296, 1596-1615, 1607-1626, 1727-1746, 1740-1759, 1751-1770, 1772-1791, 1784-1803, 1794-1813, 1838-1857, 1849-1868, 1893-1912, 2091-2110, 2101-2120, 2125-2144, 2146-2165, 2200-2219, 2213-2232, 2224-2243, 2256-2275, 2310-2329, and 2321-2340 of SEQ ID NO:1.

In another aspect, the present invention provides antisense polynucleotide agents for inhibiting expression of an angiotensinogen (AGT) gene. The agents include at least 8 contiguous nucleotides differing by no more than 3 nucleotides from any one of the target nucleotide sequences of SEQ ID NO:1 provided in Table 3 and are about 8 to about 50 nucleotides in length.

In one aspect, the present invention provides antisense polynucleotide agents for inhibiting expression of an angiotensinogen (AGT) gene. The agents include at least 8 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 1223-1296, 1596-1626, 1727-1813, 1727-1770, 1772-1813, 1838-1868, 2091-2120, 2200-2243, 2321-2340, 474-493, 981-1000, 1003-1022, 1167-1186, 1223-1242, 1234-1253, 1245-1264, 1254-1273, 1277-1296, 1596-1615, 1607-1626, 1727-1746, 1740-1759, 1751-1770, 1772-1791, 1784-1803, 1794-1813, 1838-1857, 1849-1868, 1893-1912, 2091-2110, 2101-2120, 2125-2144, 2146-2165, 2200-2219, 2213-2232, 2224-2243, 2256-2275, 2310-2329, or 2321-2340 of SEQ ID NO:1.

In some embodiments, substantially all of the nucleotides of the antisense polynucleotide agents of the invention are modified nucleotides. In other embodiments, all of the nucleotides of the antisense polynucleotide agent are modified nucleotides.

The antisense polynucleotide agent may be 10 to 40 nucleotides in length; 10 to 30 nucleotides in length; 18 to 30 nucleotides in length; 10 to 24 nucleotides in length; 18 to 24 nucleotides in length; or 20 nucleotides in length.

In one embodiment, the modified nucleotide comprises a modified sugar moiety selected from the group consisting of a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

In one embodiment, the bicyclic sugar moiety has a (—CH2—)n group forming a bridge between the 2′-oxygen and the 4′-carbon atoms of the sugar ring, wherein n is 1 or 2.

In another embodiment, the modified nucleotide is a 5-methylcytosine.

In one embodiment, the modified nucleotide comprises a modified internucleoside linkage, such as a phosphorothioate internucleoside linkage.

In one embodiment, an agent of the invention comprises one 2′-deoxynucleotide. In another embodiment, an agent of the invention comprises one 2′-deoxynucleotide flanked on each side by at least one nucleotide having a modified sugar moiety.

In one embodiment, an agent of the invention comprises a plurality, *e.g.,* more than 1, *e.g.,* 2, 3, 4, 5, 6, or 7, 2′-deoxynucleotides. In one embodiment, an agent of the invention comprises a plurality, *e.g.,* more than 1, *e.g.,* 2, 3, 4, 5, 6, or 7, 2′-deoxynucleotides flanked on each side by at least one nucleotide having a modified sugar moiety.

In one embodiment, the agent is a gapmer comprising a gap segment comprised of linked 2′-deoxynucleotides positioned between a 5′ and a 3′ wing segment.

In one embodiment, the modified sugar moiety is selected from the group consisting of a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

In one embodiment, the 5'-wing segment is 1 to 6 nucleotides in length, *e.g.,* 2, 3, 4, or 5 nucleotides in length.

In one embodiment, the 3'-wing segment is 1 to 6 nucleotides in length, *e.g.,* 2, 3, 4, or 5 nucleotides in length.

In one embodiment, the gap segment is 5 to 14 nucleotides in length, *e.g.,* 6, 7, 8, 9, 10, 11, 12, or 13 nucleotides in length. In one embodiment, the gap segment is 10 nulcoeitdes in length.

In one aspect, the present invention provides antisense polynucleotide agents for inhibiting expression of an angiotensinogen (AGT) gene. The agents include a gap segment consisting of linked deoxynucleotides; a 5'-wing segment consisting of linked nucleotides; a 3'-wing segment consisting of linked nucleotides; wherein the gap segment is positioned between the 5'-wing segment and the 3'-wing segment and wherein each nucleotide of each wing segment comprises a modified sugar.

In one embodiment, the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is five nucleotides in length.

In another embodiment, the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is four nucleotides in length.

In yet another embodiment, the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is three nucleotides in length.

In another embodiment, the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is two nucleotides in length.

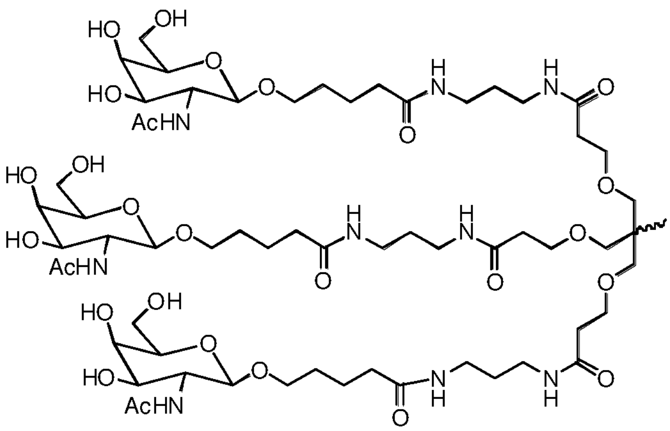
In one embodiment, the modified sugar moiety is selected from the group consisting of a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

In some embodiments, the agents of the invention further comprise a ligand.

In one embodiment, the agent is conjugated to the ligand at the 3'-terminus.

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

In one embodiment, the ligand is



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In one aspect, the present invention provides pharmaceutical compositions for inhibiting expression of an angiotensinogen (AGT) gene comprising an agent of the invention.

In one embodiment, the agent is present in an unbuffered solution, such as saline or water.

In another embodiment, the agent is is present in a buffer solution, such as a buffer comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

In one embodiment, the buffer solution is phosphate buffered saline (PBS).

In another aspect, the present invention provides pharmaceutical composition comprising an agent of the invention and a lipid formulation, such as a lipid formulation comprising an LNP or a MC3.

In one aspect, the present invention provides methods of inhibiting expression of an angiotensinogen (AGT) gene in a cell. The methods include contacting the cell with an agent of the invention or a pharmaceutical composition of the invention, thereby inhibiting expression of the AGT gene in the cell.

In one embodiment, the cell is within a subject.

In one embodiment, the subject is a human.

In one embodiment, the AGT expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In another aspect, the present invention provides methods of treating a subject having a disease or disorder that would benefit from reduction in angiotensinogen (AGT) expression. The methods include administering to the subject a therapeutically effective amount of an agent of the invention or a pharmaceutical composition of the invention, thereby treating the subject.

In yet another aspect, the present invention provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in angiotensinogen (AGT) expression. The methods include administering to the subject a prophylactically effective amount of an agent of the invention or a pharmaceutical composition of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in AGT expression.

In one embodiment, the administration of the antisense polynucleotide agent to the subject causes a decrease in hypertension and/or a decrease in AGT protein levels.

In one embodiment, the disorder is an AGT-associated disease.

In certain embodiments, the AGT-associated disease is selected from the group consisting of of hypertension, hypertensive heart disease, hypertensive nephropathy, pregnancy-associated hypertension, atherosclerosis, arteriosclerosis, chronic kidney disease, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, Cushing's syndrome, and other glucocorticoid excess states including chronic steroid therapy, pheochromocytoma, primary aldosteronism and other mineralocorticoid excess states, sleep apnea, thyroid/parathyroid disease, heart failure, myocardial infarction, stroke, diabetes mellitus, renal failure, and systemic sclerosis. In certain embodiments, the angiotensinogen-associated disease is pregnancy-associated hypertension (*e.g.*, pregnancy-induced hypertension, preeclampsia, and eclampsia) and administration of an agent of the invention to the subject results in a decrease in maternal blood pressure; a decrease in maternal albuminuria; an increase in uteroplacental unit weight; an increase in fetal weight; normalization of the fetal brain:liver ratio; a decrease in hAGT mRNA expression in the maternal liver and no significant decrease in AGT mRNA expression in the placenta; an increase in overall placenta size; an increase in the size of the villous placenta; no significant change in the size of the trophospongium of the placenta; a reduction in the ratio of sFLT1:PLGF mRNA expression in the maternal kidney; a reduction in the ratio of serum sFLT1:PLGF levels; and/or a decrease in the level of agonistic autoantibodies to AT1.

In certain embodiments the subject is human.

In certain embodiments, the methods further comprise administering to the subject an additional therapeutic agent. In some embodiments, the additional therapeutic agent 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents.

In certain embodiments, the agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

In certain embodiments, the agent is administered at a dose of about 10 mg/kg to about 30 mg/kg.

In certain embodiments, the agent is administered to the subject once a week.

In alternative embodiments, the agent is administered to the subject twice a week.

In yet other embodiments, the agent is administered to the subject twice a month.

In certain embodiments, the agent is administered to the subject subcutaneously.

In certain embodiments, the methods of the invention further include measuring blood pressure and/or AGT levels in the subject.

Brief Description of the Drawings

Figure 1 is a schematic of the renin-angiotensin-aldosterone system (RAAS) including an indication of the various points in the sytem which have been the targets for therapeutic intervention (from Zaman, *et al.* (2002) *Nat Rev Drug Disc* 1:621).

Detailed Description of the Invention

The present invention provides polynucleotide agents, *e.g.,* antisense polynucleotide agents, and compositions comprising such agents which target nucleic acids encoding angiotensinogen (AGT) (*e.g.,* mRNA encoding AGT as provided in, for example, any one of SEQ ID NOs:1-4). The polynucleotide agents bind to nucleic acids encoding AGT *via*, *e.g.,*Watson-Crick base pairing, and interfere with the normal function of the targeted nucleic acid.

The antisense polynucleotide agents of the invention include a nucleotide sequence which is about 4 to about 50 nucleotides or less in length and which is about 80% complementary to at least part of an mRNA transcript of an AGT gene. The use of these antisense polynucleotide agents enables the targeted inhibition of RNA expression and/or activity of an AGT gene in mammals.

The present inventors have demonstrated that antisense polynucleotide agents targeting AGT can mediate antisense inhibition *in vitro* resulting in significant inhibition of expression of an AGT gene. Thus, methods and compositions including these antisense polynucleotide agents are useful for treating a subject who would benefit by a reduction in the levels and/or activity of an AGT protein, such as a subject having an AGT-associated disease, such as hypertension.

The present invention also provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an AGT gene, *e.g.,* an AGT-associated disease, such as hypertension using the antisense polynucleotide agents and compositions of the invention.

The present invention also provides methods for preventing at least one symptom, *e.g.,* hypertension, in a subject having a disorder that would benefit from inhibiting or reducing the expression of an AGT gene, *e.g.,* hypertension. The present invention further provides compositions comprising antisense polynucleotide agents which effect antisense inhibition of an AGT gene. The AGT gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The combination therapies of the present invention include administering to a subject having an AGT-associated disease, an antisense polynucleotide agent of the invention and an additional therapeutic, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents. The combination therapies of the invention reduce AGT levels in the subject (*e.g.,* by about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 99%) by targeting AGT mRNA with an antisense polynucleotide agent of the invention and, accordingly, allow the therapeutically (or prophylactically) effective amount of the additional therapeutic agent(s) required to treat the subject to be reduced, thereby decreasing the costs of treatment and permitting easier and more convenient ways of administering certain agents, or decreasing side effects of certain agents.

The following detailed description discloses how to make and use antisense polynucleotide agents to inhibit the mRNA and/or protein expression of an AGT gene, as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of this gene.

I. **Definitions**

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

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

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

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term “about” is used herein to mean within the typical ranges of tolerances in the art. 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.

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:2); 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:3); 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:4).

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.

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, the entire contents of which are incorporated herein by reference. 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).

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.

As used herein, "target nucleic acid" refers to a nucleic acid molecule to which an antisense polynucleotide agent of the invention specifically hybridizes.

The terms “antisense polynucleotide agent” “antisense compound”, and “agent” as used interchangeably herein, refer to an agent comprising a single-stranded oligonucleotide that contains RNA as that term is defined herein, and which targets nucleic acid molecules encoding AGT (*e.g.,* mRNA encoding AGT as provided in, for example, any one of SEQ ID NOs:1-4). The antisense polynucleotide agents specifically bind to the target nucleic acid molecules *via* hydrogen bonding (*e.g.*, Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) and interfere with the normal function of the targeted nucleic acid (*e.g.*, by an antisense mechanism of action). This interference with or modulation of the function of a target nucleic acid by the polynucleotide agents of the present invention is referred to as “antisense inhibition.”

The functions of the target nucleic acid molecule to be interfered with may include functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA.

In some embodiments, antisense inhibition refers to "inhibiting the expression" of target nucleic acid levels and/or target protein levels in a cell, *e.g.,* a cell within a subject, such as a mammalian subject, in the presence of the antisense polynucleotide agent complementary to a target nucleic acid as compared to target nucleic acid levels and/or target protein levels in the absence of the antisense polynucleotide agent. For example, the antisense polynucleotide agents of the invention 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.

As used herein, the term "specifically hybridizes" refers to an antisense polynucleotide agent having a sufficient degree of complementarity between the antisense polynucleotide agent and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, *e.g.,* under physiological conditions in the case of *in vivo* assays and therapeutic treatments.

A target sequence may be from about 4-50 nucleotides in length, *e.g.*, 8-45, 10-45, 10-40, 10-35, 10-30, 10-20, 11-45, 11-40, 11-35, 11-30, 11-20, 12-45, 12-40, 12-35, 12-30, 12-25, 12-20, 13-45, 13-40, 13-35, 13-30, 13-25, 13-20, 14-45, 14-40, 14-35, 14-30, 14-25, 14-20, 15-45, 15-40, 15-35, 15-30, 15-25, 15-20, 16-45, 16-40, 16-35, 16-30, 16-25, 16-20, 17-45, 17-40, 17-35, 17-30, 17-25, 17-20, 18-45, 18-40, 18-35, 18-30, 18-25, 18-20, 19-45, 19-40, 19-35, 19-30, 19-25, 19-20, *e.g.,* 4, 5, 6, 7, 8, 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 contiguous nucleotides of the nucleotide sequence of an mRNA molecule formed during the transcription of an AGT gene. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The terms "complementary," "fully complementary" and "substantially complementary" are used herein with respect to the base matching between an antisense polynucleotide agent and a target sequence. The term"complementarity" refers to the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

As used herein, an antisense polynucleotide agent that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to an antisense polynucleotide agent that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding AGT). 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 AGT.

As used herein, the term "region of complementarity" refers to the region of the antisense polynucletiode agent 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, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the antisense polynucleotide.

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 a polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with 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 nucleotides.

Complementary sequences include those nucleotide sequences of an antisense polynucleotide agent of the invention that base-pair to 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.*, antisense inhibition of target gene expression.

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

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

"G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the terms "deoxyribonucleotide", "ribonucleotide" and "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 the agents featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

A "nucleoside" is a base-sugar combination. The "nucleobase" (also known as "base") portion of the nucleoside is normally a heterocyclic base moiety. "Nucleotides" are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2′, 3′ or 5′ hydroxyl moiety of the sugar. "Polynucleotides," also referred to as "oligonucleotides," are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the polynucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the polynucleotide.

In general, the majority of nucleotides of the antisense polynucleotide agents are ribonucleotides, but as described in detail herein, the agents may also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide. In addition, as used in this specification, an "antisense polynucleotide agent" may include nucleotides (*e.g.,* ribonucleotides or deoxyribonucleotides) with chemical modifications; an antisense polynucleotide agent 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, and/or modified nucleobase. 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 antisense polynucleiotde agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in nucleotides, are encompassed by "antisense polynucleotide agent" for the purposes of this specification and claims.

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 camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in AGT expression; a human at risk for a disease, disorder or condition that would benefit from reduction in AGT expression; a human having a disease, disorder or condition that would benefit from reduction in AGT expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in AGT expression as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with 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 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) whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of AGT 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 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more. In certain embodiments, a decrease is at least 20%. ”Lower” in the context of the level of AGT in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder.

As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of an AGT gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom associated with a disease or disorder that is caused by, or associated with renin-angiotensin-aldosterone system (RAAS) activation, such as hypertension. The likelihood of developing hypertension is reduced, for example, when an individual having one or more risk factors for hypertension either fails to develop hypertension or develops a hypertension with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g*., by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g*., by days, weeks, months or years) is considered effective prevention.

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 whose symptoms or progression respond 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), hypertensive emergency (also known as malignant hypertension), hypertensive urgency, isolated systolic or diastolic hypertension, pregnancy-associated hypertension (*e.g.,* preeclampsia, eclampsia, and post-partum preeclampsia), 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, Cushing’s syndrome, and other glucocorticoid excess states including chronic steroid therapy, pheochromocytoma, reninoma, secondary aldosteronism and other mineralocorticoid excess states, sleep apnea, thyroid/parathyroid disease, heart failure (*e.g.,* left ventricular systolic dysfunction), myocardial infarction, angina, stroke, diabetes mellitus (*e.g.,* diabetic nephropathy), renal disease *e.g.*, chronic kidney disease or diabetic nephropathy optionally in the context of pregnancy, renal failure, *e.g.,* chronic renal failure, cognitive dysfunction (such as Alzheimer's), and systemic sclerosis (*e.g.,* scleroderma renal crisis). In certain embodiments, AGT- associated disease includes intrauterine growth restriction (IUGR) or fetal growth restriction.

Based on the average of seated blood pressure readings that are properly measured during two or more office visits, a subject having a normal blood pressure is one having a systolic pressure of about 90–119 mmHg (about 12–15.9 kPa (kN/m^2)) and a diastolic pressure of about 60–79 mmHg (about 8.0–10.5 kPa (kN/m^2)); a subject having prehypertension is one having a systolic pressure of about 120–139 mmHg (about 16.1–18.5 kPa (kN/m^2)) and a diastolic pressure of about 60–79 mmHg (about 8.0–10.5 kPa (kN/m^2)); a subject having hypertension (*e.g.,* Stage I hypertension) is one having a systolic pressure of about 140–159 mmHg (about 18.7–21.2 kPa (kN/m^2)) and a diastolic pressure of about 90–99 mmHg (about 12.0–13.2 kPa (kN/m^2)); and a subject having hypertension (*e.g.,* Stage II hypertension) is one having a systolic pressure of about ≥160 mmHg (about ≥21.3 kPa (kN/m^2)) and a diastolic pressure of about ≥100 mmHg (about ≥13.3 kPa (kN/m^2)). Subjects with blood pressures over 130/80 mmHg along with type 1 or type 2 diabetes, or kidney disease are considered as having hypertension.

In certain embodiments, 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).

In certain embodiments, 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.

In certain embodiments, an angiotensinogen-associated disease is a hypertensive emergency, *e.g.,* malignant hypertension and accelerated hypertension. "Accelerated hypertension" is severely elevated blood pressure (*i.e.,* equal to or greater than a systolic 180 mmHg or diastolic of 110 mmHg) with direct damage to one or more end organs. Blood pressure must be reduced immediately to prevent further organ damage. "Malignant hypertension" is severely elevated blood pressure (*i.e.,* equal to or greater than a systolic 180 mmHg or diastolic of 110 mmHg) with direct damage to one or more end organs and papilledema. Blood pressure must be reduced immediately to prevent further organ damage. Neurologic end-organ damage due to uncontrolled blood pressure may include hypertensive encephalopathy, cerebral vascular accident/cerebral infarction; subarachnoid hemorrhage, and/or intracranial hemorrhage. Cardiovascular end-organ damage may include myocardial ischemia/infarction, acute left ventricular dysfunction, acute pulmonary edema, and/or aortic dissection. Other organ systems may also be affected by uncontrolled hypertension, which may lead to acute renal failure/insufficiency, retinopathy, eclampsia, or microangiopathic hemolytic anemia.

In certain embodiments, an angiotensinogen-associated disease is hypertensive urgency. “Hypertensive urgency” is severely elevated blood pressure (*i.e.,* equal to or greater than a systolic 180 mmHg or diastolic of 110 mmHg) with no direct damage to one or more organs. Blood pressure can be brought down safely within a few hours.

In certain embodiments, 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)). A subject having “chronic hypertension of pregnancy” is one having a blood pressure exceeding 140/90 mm Hg before pregnancy or before 20 weeks' gestation. “Gestational hypertension” or “pregnancy-induced hypertension” refers to hypertension with onset in the latter part of pregnancy (>20 weeks' gestation) without any other features of preeclampsia, and followed by normalization of the blood pressure postpartum. “Mild preeclampsia” is defined as the presence of hypertension (blood pressure ≥140/90 mm Hg) on two occasions, at least six hours apart, but without evidence of end-organ damage, in a woman who was normotensive before 20 weeks' gestation. In a subject with preexisting essential hypertension, preeclampsia is diagnosed if systolic blood pressure has increased by 30 mm Hg or if diastolic blood pressure has increased by 15 mm Hg. “Severe preeclampsia” is defined as the presence of 1 of the following symptoms or signs in the presence of preeclampsia; asystolic blood pressure of 160 mm Hg or higher or diastolic blood pressure of 110 mm Hg or higher on two occasions at least six hours apart; proteinuria of more than 5g in a 24-hour collection or more than 3+ on two random urine samples collected at least four hours apart, pulmonary edema or cyanosis, oliguria (< 400 mL in 24 hours), persistent headaches, epigastric pain and/or impaired liver function, thrombocytopenia, oligohydramnios, decreased fetal growth, or placental abruption. “Eclampsia” is defined as seizures that cannot be attributable to other causes in a woman with preeclampsia. “HELLP syndrome” (also known as edema-proteinuria-hypertension gestosis type B) is Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels in a pregnant subject.

In certain embodiments, an angiotensinogen-associated disease is resistant hypertension. "Resistant hypertension" is blood pressure that remains above goal (*e.g.*, 140/90 mmHg) 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.

II. Antisense Polynucleotide Agents of the Invention

The present invention provides antisense polynucleotide agents, and compositions comprising such agents, which target an AGT gene and inhibit the expression of the AGT gene. In one embodiment, the antisense polynucleotide agents inhibit the expression of an AGT gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having an AGT-associated disease, *e.g.*, hypertension.

The antisense polynucleotide agents of the invention include 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 may be about 50 nucleotides or less in length (*e.g.*, about 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, or 4 nucleotides or less in length). Upon contact with a cell expressing the AGT gene, the antisense polynucleotide agent inhibits the expression of the AGT gene (*e.g.*, a human, a primate, a non-primate, or a bird AGT gene) by at least about 10% 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.

The region of complementarity between an antisense polynucleotide agent and a target sequence may be substantially complementary (*e.g.*, there is a sufficient degree of complementarity between the antisense polynucleotide agent and a target nucleic acid to so that they specifically hybridize and induce a desired effect), but is generally fully complementary to the target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a AGT gene.

Accordingly, in one aspect, an antisense polynucleotide agent of the invention specifically hybridizes to a target nucleic acid molecule, such as the mRNA encoding AGT, and comprises a contiguous nucleotide sequence which corresponds to the reverse complement of a nucleotide sequence of any one of SEQ ID NOs:1-4, or a fragment of any one of SEQ ID NOs:1-4.

In some embodiments, the antisense polynucleotide agents of the invention may be substantially complementary to the target sequence. For example, an antisense polynucleotide agent that is substantially complementary to the target sequence may include a contiguous nucleotide sequence comprising no more than 5 mismatches (*e.g.*, no more than 1, no more than 2, no more than 3, no more than 4, or no more than 5 mismatches) when hybridizing to a target sequence, such as to the corresponding region of a nucleic acid which encodes a mammalian AGT mRNA. In some embodiments, the contiguous nucleotide sequence comprises no more than a single mismatch when hybridizing to the target sequence, such as the corresponding region of a nucleic acid which encodes a mammalian AGT mRNA.

In some embodiments, the antisense polynucleotide agents of the invention that are substantially complementary to the target sequence comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1-4, or a fragment of any one of SEQ ID NOs:1-4, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, an antisense polynucleotide agent comprises a contiguous nucleotide sequence which is fully complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1-4 (or a fragment of any one of SEQ ID NOs:1-4). For example, the nucleotide sequence of the antisense polynucleotide agent (ASO), A-134986.1, is fully complementary over its entire length to the equivalent region of nucleotides 275-294 of NM_000029.3 (SEQ ID NO:1) (see, *e.g.,* Table 3). Similarly, the nucleotide sequence of the antisense polynucleotide agent (ASO), A-135032.1, is fully complementary over its entire length to the equivalent region of nucleotides 981-1000 of NM_000029.3 (SEQ ID NO:1) (see, *e.g.,* Table 3) and the nucleotide sequence of the antisense polynucleotide agent (ASO), A-135034.1, is fully complementary over its entire length to the equivalent region of nucleotides 1003-1022 of NM_000029.3 (SEQ ID NO:1) (see, *e.g.,* Table 3).

An antisense polynucleotide agent may comprise a contiguous nucleotide sequence of about 4 to about 50 nucleotides in length, *e.g.*, 8-49, 8-48, 8-47, 8-46, 8-45, 8-44, 8-43, 8-42, 8-41, 8-40, 8-39, 8-38, 8-37, 8-36, 8-35, 8-34, 8-33, 8-32, 8-31, 8-30, 8-29, 8-28, 8-27, 8-26, 8-25, 8-24, 8-23, 8-22, 8-21, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 10-49, 10-48, 10-47, 10-46, 10-45, 10-44, 10-43, 10-42, 10-41, 10-40, 10-39, 10-38, 10-37, 10-36, 10-35, 10-34, 10-33, 10-32, 10-31, 10-30, 10-29, 10-28, 10-27, 10-26, 10-25, 10-24, 10-23, 10-22, 10-21, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11,11-49, 11-48, 11-47, 11-46, 11-45, 11-44, 11-43, 11-42, 11-41, 11-40, 11-39, 11-38, 11-37, 11-36, 11-35, 11-34, 11-33, 11-32, 11-31, 11-30, 11-29, 11-28, 11-27, 11-26, 11-25, 11-24, 11-23, 11-22, 11-21, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-49, 12-48, 12-47, 12-46, 12-45, 12-44, 12-43, 12-42, 12-41, 12-40, 12-39, 12-38, 12-37, 12-36, 12-35, 12-34, 12-33, 12-32, 12-31, 12-30, 12-29, 12-28, 12-27, 12-26, 12-25, 12-24, 12-23, 12-22, 12-21, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-49, 13-48, 13-47, 13-46, 13-45, 13-44, 13-43, 13-42, 13-41, 13-40, 13-39, 13-38, 13-37, 13-36, 13-35, 13-

34, 13-33, 13-32, 13-31, 13-30, 13-29, 13-28, 13-27, 13-26, 13-25, 13-24, 13-23, 13-22, 13-21, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-49, 14-48, 14-47, 14-46, 14-45, 14-44, 14-43, 14-42, 14-41, 14-40, 14-39, 14-38, 14-37, 14-36, 14-35, 14-34, 14-33, 14-32, 14-31, 14-30, 14-29, 14-28, 14-27, 14-26, 14-25, 14-24, 14-23, 14-22, 14-21, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-49, 15-48, 15-47, 15-46, 15-45, 15-44, 15-43, 15-42, 15-41, 15-40, 15-39, 15-38, 15-37, 15-36, 15-35, 15-34, 15-33, 15-32, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 15-16,16-49, 16-48, 16-47, 16-46, 16-45, 16-44, 16-43, 16-42, 16-41, 16-40, 16-39, 16-38, 16-37, 16-36, 16-35, 16-34, 16-33, 16-32, 16-31, 16-30, 16-29, 16-28, 16-27, 16-26, 16-25, 16-24, 16-23, 16-22, 16-21, 16-20, 16-19, 16-18, 16-17, 17-49, 17-48, 17-47, 17-46, 17-45, 17-44, 17-43, 17-42, 17-41, 17-40, 17-39, 17-38, 17-37, 17-36, 17-35, 17-34, 17-33, 17-32, 17-31, 17-30, 17-29, 17-28, 17-27, 17-26, 17-25, 17-24, 17-23, 17-22, 17-21, 17-20, 17-19, 17-18, 18-49, 18-48, 18-47, 18-46, 18-45, 18-44, 18-43, 18-42, 18-41, 18-40, 18-39, 18-38, 18-37, 18-36, 18-35, 18-34, 18-33, 18-32, 18-31, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-49, 19-48, 19-47, 19-46, 19-45, 19-44, 19-43, 19-42, 19-41, 19-40, 19-39, 19-38, 19-37, 19-36, 19-35, 19-34, 19-33, 19-32, 19-31, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-49, 20-48, 20-47, 20-46, 20-45, 20-44, 20-43, 20-42, 20-41, 20-40, 20-39, 20-38, 20-37, 20-36, 20-35, 20-34, 20-33, 20-32, 20-31, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-49, 21-48, 21-47, 21-46, 21-45, 21-44, 21-43, 21-42, 21-41, 21-40, 21-39, 21-38, 21-37, 21-36, 21-35, 21-34, 21-33, 21-32, 21-31, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, 21-22, 22-49, 22-48, 22-47, 22-46, 22-45, 22-44, 22-43, 22-42, 22-41, 22-40, 22-39, 22-38, 22-37, 22-36, 22-35, 22-34, 22-33, 22-32, 22-31, 22-30, 22-29, 22-28, 22-27, 22-26, 22-25, 22-24, 22-23, 23-49, 23-48, 23-47, 23-46, 23-45, 23-44, 23-43, 23-42, 23-41, 23-40, 23-39, 23-38, 23-37, 23-36, 23-35, 23-34, 23-33, 23-32, 23-31, 23-30, 23-29, 23-28, 23-27, 23-26, 23-25, 23-24, 24-49, 24-48, 24-47, 24-46, 24-45, 24-44, 24-43, 24-42, 24-41, 24-40, 24-39, 24-38, 24-37, 24-36, 24-35, 24-34, 24-33, 24-32, 24-31, 24-30, 24-29, 24-28, 24-27, 24-26, 24-25, 25-49, 25-48, 25-47, 25-46, 25-45, 25-44, 25-43, 25-42, 25-41, 25-40, 25-39, 25-38, 25-37, 25-36, 25-35, 25-34, 25-33, 25-32, 25-31, 25-30, 25-29, 25-28, 25-27, 25-26,26-49, 26-48, 26-47, 26-46, 26-45, 26-44, 26-43, 26-42, 26-41, 26-40, 26-39, 26-38, 26-37, 26-36, 26-35, 26-34, 26-33, 26-32, 26-31, 26-30, 26-29, 26-28, 26-27, 27-49, 27-48, 27-47, 27-46, 27-45, 27-44, 27-43, 27-42, 27-41, 27-40, 27-39, 27-38, 27-37, 27-36, 27-35, 27-34, 27-33, 27-32, 27-31, 27-30, 27-29, 27-28, 28-49, 28-48, 28-47, 28-46, 28-45, 28-44, 28-43, 28-42, 28-41, 28-40, 28-39, 28-38, 28-37, 28-36, 28-35, 28-34, 28-33, 28-32, 28-31, 28-30, 28-29, 29-49, 29-48, 29-47, 29-46, 29-45, 29-44, 29-43, 29-42, 29-41, 29-40, 29-39, 29-38, 29-37, 29-36, 29-35, 29-34, 29-33, 29-32, 29-31, 29-30, 30-49, 30-48, 30-47, 30-46, 30-45, 30-44, 30-43, 30-42, 30-41, 30-40, 30-39, 30-38, 30-37, 30-36, 30-35, 30-34, 30-33, 30-32, or 30-31 nucleotides in length, *e.g.*, 4, 5, 6, 7, 8, 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length.

In some embodiments, an antisense polynucleotide agent may comprise a contiguous nucleotide sequence of no more than 22 nucleotides, such as no more than 21 nucleotides, 20 nucleotides, 19 nucleotides, or no more than 18 nucleotides. In some embodiments the antisense polynucleotide agenst of the invention comprises less than 20 nucleotides. In other emabodiments, the antisense polynucleotide agents of the invention comprise 20 nucleotides.

In certain aspects, an antisense polynucleotide agent of the invention includes a sequence selected from the group of sequences provided in Table 3. It will be understood that, although some of the sequences in Table 3 are described as modified and/or conjugated sequences, an antisense polynucleotide agent of the invention, may also comprise any one of the sequences set forth in Table 3 that is un-modified, un-conjugated, and/or modified and/or conjugated differently than described therein.

By virtue of the nature of the nucleotide sequences provided in Table 3, antisense polynucleotide agents of the invention may include one of the sequences of Table 3 minus only a few nucleotides on one or both ends and yet remain similarly effective as compared to the antisense polynucleotide agents described above. Hence, antisense polynucleotide agents having a sequence of at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of Table 3 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 an antisense polynucleotide agent comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the antisense polynucleotide agents provided in Table 3 identify a region(s) in an AGT transcript that is susceptible to antisense inhibition (*e.g.*, the regions encompassed by the start and end positions relative to NM_000029.3in Table 3). As such, the present invention further features antisense polynucleotide agents that target within one of these sites. As used herein, an antisense polynucleotide agent is said to target within a particular site of an RNA transcript if the antisense polynucleotide agent promotes antisense inhibition of the target at that site. Such an antisense polynucleotide agent will generally include at least about 15 contiguous nucleotides from one of the sequences provided in Table 3 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an AGT gene.

While a target sequence is generally about 4-50 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing antisense inhibition of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a “window” or “mask” of a given size (as a non-limiting example, 20 nucleotides) is literally or figuratively (including, *e.g.*, *in silico*) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an antisense polynucleotide agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in Table 3 represent effective target sequences, it is contemplated that further optimization of antisense inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, in Table 3, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of antisense polynucleotide agents based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in length, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

III. Modified Antisense Polynucleotide Agents of the Invention

In one embodiment, the nucleotides of an antisense polynucleotide agent of the invention are un-modified, and do not comprise, *e.g.,* chemical modifications and/or conjugations known in the art and described herein. In another embodiment, at least one of the nucleotides of an antisense polynucleotide agent of the invention is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an antisense polynucleotide agent of the invention are modified. In other embodiments of the invention, all of the nucleotides of an antisense polynucleotide agent of the invention are modified. Antisense polynucleotide agents of the invention in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

The nucleic acids featured in the invention can be synthesized and/or modified by standard methods known in the art as further discussed below, *e.g.*, solution-phase or solid-phase organic synthesis or both, *e.g.,* by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems®, Inc. Well-established methods for the synthesis and/or modification of the nucleic acids featured in the invention are described in, for example, "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, 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; and/or backbone modifications, including modification or replacement of the phosphodiester linkages.

Specific examples of modified nucleotides useful in the embodiments described herein include, but are not limited to nucleotides containing modified backbones or no natural internucleoside linkages. Nucleotides 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 nucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified antisense polynucleotide agent will have a phosphorus atom in its internucleoside backbone.

Modified nucleotide 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.

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 US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified nucleotide 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.

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, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable nucleotide mimetics are contemplated for use in antisense polynucleotide agents, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the antisense polynucleotide agents of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include polynucleotides 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 embodiments, the antisense polynucleotide agents featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified nucleotides can also contain one or more modified or substituted sugar moieties. The antisense polynucleotide agents 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 embodiments, antisense polynucleotide agents 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 antisense polynucleotide, or a group for improving the pharmacodynamic properties of an antisense polynucleotide agent, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al*., *Helv. Chim. Acta*, 1995, 78:486-504) *i.e*., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e*., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e*., 2'-O--CH_2--O--CH_2--N(CH_2)$_2$.

Other modifications include 2'-methoxy (2'-O-CH_3) also referred to as 2'-OMe, 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on a nucleotide of an antisense polynucleotide agent, particularly the 3' position of the sugar on the 3' terminal nucleotide. Antisense polynucleotide agents can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. 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. The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional nucleotides having modified or substituted sugar moieties for use in the polynucleotide agents of the invention include nucleotides comprising a bicyclic sugar. 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 embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring. Thus, in some embodiments an antisense polynucleotide agent may include one or more locked nucleic acids. A "locked nucleic acid" ("LNA") 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 santisense polynucleotide agents has been shown to increase santisense polynucleotide agent stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

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

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

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

In one particular embodiment of the invention, an antisense polynucleotide agent can 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 embodiment, a constrained ethyl nucleotide is in an S conformation and is referred to as an "*S*-constrained ethyl nucleotide" or "*S*-cEt."

Modified nucleotides included in the antisense polynucleotide agents of the invention can also contain one or more sugar mimetics. For example, the antisense polynucleotide agent may include a "modified tetrahydropyran nucleotide" or "modified THP nucleotide." A "modified tetrahydropyran nucleotide" has a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleotides (a sugar surrogate). Modified THP nucleotides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see, *e.g.,* Leumann, *Bioorg. Med. Chem*., 2002, 10, 841-854), or fluoro HNA (F-HNA).

In some embodiments of the invention, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleotides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al., Biochemistry*, 2002, 41, 4503-4510; and U.S. Patent Nos. 5,698,685; 5,166,315; 5,185,444; and 5,034,506). Morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modified morpholinos."

Combinations of modifications are also provided without limitation, such as 2′-F-5′-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on Aug. 21, 2008 for other disclosed 5′, 2′-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2′-position (see published U.S. Patent Application US2005-0130923, published on Jun. 16, 2005) or alternatively 5′-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4′-CH2-0-2′ bicyclic nucleoside is further substituted at the 5′ position with a 5′-methyl or a 5′-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (see, e.g., Srivastava et al., J. Am. Chem. Soc. 2007, 129(26), 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on Apr. 10, 2010, Robeyns et al., J. Am. Chem. Soc., 2008, 130(6), 1979-1984; Horvath et al., Tetrahedron Letters, 2007, 48, 3621-3623; Nauwelaerts et al., J. Am. Chem. Soc., 2007, 129(30), 9340-9348; Gu et al., Nucleosides, Nucleotides & Nucleic Acids, 2005, 24(5-7), 993-998; Nauwelaerts et al., Nucleic Acids Research, 2005, 33(8), 2452-2463; Robeyns et al., Acta Crystallographica, Section F: Structural Biology and Crystallization Communications, 2005, F61(6), 585-586; Gu et al., Tetrahedron, 2004, 60(9), 2111-2123; Gu et al., Oligonucleotides, 2003, 13(6), 479-489; Wang et al., J. Org. Chem., 2003, 68, 4499-4505; Verbeure et al., Nucleic Acids Research, 2001, 29(24), 4941-4947; Wang et al., J. Org. Chem., 2001, 66, 8478-82; Wang et al., Nucleosides, Nucleotides & Nucleic Acids, 2001, 20(4-7), 785-788; Wang et al., J. Am. Chem., 2000, 122, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety).

An antisense polynucleotide agent can also include nucleobase 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. Patent 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, *antisense polynucleotide agent 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 agents featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., *antisense polynucleotide agent 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.

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, the entire contents of each of which are hereby incorporated herein by reference.

One or more of the nucleotides of an antisense polynucleotide agent of the invention may also include a hydroxymethyl substituted nucleotide. A “hydroxymethyl substituted nucleotide” is an acyclic 2’-3’-seco-nucleotide, also referred to as an “unlocked nucleic acid” (“UNA”) modification. Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Additional modifications which may potentially stabilize the ends of antisense polynucleotide agents 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 US Patent Publication No. 2012/0142101.

Any of the antisense polynucleotide agents of the invention may be optionally conjugated with a GalNAc derivative ligand, as described in Section IV, below.

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

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The antisense polynucleotide agents may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In certain specific embodiments, the antisense polynucleotide agent for use in the methods of the invention is an agent selected from the group of agents listed in Table 3. These agents may further comprise a ligand, as described in Section IV, below.

A. Antisense Polynucleotide Agents Comprising Motifs

In certain embodiments of the invention, at least one of the contiguous nucleotides of the antisense polynucleotide agents of the invention may be a modified nucleotide. In one embodiment, the modified nucleotide comprises one or more modified sugars. In other embodiments, the modified nucleotide comprises one or more modified nucleobases. In yet other embodiments, the modified nucleotide comprises one or more modified internucleoside linkages. In some embodiments, the modifications (sugar modifications, nucleobase modifications, and/or linkage modifications) define a pattern or motif. In one embodiment, the patterns of modifications of sugar moieties, internucleoside linkages, and nucleobases are each independent of one another.

Antisense polynucleotide agents having modified oligonucleotides arranged in patterns, or motifs may, for example, confer to the agents properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases. For example, such agents may contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of such agents may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

An exemplary antisense polynucleotide agent having modified oligonucleotides arranged in patterns, or motifs is a gapmer. In a “gapmer”, an internal region or "gap" having a plurality of linked nucleotides that supports RNaseH cleavage is positioned between two external flanking regions or "wings" having a plurality of linked nucleotides that are chemically distinct from the linked nucleotides of the internal region. The gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleotides.

The three regions of a gapmer motif (the 5 '-wing, the gap, and the 3 '-wing) form a contiguous sequence of nucleotides and may be described as “X-Y-Z”, wherein “X” represents the length of the 5-wing, “Y” represents the length of the gap, and “Z” represents the length of the 3’-wing. In one embodiment, a gapmer described as “X-Y-Z” has a configuration such that the gap segment is positioned immediately adjacent to each of the 5′ wing segment and the 3′ wing segment. Thus, no intervening nucleotides exist between the 5′ wing segment and gap segment, or the gap segment and the 3′ wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different.

In certain embodiments, the regions of a gapmer are differentiated by the types of modified nucleotides in the region. The types of modified nucleotides that may be used to differentiate the regions of a gapmer, in some embodiments, include β-D-ribonucleotides, β-D-deoxyribonucleotides, 2′-modified nucleotides, *e.g.,* 2′-modified nucleotides (*e.g.,* 2′-MOE, and 2′-O—CH3), and bicyclic sugar modified nucleotides (*e.g.,* those having a 4′-(CH2)n-O-2′ bridge, where n=1 or n=2).

In one embodiment, at least some of the modified nucleotides of each of the wings may differ from at least some of the modified nucleotides of the gap. For example, at least some of the modified nucleotides of each wing that are closest to the gap (the 3 '-most nucleotide of the 5'-wing and the 5'-most nucleotide of the 3 -wing) differ from the modified nucleotides of the neighboring gap nucleotides, thus defining the boundary between the wings and the gap. In certain embodiments, the modified nucleotides within the gap are the same as one another. In certain embodiments, the gap includes one or more modified nucleotides that differ from the modified nucleotides of one or more other nucleotides of the gap.

The length of the 5'- wing (X) of a gapmer may be 1 to 6 nucleotides in length, *e.g.,* 2 to 6, 2 to 5, 3 to 6, 3 to 5, 1 to 5, 1 to 4, 1 to 3, 2 to 4 nucleotides in length, *e.g.,* 1, 2, 3, 4, 5, or 6 nucleotides in length.

The length of the 3'- wing (Z) of a gapmer may be 1 to 6 nucleotides in length, *e.g.,* 2 to 6, 2-5, 3 to 6, 3 to 5, 1 to 5, 1 to 4, 1 to 3, 2 to 4 nucleotides in length, *e.g.,* 1, 2, 3, 4, 5, or 6 nucleotides in length.

The length of the gap (Y) of a gapmer may be 5 to 14 nucleotides in length, *e.g.,* 5 to 13, 5 to 12, 5 to 11, 5 to 10, 5 to 9, 5 to 8, 5 to 7, 5 to 6, 6 to 14, 6 to 13, 6 to 12, 6 to 11, 6 to 10, 6 to 9, 6 to 8, 6 to 7, 7 to 14, 7 to 13, 7 to 12, 7 to 11, 7 to 10, 7 to 9, 7 to 8, 8 to 14, 8 to 13, 8 to 12, 8 to 11, 8 to 10, 8 to 9, 9 to 14, 9 to 13, 9 to 12, 9 to 11, 9 to 10, 10 to 14, 10 to 13, 10 to 12, 10 to 11, 11 to 14, 11 to 13, 11 to 12, 12 to 14, 12 to 13, or 13 to 14 nucleotides in length, *e.g.,* 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 nucleotides in length.

In some embodiments of the invention X consists of 2, 3, 4, 5 or 6 nucleotides, Y consists of 7, 8, 9, 10, 11, or 12 nucleotides, and Z consists of 2, 3, 4, 5 or 6 nucleotides. Such gapmers include (X-Y-Z) 2-7-2, 2-7-3, 2-7-4, 2-7-5, 2-7-6, 3-7-2, 3-7-3, 3-7-4, 3-7-5, 3-7-6, 4-7-3, 4-7-4, 4-7-5, 4-7-6, 5-7-3, 5-7-4, 5-7-5, 5-7-6, 6-7-3, 6-7-4, 6-7-5, 6-7-6, 3-7-3, 3-7-4, 3-7-5, 3-7-6, 4-7-3, 4-7-4, 4-7-5, 4-7-6, 5-7-3, 5-7-4, 5-7-5, 5-7-6, 6-7-3, 6-7-4, 6-7-5, 6-7-6, 2-8-2, 2-8-3, 2-8-4, 2-8-5, 2-8-6, 3-8-2, 3-8-3, 3-8-4, 3-8-5, 3-8-6, 4-8-3, 4-8-4, 4-8-5, 4-8-6, 5-8-3, 5-8-4, 5-8-5, 5-8-6, 6-8-3, 6-8-4, 6-8-5, 6-8-6, 2-9-2, 2-9-3, 2-9-4, 2-9-5, 2-9-6, 3-9-2, 3-9-3, 3-9-4, 3-9-5, 3-9-6, 4-9-3, 4-9-4, 4-9-5, 4-9-6, 5-9-3, 5-9-4, 5-9-5, 5-9-6, 6-9-3, 6-9-4, 6-9-5, 6-9-6, 2-10-2, 2-10-3, 2-10-4, 2-10-5, 2-10-6, 3-10-2, 3-10-3, 3-10-4, 3-10-5, 3-10-6, 4-10-3, 4-10-4, 4-10-5, 4-10-6, 5-10-3, 5-10-4, 5-10-5, 5-10-6, 6-10-3, 6-10-4, 6-10-5, 6-10-6, 2-11-2, 2-11-3, 2-11-4, 2-11-5, 2-11-6, 3-11-2, 3-11-3, 3-11-4, 3-11-5, 3-11-6, 4-11-3, 4-11-4, 4-11-5, 4-11-6, 5-11-3, 5-11-4, 5-11-5, 5-11-6, 6-11-3, 6-11-4, 6-11-5, 6-11-6, 2-12-2, 2-12-3, 2-12-4, 2-12-5, 2-12-6, 3-12-2, 3-12-3, 3-12-4, 3-12-5, 3-12-6, 4-12-3, 4-12-4, 4-12-5, 4-12-6, 5-12-3, 5-12-4, 5-12-5, 5-12-6, 6-12-3, 6-12-4, 6-12-5, or 6-12-6.

In some embodiments of the invention, antisense polynucleotide agents targeting AGT include a 5-10-5 gapmer motif. In other embodiments of the invention, antisense polynucleotide agents targeting AGT include a 4-10-4 gapmer motif. In another embodiment of the invention, antisense polynucleotide agents targeting AGT include a 3-10-3 gapmer motif. In yet other embodiments of the invention, antisense polynucleotide agents targeting AGT include a 2-10-2 gapmer motif.

The 5'- wing and/or 3'-wing of a gapmer may independently include 1-6 modified nucleotides, *e.g.,* 1, 2, 3, 4, 5, or 6 modified nucleotides.

In some embodiment, the 5’-wing of a gapmer includes at least one modified nucleotide. In one embodiment, the 5'- wing of a gapmer comprises at least two modified nucleotides. In another embodiment, the 5'- wing of a gapmer comprises at least three modified nucleotides. In yet another embodiment, the 5'- wing of a gapmer comprises at least four modified nucleotides. In another embodiment, the 5'- wing of a gapmer comprises at least five modified nucleotides. In certain embodiments, each nucleotide of the 5'-wing of a gapmer is a modified nucleotide.

In some embodiments, the 3’-wing of a gapmer includes at least one modified nucleotide. In one embodiment, the 3'- wing of a gapmer comprises at least two modified nucleotides. In another embodiment, the 3'- wing of a gapmer comprises at least three modified nucleotides. In yet another embodiment, the 3'- wing of a gapmer comprises at least four modified nucleotides. In another embodiment, the 3'- wing of a gapmer comprises at least five modified nucleotides. In certain embodiments, each nucleotide of the 3'-wing of a gapmer is a modified nucleotide.

In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties of the nucleotides. In one embodiment, the nucleotides of each distinct region comprise uniform sugar moieties. In other embodiments, the nucleotides of each distinct region comprise different sugar moieties. In certain embodiments, the sugar nucleotide modification motifs of the two wings are the same as one another. In certain embodiments, the sugar nucleotide modification motifs of the 5'-wing differs from the sugar nucleotide modification motif of the 3'-wing.

The 5’-wing of a gapmer may include 1-6 modified nucleotides, *e.g.,* 1, 2, 3, 4, 5, or 6 modified nucleotides.

In one embodiment, at least one modified nucleotide of the 5'-wing of a gapmer is a bicyclic nucleotide, such as a constrained ethyl nucleotide, or an LNA. In another embodiment, the 5’-wing of a gapmer includes 2, 3, 4, or 5 bicyclic nucleotides. In some embodiments, each nucleotide of the 5'- wing of a gapmer is a bicyclic nucleotide.

In one embodiment, the 5’-wing of a gapmer includes at least 1, 2, 3, 4, or 5 constrained ethyl nucleotides. In some embodiments, each nucleotide of the 5'- wing of a gapmer is a constrained ethyl nucleotide.

In one embodiment, the 5'-wing of a gapmer comprises at least one LNA nucleotide. In another embodiment, the 5’-wing of a gapmer includes 2, 3, 4, or 5 LNA nucleotides. In other embodiments, each nucleotide of the 5'- wing of a gapmer is an LNA nucleotide.

In certain embodiments, at least one modified nucleotide of the 5'- wing of a gapmer is a non-bicyclic modified nucleotide, *e.g.,* a 2 '-substituted nucleotide. A “2 '-substituted nucleotide” is a nucleotide comprising a modification at the 2’-position which is other than H or OH, such as a 2’-OMe nucleotide, or a 2’-MOE nucleotide. In one embodiment, the 5’-

wing of a gapmer comprises 2, 3, 4, or 5 2 '-substituted nucleotides. In one embodiment, each nucleotide of the 5'-wing of a gapmer is a 2 '-substituted nucleotide.

In one embodiment, the 5'- wing of a gapmer comprises at least one 2'-OMe nucleotide. In one embodiment, the 5'- wing of a gapmer comprises at least 2, 3, 4, or 5 2'-OMe nucleotides. In one embodiment, each of the nucleotides of the 5'- wing of a gapmer comprises a 2'-OMe nucleotide.

In one embodiment, the 5'- wing of a gapmer comprises at least one 2'- MOE nucleotide. In one embodiment, the 5'- wing of a gapmer comprises at least 2, 3, 4, or 5 2'-MOE nucleotides. In one embodiment, each of the nucleotides of the 5'- wing of a gapmer comprises a 2'- MOE nucleotide.

In certain embodiments, the 5'- wing of a gapmer comprises at least one 2'-deoxynucleotide. In certain embodiments, each nucleotide of the 5'- wing of a gapmer is a 2'-deoxynucleotide. In a certain embodiments, the 5'- wing of a gapmer comprises at least one ribonucleotide. In certain embodiments, each nucleotide of the 5'- wing of a gapmer is a ribonucleotide.

The 3'-wing of a gapmer may include 1-6 modified nucleotides, *e.g.,* 1, 2, 3, 4, 5, or 6 modified nucleotides.

In one embodiment, at least one modified nucleotide of the 3'-wing of a gapmer is a bicyclic nucleotide, such as a constrained ethyl nucleotide, or an LNA. In another embodiment, the 3'-wing of a gapmer includes 2, 3, 4, or 5 bicyclic nucleotides. In some embodiments, each nucleotide of the 3'-wing of a gapmer is a bicyclic nucleotide.

In one embodiment, the 3'-wing of a gapmer includes at least one constrained ethyl nucleotide. In another embodiment, the 3'-wing of a gapmer includes 2, 3, 4, or 5 constrained ethyl nucleotides. In some embodiments, each nucleotide of the 3'-wing of a gapmer is a constrained ethyl nucleotide.

In one embodiment, the 3'-wing of a gapmer comprises at least one LNA nucleotide. In another embodiment, the 3'-wing of a gapmer includes 2, 3, 4, or 5 LNA nucleotides. In other embodiments, each nucleotide of the 3'-wing of a gapmer is an LNA nucleotide.

In certain embodiments, at least one modified nucleotide of the 3'-wing of a gapmer is a non-bicyclic modified nucleotide, *e.g.,* a 2 '-substituted nucleotide. In one embodiment, the 3'-wing of a gapmer comprises 2, 3, 4, or 5 2 '-substituted nucleotides. In one embodiment, each nucleotide of the 3'-wing of a gapmer is a 2 '-substituted nucleotide.

In one embodiment, the 3'-wing of a gapmer comprises at least one 2'-OMe nucleotide. In one embodiment, the 3'-wing of a gapmer comprises at least 2, 3, 4, or 5 2'-OMe nucleotides. In one embodiment, each of the nucleotides of the 3'-wing of a gapmer comprises a 2'-OMe nucleotide.

In one embodiment, the 3'-wing of a gapmer comprises at least one 2'- MOE nucleotide. In one embodiment, the 3'-wing of a gapmer comprises at least 2, 3, 4, or 5 2'-MOE nucleotides. In one embodiment, each of the nucleotides of the 3'-wing of a gapmer comprises a 2'- MOE nucleotide.

In certain embodiments, the 3'-wing of a gapmer comprises at least one 2'-deoxynucleotide. In certain embodiments, each nucleotide of the 3'-wing of a gapmer is a 2'-deoxynucleotide. In a certain embodiments, the 3'-wing of a gapmer comprises at least one ribonucleotide. In certain embodiments, each nucleotide of the 3'-wing of a gapmer is a ribonucleotide.

The gap of a gapmer may include 5-14 modified nucleotides, *e.g.,* 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 modified nucleotides.

In one embodiment, the gap of a gapmer comprises at least one 5-methylcytosine. In one embodiment, the gap of a gapmer comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 5-methylcytosines. In one embodiment, all of the nucleotides of the the gap of a gapmer are 5-methylcytosines.

In one embodiment, the gap of a gapmer comprises at least one 2'-deoxynucleotide. In one embodiment, the gap of a gapmer comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 2'-deoxynucleotides. In one embodiment, all of the nucleotides of the the gap of a gapmer are 2'-deoxynucleotides.

A gapmer may include one or more modified internucleotide linkages. In some embodiments, a gapmer includes one or more phosphodiester internucleotide linkages. In other embodiments, a gapmer includes one or more phosphorothioate internucleotide linkages.

In one embodiment, each nucleotide of a 5'-wing of a gapmer are linked *via* a phosphorothioate internucleotide linkage. In another embodiment, each nucleotide of a 3'-wing of a gapmer are linked *via* a phosphorothioate internucleotide linkage. In yet another embodiment, each nucleotide of a gap segment of a gapmer is linked *via* a phosphorothioate internucleotide linkage. In one embodiment, all of the nucleotides in a gapmer are linked *via* phosphorothioate internucleotide linkages.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising five nucleotides and a 3'-wing segment comprising 5 nucleotides.

In another embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising four nucleotides and a 3'-wing segment comprising four nucleotides.

In another embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising three nucleotides and a 3'-wing segment comprising three nucleotides.

In another embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising two nucleotides and a 3'-wing segment comprising two nucleotides.

In one embodiment, each nucleotide of a 5-wing flanking a gap segment of 10 2′-deoxyribonucleotides comprises a modified nucleotide. In another embodiment, each nucleotide of a 3-wing flanking a gap segment of 10 2′-deoxyribonucleotides comprises a modified nucleotide. In one embodiment, each of the modified 5'-wing nucleotides and each of the modified 3'-wing nucleotides comprise a 2′-sugar modification. In one embodiment, the 2′-sugar modification is a 2'-OMe modification. In another embodiment, the 2′-sugar modification is a 2'-MOE modification. In one embodiment, each of the modified 5'-wing nucleotides and each of the modified 3'-wing nucleotides comprise a bicyclic nucleotide. In one embodiment, the bicyclic nucleotide is a constrained ethyl nucleotide. In another embodiment, the bicyclic nucleotide is an LNA nucleotide. In one embodiment, each cytosine in an antisense polynucleotide agent targeting an AGT gene is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising five nucleotides comprising a 2'OMe modification and a 3'-wing segment comprising five nucleotides comprising a 2'OMe modification, wherein each internucleotde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine. In one embodiment, the agent further comprises a ligand.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising five nucleotides comprising a 2'MOE modification and a 3'-wing segment comprising five nucleotides comprising a 2'MOE modification, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine. In one embodiment, the agent further comprises a ligand.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising five constrained ethyl nucleotides and a 3'-wing segment comprising five constrained ethyl nucleotides, wherein each internucleoitde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising five LNA nucleotides and a 3’-wing segment comprising five LNA nucleotides, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising four nucleotides comprising a 2’OMe modification and a 3’-wing segment comprising four nucleotides comprising a 2’OMe modification, wherein each internucleotde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising four nucleotides comprising a 2’MOE modification and a 3’-wing segment comprising four nucleotides comprising a 2’MOE modification, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising four constrained ethyl nucleotides and a 3’-wing segment comprising four constrained ethyl nucleotides, wherein each internucleoitde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising four LNA nucleotides and a 3’-wing segment comprising four LNA nucleotides, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising three nucleotides comprising a 2’OMe modification and a 3’-wing segment comprising three nucleotides comprising a 2’OMe modification, wherein each internucleotde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising three nucleotides comprising a 2’MOE modification and a 3’-wing segment comprising three nucleotides comprising a 2’MOE modification, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising three constrained ethyl nucleotides and a 3’-wing segment comprising three constrained ethyl nucleotides, wherein each internucleoitde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising three LNA nucleotides and a 3’-wing segment comprising three LNA nucleotides, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising two nucleotides comprising a 2’OMe modification and a 3’-wing segment comprising two nucleotides comprising a 2’OMe modification, wherein each internucleotde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising two nucleotides comprising a 2’MOE modification and a 3’-wing segment comprising two nucleotides comprising a 2’MOE modification, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5’-wing segment comprising two constrained ethyl nucleotides and a 3’-wing segment comprising two constrained ethyl nucleotides, wherein each internucleoitde linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

In one embodiment, an antisense polynucleotide agent targeting an AGT gene comprises a gap segment of ten 2′-deoxyribonucleotides positioned immediately adjacent to and between a 5'-wing segment comprising two LNA nucleotides and a 3'-wing segment comprising two LNA nucleotides, wherein each internucleotide linkage of the agent is a phosphorothioate linkage. In one embodiment, each cytosine of the agent is a 5-methylcytosine.

Further gapmer designs suitable for use in the agents, compositions, and methods of the invention are disclosed in, for example, U.S. Patent Nos. 7,687,617 and 8,580,756; U.S. Patent Publication Nos. 20060128646, 20090209748, 20140128586, 20140128591, 20100210712, and 20080015162A1; and International Publication No. WO 2013/159108, the entire content of each of which are incorporated herein by reference.

IV. Antisense Polynucleotide Agents Conjugated to Ligands

Another modification of the polynucleotide agents of the invention involves chemically linking to the agent one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the antisense polynucleotide agent. 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), 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).

In one embodiment, a ligand alters the distribution, targeting or lifetime of an antisense polynucleotide agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in hybridization of an antisense polynucleotide agent to the targeted mRNA.

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

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucoseamine 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.

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

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

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

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

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

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

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

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

In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

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

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

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

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

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

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 5). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 6) 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: 7) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 8) 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 an antisens epolynucleotide agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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

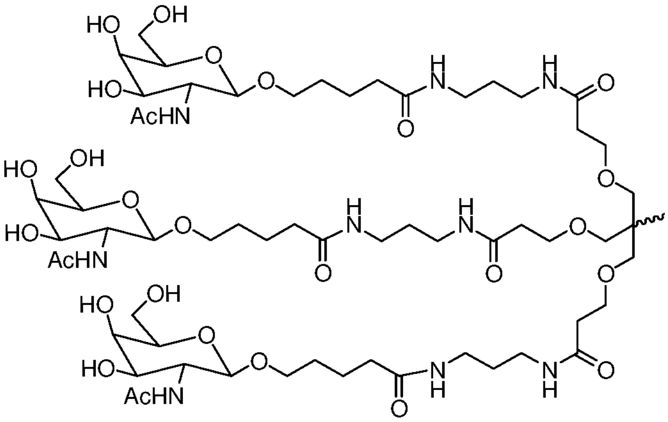
A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing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

In some embodiments of the compositions and methods of the invention, an antisense polynucleotide agent further comprises a carbohydrate. The carbohydrate conjugated agents are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein (see, *e.g.,* Prakash, *et al.* (2014) *Nuc Acid Res* doi 10.1093/nar/gku531). As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.,* C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.,* C5, C6, C7, or C8).

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

from the group consisting of:



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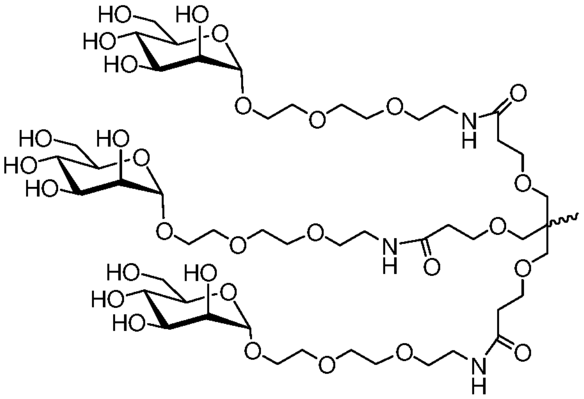

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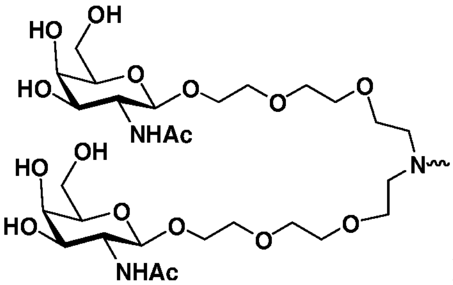

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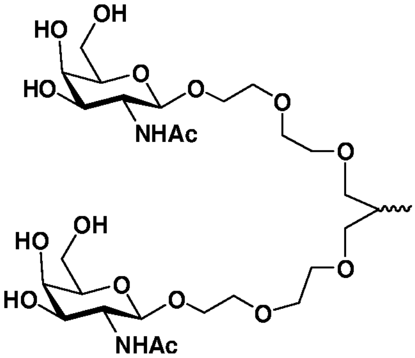

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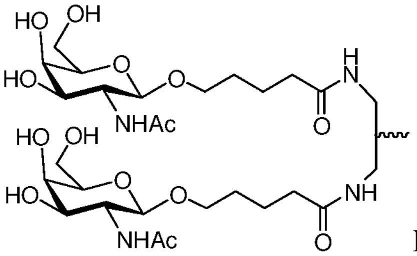

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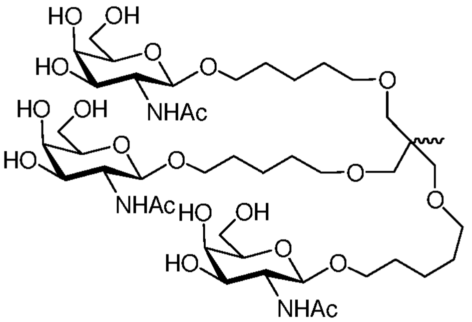

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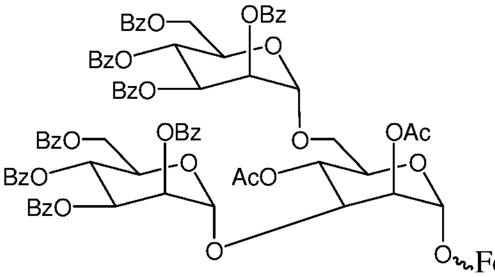

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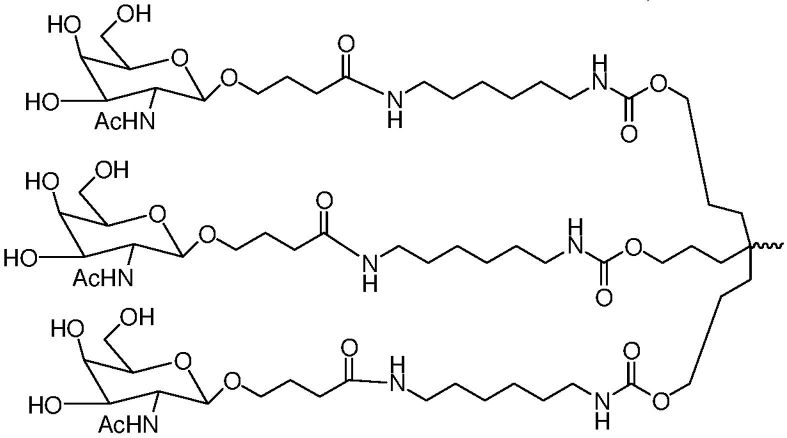

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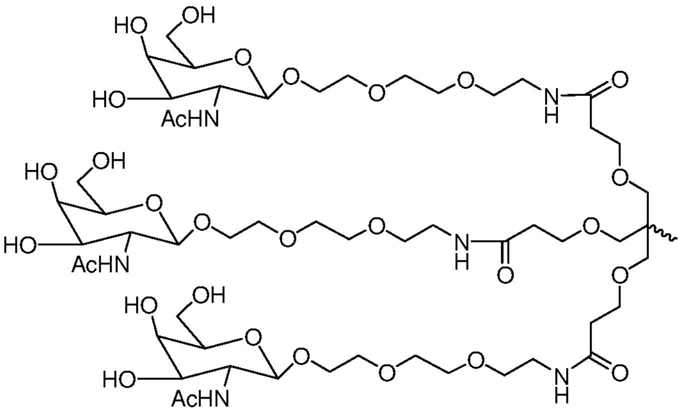

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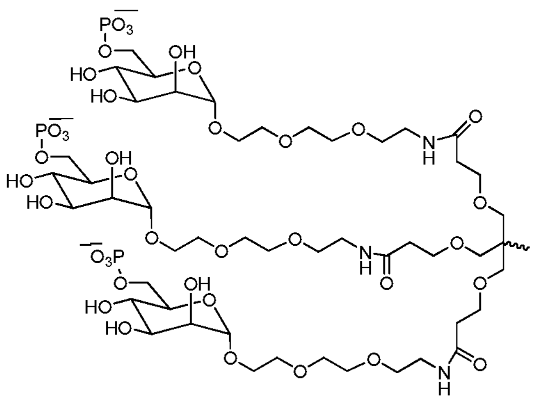

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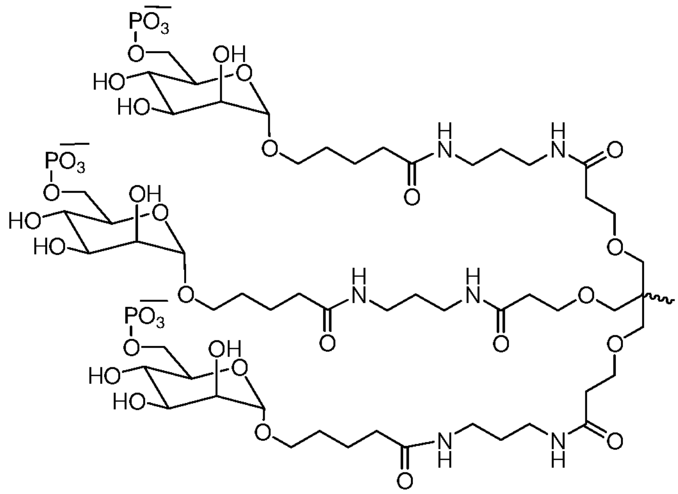

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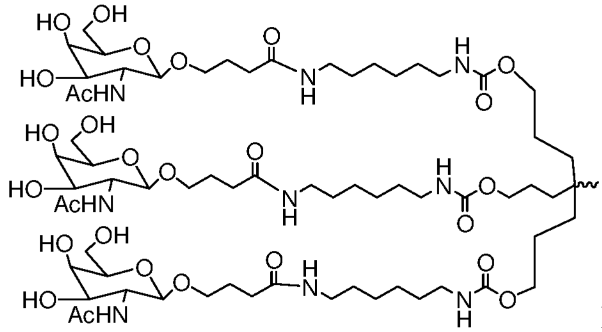

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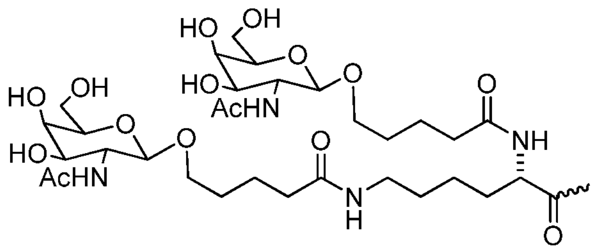

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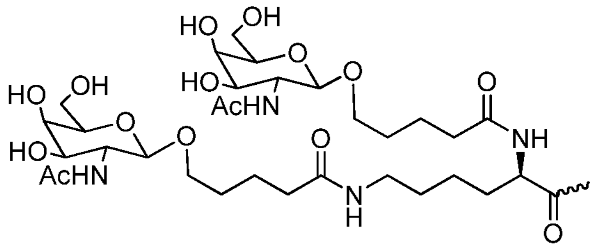

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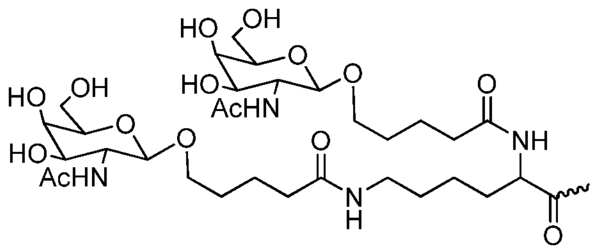

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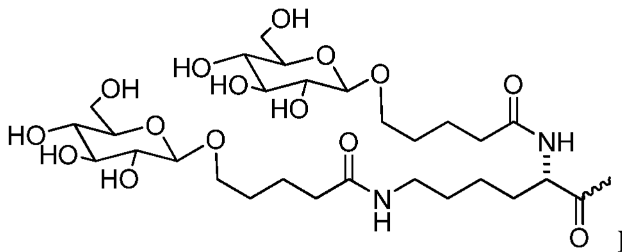

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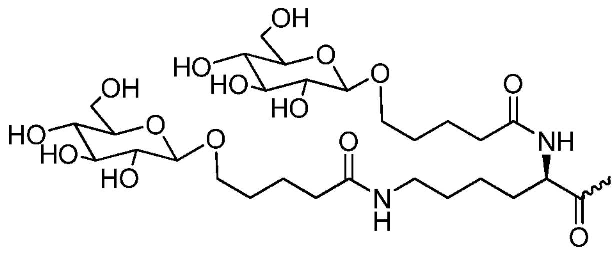

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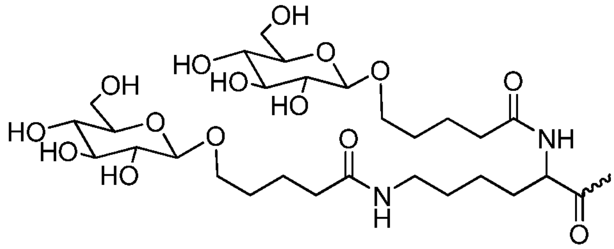

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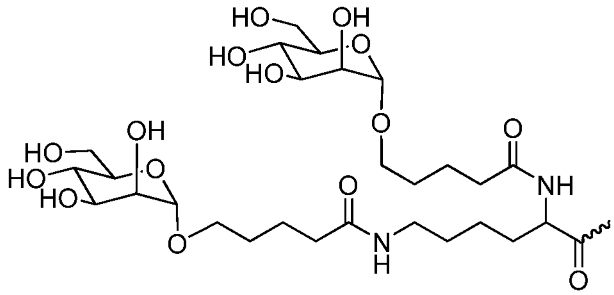
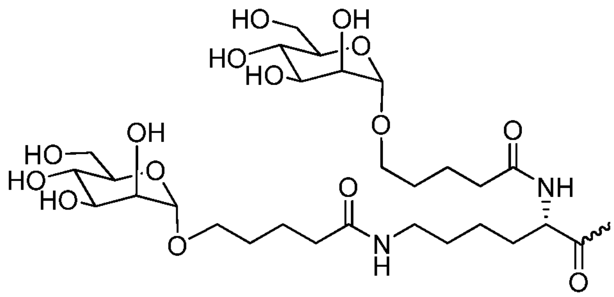
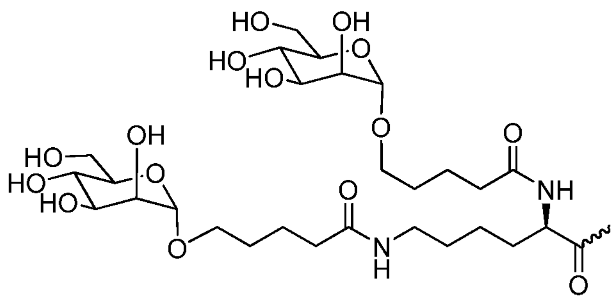


Formula XX,

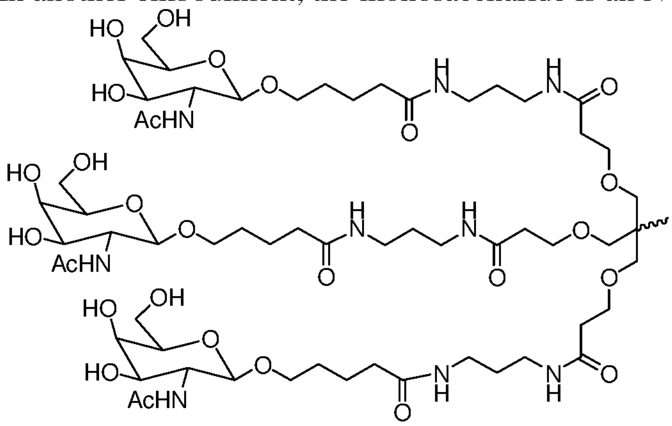


Formula XXI,



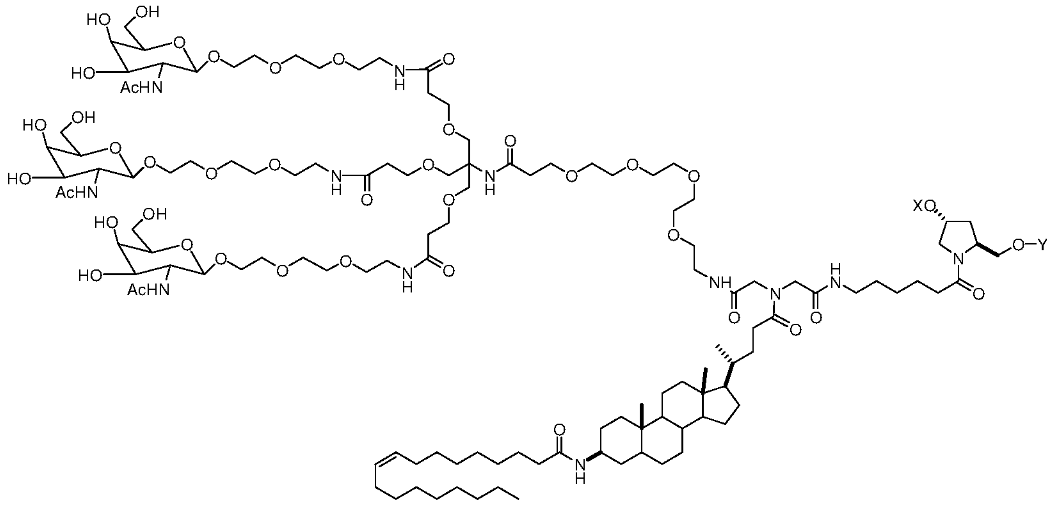
Formula XXII.

In another embodiment, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

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



(Formula XXIII),

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

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

In one embodiment, the agents of the invention comprise one GalNAc or GalNAc derivative attached to the agent. In another embodiment, the agents of the invention 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 agent through a plurality of monovalent linkers.

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

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

D. Linkers

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

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise 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, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

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

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

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

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

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

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

ii. Phosphate-based cleavable linking groups

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

iii. Acid cleavable linking groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

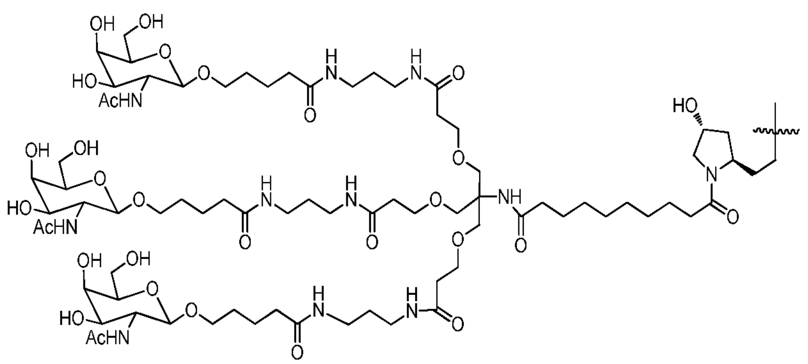
iv. Ester-based linking groups

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

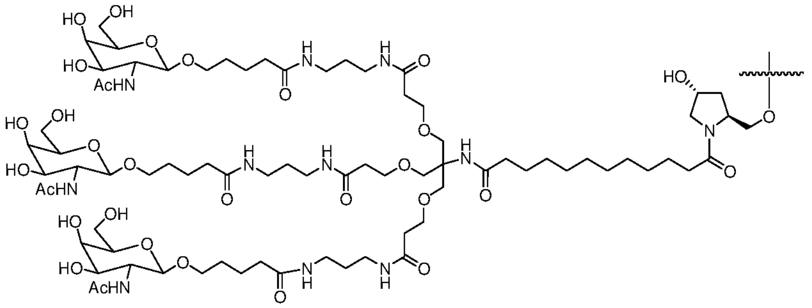
v. Peptide-based cleaving groups

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

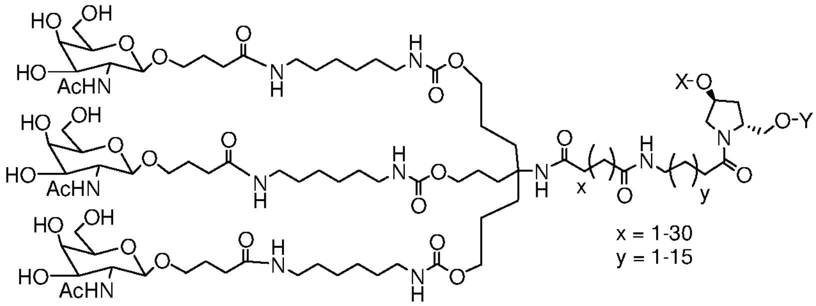
In one embodiment, an antisense polynucleotide agent of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of antisense polynucleotide agent carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



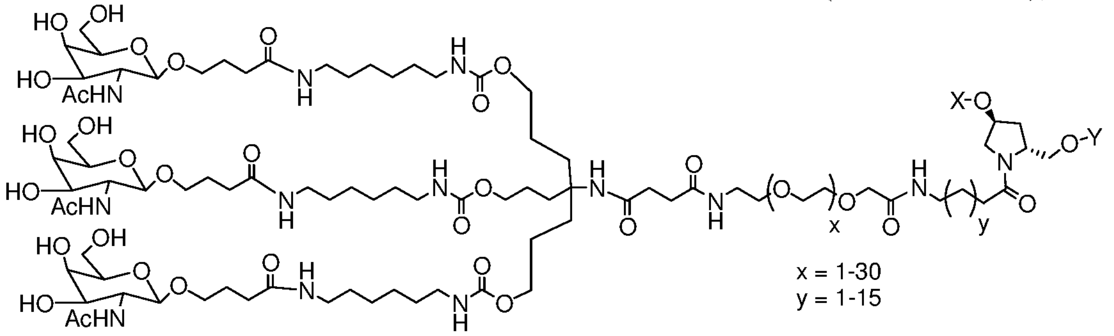
(Formula XXIV),



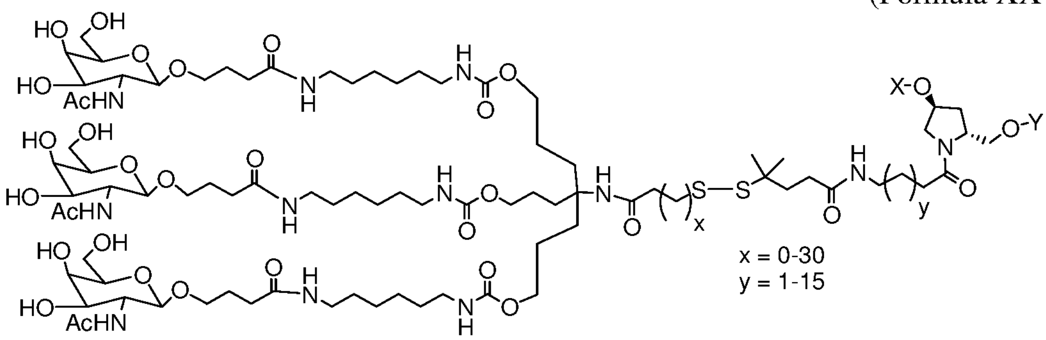
(Formula XXV),



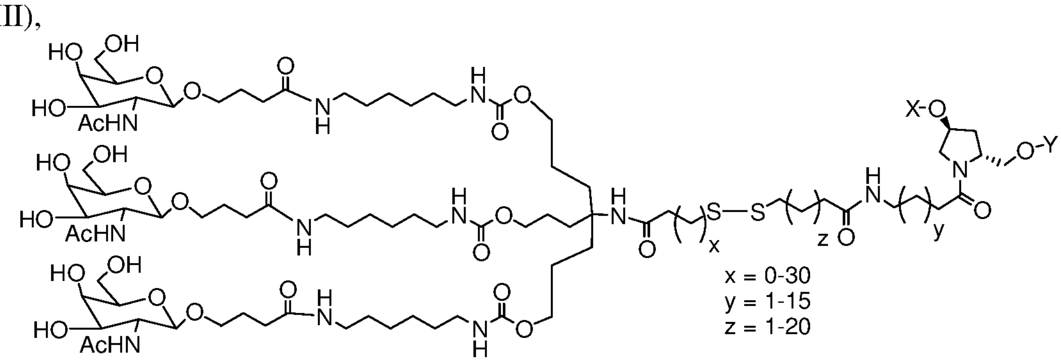
(Formula XXVI),



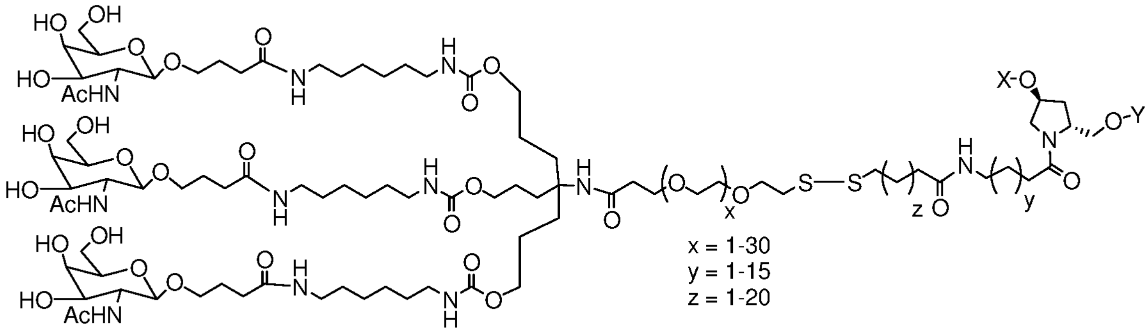
(Formula XXVII),



(Formula XXVIII),

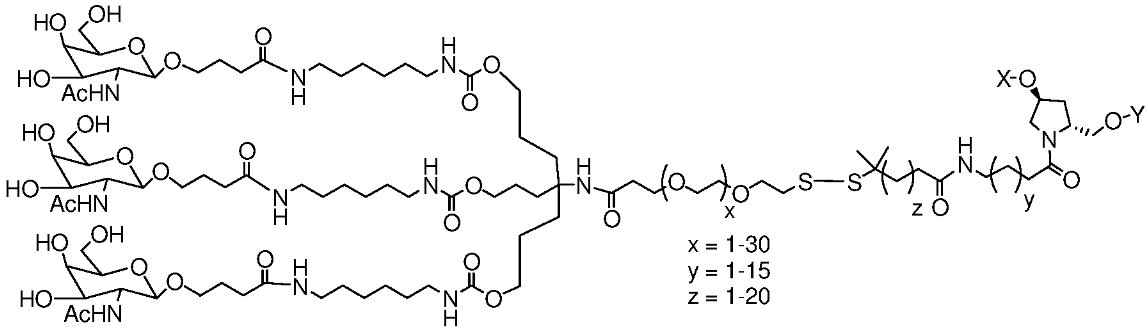


(Formula XXIX),



(Formula XXX),

and



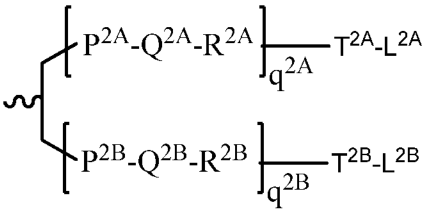
(Formula XXXI),

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

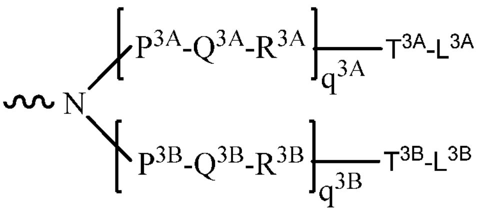
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

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

Formula XXXII



Formula XXXIII



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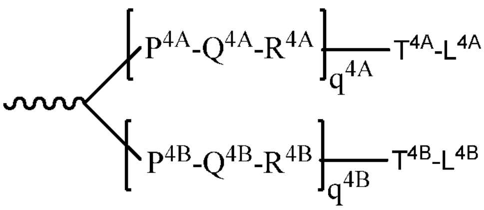
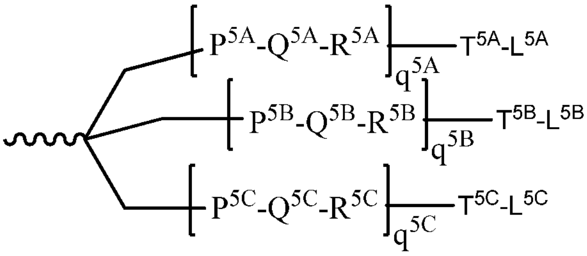
,



, or



;

Formula XXXIV

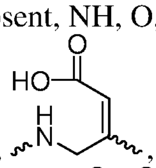
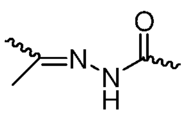
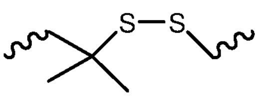
Formula XXXV

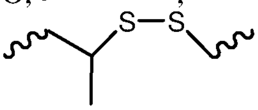
wherein:

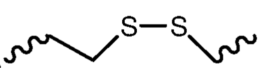
q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

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 wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

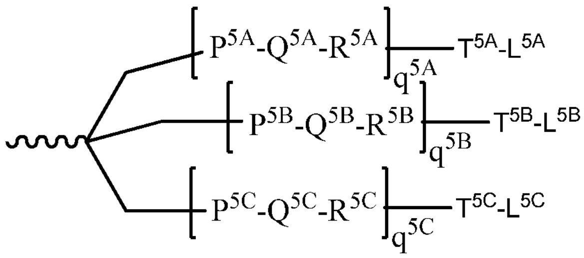
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, $-C(O)-CH(R^a)-NH-$, CO, CH=N-O, , , ,



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

Formula XXXVI



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

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

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

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

“Chimeric” antisense polynucleotide agents or “chimeras,” in the context of this invention, are antisense polynucleotide agent compounds, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of an antisense polynucleotide agent. These antisense polynucleotide agents typically contain at least one region wherein the RNA is modified so as to confer upon the antisense polynucleotide agent increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the antisense polynucleotide agent 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 antisense polynucleotide agent inhibition of gene expression. Consequently, comparable results can often be obtained with shorter antisense polynucleotide agents when chimeric antisense polynucleotide agents are used, compared to phosphorothioate deoxy antisense polynucleotide agents hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

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

V. Delivery of an Antisense Polynucleotide Agent of the Invention

The delivery of an antisense polynucleotide agent of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having an AGT-associated disease) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an antisense polynucleotide agent of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an antisense polynucleotide agent to a subject.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an antisense polynucleotide agent of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an antisense polynucleotide agent include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an antisense polynucleotide agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the antisense polynucleotide agent to be administered. Several studies have shown successful knockdown of gene products when an antisense polynucleotide agent is administered locally. For example, intraocular delivery of a VEGF antisense polynucleotide agent by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., *et al* (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ., *et al* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a antisense polynucleotide agent in mice reduces tumor volume (Pille, J., *et al* (2005) *Mol. Ther.*11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ., *et al* (2006) *Mol. Ther.* 14:343-350; Li, S., *et al* (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA., *et al* (2006) *Mol. Ther.* 14:476-484; Zhang, X., *et al* (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., *et al* (2005) *Nat. Med.* 11:50-55). For administering an antisense polynucleotide agent systemically for the treatment of a disease, the agent can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the antisense polynucleotide agent by endo- and exo-nucleases *in vivo*. Modification of the agent or the pharmaceutical carrier can also permit targeting of the antisense polynucleotide agent composition to the target tissue and avoid undesirable off-target effects. Antisense polynucleotide agent can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. In an alternative embodiment, the antisense polynucleotide agent 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 antisense polynucleotide agent molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an antisense polynucleotide agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an antisense polynucleotide agent, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., *et al* (2008) J*ournal of Controlled Release* 129(2):107-116) that encases an antisense polynucleotide agent. The formation of vesicles or micelles further prevents degradation of the antisense polynucleotide agent when administered systemically. Methods for making and administering cationic- antisense polynucleotide agent complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN, *et al* (2003) Clin. *Cancer Res*. 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens*. 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of antisense polynucleotide agents include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an antisense polynucleotide agent forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of antisense polynucleotide agents and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

VI. Pharmaceutical Compositions of the Invention

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

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

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

The pharmaceutical compositions containing the antisense polynucleotide agents are useful for treating a disease or disorder associated with the expression or activity of an AGT gene, *e.g.* an AGT-associated disease. 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) or intravenous (IV) delivery. Another example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an AGT gene. In general, a suitable dose of an antisense polynucleotide agent of the invention 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. For example, the antisense polynucleotide agent can be administered at about 0.01 mg/kg, about 0.05 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 3 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, or about 50 mg/kg per single dose.

For example, the antisense polynucleotide agent may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 2, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the antisense polynucleotide agent is administered at a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/mg, about 1.5 to about 50 mg/kb, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/mg, about 1.5 to about 45 mg/kb, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/mg, about 1.5 to about 40 mg/kb, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/mg, about 1.5 to about 30 mg/kb, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/mg, about 1.5 to about 20 mg/kb, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the antisense polynucleotide agent may be administered at a dose of about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 2, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the antisense polynucleotide agent is administered at a dose of about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/mg, about 1.5 to about 50 mg/kgb, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/mg, about 1.5 to about 45 mg/kb, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/mg, about 1.5 to about 40 mg/kb, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/mg, about 1.5 to about 30 mg/kb, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/mg, about 1.5 to about 20 mg/kb, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. In one embodiment, the antisense polynucleotide agent is administered at a dose of about 10mg/kg to about 30 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered, *e.g.,* subcutaneously or intravenously, a single therapeutic amount of antisense polynucleotide agent, such as about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In some embodiments, subjects are administered, *e.g.,* subcutaneously or intravenously, multiple doses of a therapeutic amount of antisense polynucleotide agent, such as a dose about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. A multi-dose regimine may include administration of a therapeutic amount of antisense polynucleotide agent daily, such as for two days, three days, four days, five days, six days, seven days, or longer.

In other embodiments, subjects are administered, *e.g.,* subcutaneously or intravenously, a repeat dose of a therapeutic amount of antisense polynucleotide agent, such as a dose about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. A repeat-dose regimine may include administration of a therapeutic amount of antisense polynucleotide agent on a regular basis, such as every other day, every third day, every fourth day, twice a week, once a week, every other week, or once a month.

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

The pharmaceutical composition can be administered once daily, or the pharmaceutical composition can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the antisense polynucleotide agent contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the antisense polynucleotide agent over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per week. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bi-monthly.

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

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as a disorder that would benefit from reduction in the expression of AGT. Such models can be used for *in vivo* testing of an antisense polynucleotide agent, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, transgenic female Sprague-Dawley rats harboring the complete genomic human AGT gene (*e.g.,* [TGR(hAGT)L1623] (see, *e.g.,* Bohlender J, , *et al.* (1996) *Hypertension* 27: 535–540 and Bohlender J, , *et al.* (2000) *J Am Soc Nephrol* 11: 2056–2061)) are surgically implemented with a device for measuring blood pressure by telemetry. Subsequent to recovery from the procedure, these rats are mated with transgenic male Sprague-Dawley rats harboring the entire genomic human REN gene (*e.g.,* [TGR(hREN)L10J] (see, *e.g.*, Bohlender J, *et al.* (1997) *Hypertension* 29: 428–434 and Bohlender J, , *et al.* (2000) *J Am Soc Nephrol* 11: 2056–2061)). A number of models for hypertension, both genetic and induced are well known in the art. Appropriate animal models to test knockdown of AGT expression are also provided herein.

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

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

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the antisense polynucleotide agents featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). Antisense polynucleotide agents featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, antisense polynucleotide agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

A. Antisense Polynucleotide Agent Formulations Comprising Membranous Molecular Assemblies

An antisense polynucleotide agent for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the antisense polynucleotide agent composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the antisense polynucleotide agent composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the antisense polynucleotide agent are delivered into the cell where the antisense polynucleotide agent can specifically bind to a target RNA and can mediate antisense inhibition. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the antisense polynucleotide agent to particular cell types.

A liposome containing an antisense polynucleotide agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The antisense polynucleotide agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the antisense polynucleotide agent and condense around the antisense polynucleotide agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of antisense polynucleotide agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also be adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, *e.g.*, WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al., Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham, *et al. M. Mol. Biol.* 23:238, 1965; Olson, *et al. Biochim. Biophys. Acta* 557:9, 1979; Szoka, *et al. Proc. Natl. Acad. Sci.* 75: 4194, 1978; Mayhew, e*t al. Biochim. Biophys. Acta* 775:169, 1984; Kim, *et al. Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, *et al. Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.,* Mayer, *et al. Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging antisense polynucleotide agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al. S.T.P.Pharma. Sci.*, 1994, 4(6) 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al*).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages in vivo and can be used to deliver antisense polynucleotide agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated antisense polynucleotide agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of Antisense polynucleotide agent (see, *e.g.*, Felgner, P. L. *et al., Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987 and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration; liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer an antisense polynucleotide agent into the skin. In some implementations, liposomes are used for delivering antisense polynucleotide agenst to epidermal cells and also to enhance the penetration of antisense polynucleotide agenst into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al., Journal of Drug Targeting,* 1992, vol. 2,405-410 and du Plessis *et al., Antiviral Research*, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. *et al. Gene* 56:267-276. 1987; Nicolau, C. *et al. Meth. Enz.* 149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., P*roc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with antisense polynucleotide agents are useful for treating a dermatological disorder.

Liposomes that include antisense polynucleotide agent can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include antisense polynucleotide agents can be delivered, for example, subcutaneously by infection in order to deliver antisense polynucleotide agents to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in United States provisional application serial Nos. 61/018,616, filed January 2, 2008; 61/018,611, filed January 2, 2008; 61/039,748, filed March 26, 2008; 61/047,087, filed April 22, 2008 and 61/051,528, filed May 8, 2008. PCT application no PCT/US2007/080331, filed October 3, 2007 also describes formulations that are amenable to the present invention.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.*, they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The antisense polynucleotide agent for use in the compositions and methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the antisense polynucleotide agent composition, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compounds.

Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the antisense polynucleotide agent composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the antisense polynucleotide agent composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol and/or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol and/or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.,* there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.,* through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, *e.g.,* at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. *Lipid particles*

Antisense polynucleotide agents of in the invention may be fully encapsulated in a lipid formulation, *e.g.,* a LNP, or other nucleic acid-lipid particle.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle comprising a lipid layer encapsulating a pharmaceutically active molecule. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; 6,858,225; 8,158,601; and 8,058,069; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to antisense polynucleotide agent ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In another embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-santisense polynucleotide agent nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

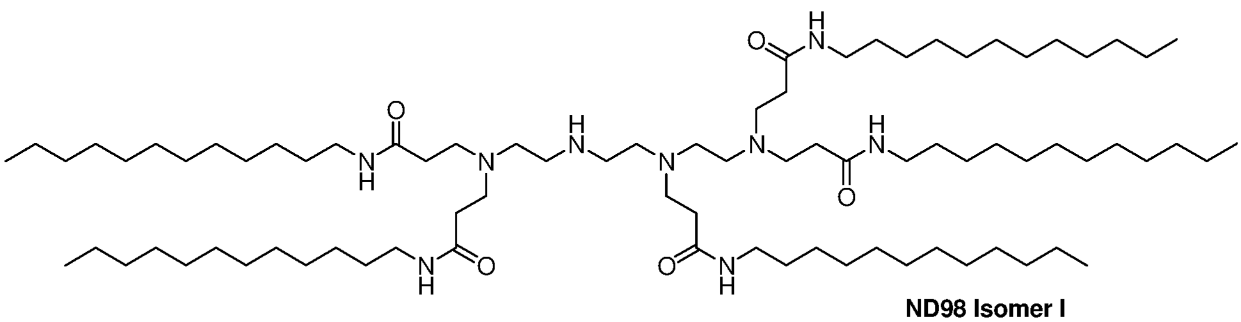
In one embodiment, the lipid-antisense polynucleotide agent particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 antisense polynucleotide agent/Lipid Ratio.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG-distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-antisense polynucleotide agent nanoparticles (*i.e.,* LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous antisense polynucleotide agent (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-antisense polynucleotide agent nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-antisense polynucleotide agent formulations are described in Table 1.

Table 1

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:santisense polynucleotide agent ratio**
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:santisense polynucleotide agent ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:santisense polynucleotide agent ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:santisense polynucleotide agent ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:santisense polynucleotide agent ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:santisense polynucleotide agent ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:santisense polynucleotide agent ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5

	hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Lipid:santisense polynucleotide agent 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:santisense polynucleotide agent: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:santisense polynucleotide agent: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:santisense polynucleotide agent: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:santisense polynucleotide agent: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. filed June 10, 2009; U.S. Provisional Serial No. 61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No. PCT/US2010/022614, filed January 29, 2010, which are hereby incorporated by reference.

MC3 comprising formulations are described, *e.g.*, in U.S. Publication No. 2010/0324120, filed June 10, 2010, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described, *e.g.*, International patent application number PCT/US09/63933, filed on November 10, 2009, which is hereby incorporated by reference.

C12-200 comprising formulations are described in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009 and International Application No. PCT/US10/33777, filed May 5, 2010, which are hereby incorporated by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which the antisense polynucleotide agents featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.*, sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Antisense polynucleotide agents featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Antisense polynucleotide agent complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for antisense polynucleotide agents and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

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

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

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

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

C. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

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

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

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

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

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

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

ii. Microemulsions

In one embodiment of the present invention, the compositions of antisense polynucleotide agents 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). Microemulsions commonly are prepared *via* a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

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

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

iii. Microparticles

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

iv. Penetration Enhancers

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

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

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

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

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, *J. Pharm. Exp. Ther.*, 1992, 263, 25; Yamashita *et al.*, *J. Pharm. Sci.*, 1990, 79, 579-583).

Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of antisense polynucleotide agents through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(see *e.g.*, Katdare, A. *et al.*, Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, *J. Control Rel.*, 1990, 14, 43-51).

As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of antisense polynucleotide agents through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, *J. Pharm. Pharmacol.*, 1987, 39, 621-626).

Agents that enhance uptake of antisense polynucleotide agents at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of antisense polynucleotide agents. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass[a] D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

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

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioated antisense polynucleotide agent in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, Antisense polynucleotide agent Res. Dev., 1995, 5, 115-121; Takakura *et al.*, Antisense polynucleotide agent & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. Excipients

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

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

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

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

vii. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

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

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more antisense polynucleotide agents and (b) one or more agents which function by a non-antisense inhibition mechanism and which are useful in treating a hemolytic disorder. Examples of such agents include, but are not lmited to an anti-inflammatory agent, anti-steatosis agent, anti-viral, and/or anti-fibrosis agent. In addition, other substances commonly used to protect the liver, such as silymarin, can also be used in conjunction with the antisense polynucleotide agents described herein. Other agents useful for treating liver diseases include telbivudine, entecavir, and protease inhibitors such as telaprevir and other disclosed, for example, in Tung *et al.*, U.S. Application Publication Nos. 2005/0148548, 2004/0167116, and 2003/0144217; and in Hale *et al.*, U.S. Application Publication No. 2004/0127488.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit high therapeutic indices are preferred.

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 invention lies generally within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated 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 IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

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

VII. Methods For Inhibiting AGT Expression

The present invention provides methods of inhibiting expression of AGT in a cell. The methods include contacting a cell with an antisense polynucleotide agent of the invention in an amount effective to inhibit expression of the AGT in the cell, thereby inhibiting expression of the AGT in the cell.

Contacting of a cell with an antisense polynucleotide agent may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the antisense polynucleotide agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the antisense polynucleotide agent. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. Contacting may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished *via* a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc$_3$ ligand, or any other ligand that directs the antisense polynucleotide agent to a site of interest, *e.g.*, the liver of a subject.

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

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 AGT gene. 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.

"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, AGT protein level, or for example, blood pressure can be measured in a subject with hypertension to assess AGT expression. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

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

In some embodiments of the methods of the invention, expression of an AGT gene is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%. Prefereably expression is inhibited by at least about 20%.

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 antisense polynucleotide agent of the invention, or by administering an antisense polynucleotide agent of the invention 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)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

Alternatively, inhibition of the expression of 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 expression, mRNA or protein levels of AGT in tissues or serum, blood pressure. AGT gene silencing may be determined in any cell expressing AGT, either constitutively or by genomic engineering, and by any assay known in the art. The liver is the major site of AGT expression.

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

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

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

In one embodiment, 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.

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 embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of AGT mRNA.

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

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. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of AGT expression level may also comprise using nucleic acid probes in solution.

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

The level of 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.

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

In some embodiments of the methods of the invention, the antisense polynucleotide agent is administered to a subject such that the antisense polynucleotide agent 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. In preferred embodiments, the site is sthe liver. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

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

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the antisense polynucleotide agent. Contacting a cell *in vivo* may be done, for example, by injecting the antisense polynucleotide agent into or near the tissue where the cell is located, or by injecting the antisense polynucleotide agent 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 antisense polynucleotide agent may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that directs the antisense polynucleotide agent 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 antisense polynucleotide agent and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an antisense polynucleotide agent includes "introducing" or "delivering the antisense polynucleotide agent into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an antisense polynucleotide agent can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an antisense polynucleotide agent into a cell may be *in vitro* and/or *in vivo*. For example, for *in vivo* introduction, antisense polynucleotide agent can be injected into a tissue site or administered systemically. *In vivo* delivery can also be done by a beta-glucan delivery system, such as those described in U.S. Patent Nos. 5,032,401 and 5,607,677, and U.S. Publication No. 2005/0281781, the entire contents of which are hereby incorporated herein by reference. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

VIII. Methods for Treating or Preventing an AGT-Associated Disorders

The present invention also provides therapeutic and prophylactic methods which include administering to a subject having an AGT-associated disease, *e.g.,* hypertension, an antisense polynucleotide agent or a pharmaceutical composition comprising an antisense polynucleotide agent of the invention. In some aspects of the invention, the methods further include administering to the subject an additional therapeutic agent, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension. The treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an antisense polynucleotide agent targeting an AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene, thereby treating the subject having a disorder that would benefit from reduction in AGT expression.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension, which include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an antisense polynucleotide agent targeting a AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene, and an additional therapeutic agent, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, thereby treating the subject having a disorder that would benefit from reduction in AGT expression.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension. The methods include administering to the subject a prohpylactically effective amount of an antisense polynucleotide agent targeting an AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in AGT expression. For example, the invention provides methods for preventing hypertension in a subject suffering from a disorder that would benefit from reduction in AGT expression.

In certain aspects, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension. The methods include administering to the subject a prohpylactically effective amount of an antisense polynucleotide agent targeting an AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene, and an additional therapeutic agent, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in AGT expression.

"Therapeutically effective amount," as used herein, is intended to include the amount of an antisense polynucleotide agent or another agent for treatment of an AGT-associated disease, e.g., 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, that, when administered to a subject having an AGT-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the antisense polynucleotide agent or the other agent(s) for treatment of the AGT-associated disease, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an antisense polynucleotide agent other agent(s) for treatment of the AGT-associated disease, that, when administered to a subject having an AGT-associated disease but not yet (or currently) experiencing or displaying symptoms of the disease, and/or a subject at risk of developing an AGT-associated disease, *e.g.,* a subject who is pregnant, *e.g.,* a subject who is overweight, and/or a subject having artherosclerosis, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the antisense polynucleotide agent or agent(s) for treatment of the AGT-associated disease, how the agent(s) is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically effective amount" or "prophylactically effective amount" also includes an amount of an antisense polynucleotide agent or other agent(s) for treatment of the AGT-associated disease, that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. Antisense polynucleotide agents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

In another aspect, the present invention provides uses of a therapeutically effective amount of an antisense polynucleotide agent of the invention for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of AGT expression.

In another aspect, the present invention provides uses of a therapeutically effective amount of an antisense polynucleotide agent of the invention and an additional therapeutic agent(s) for treatment of the AGT-associated disease for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of AGT expression.

In yet another aspect, the present invention provides use of an antisense polynucleotide agent of the invention targeting an AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of AGT expression, such as a subject having a disorder that would benefit from reduction in AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension.

In another aspect, the present invention provides uses of an antisense polynucleotide agent of the invention targeting an AGT gene or a pharmaceutical composition comprising an antisense polynucleotide agent targeting an AGT gene in the manufacture of a medicament for use in combination with an additional therapeutic agent for treatment of the AGT-associated diseases, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of AGT expression, *e.g.,* an AGT-associated disease, *e.g.,* hypertension.

In another aspect, the invention provides uses of an antisense polynucleotide agent of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of AGT expression, such as an AGT-associated disease, *e.g.,* hypertension.

In yet another aspect, the invention provides uses of an antisense polynucleotide agent of the invention, and an additional therapeutic agent for treatment of the AGT-associated diseases, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of AGT, such as and AGT-associated disease, *e.g.,* hypertension.

In a further aspect, the present invention provides uses of an antisense polynucleotide agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of AGT expression, such as an AGT-associated disease, *e.g.,* hypertension.

In a further aspect, the present invention provides uses of an antisense polynucleotide agent of the invention in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents, for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of AGT expression, such as an AGT-associated disease, *e.g.,* hypertension.

In one embodiment, an antisense polynucleotide agent targeting AGT is administered to a subject having an AGT-associated disease such that AGT levels, *e.g.*, in a cell, tissue, blood, urine or other tissue or fluid of the subject are reduced by at least about 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%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more and, subsequently, an additional therapeutic (as described below) is administered to the subject.

The additional therapeutic agent for the treatment of an AGT-associated disease may be, for example, 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 alpha$_2$-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, steroidal antimineralocorticoid agent. In certain embodiments, the additional agent is safe for use in pregnant women. In certain embodiments, the additional agent does not substantially traverse the placenta, *i.e.*, does not traverse the placenta in sufficient amounts to harm the fetus.

In exemplary methods of the invention for treating an AGT-associated disease, *e.g.,* hypertension, an antisense polynucleotide agent targeting AGT is administered (*e.g.,* subcutaneously) to the subject first, such that the AGT levels in the subject are reduced (*e.g.,* by at least about 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more) and subsequently for treatment of the AGT-associated diseases is administered at doses lower than the ones described in the product insert for the agent. In certain embodiments, the agent for treatment of the AGT-associated disease can be administered at a sufficiently low dose to decrease side effects to an acceptable level. In certain embodiments, the agent for treatment of the AGT-associated disease can be administered at a sufficiently low dose to be acceptable for administration to a pregnant woman.

The methods and uses of the invention include administering a composition described herein such that expression of the target AGT gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In certain embodiments, expression of the target AGT gene is decreased for an extended duration, *e.g.*, at least about two, three, four, five, six, seven days or more, *e.g.*, about one week, two weeks, three weeks, or about four weeks or longer.

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

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of an AGT-associated disorder may be assessed, for example, by periodic monitoring of blood pressurelevels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring such a parameter, or any combination of parameters. In connection with the administration of an antisense polynucleotide agent targeting AGT or pharmaceutical composition thereof, "effective against" an AGT-associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating an AGT-associated disease and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given antisense polynucleotide agent drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an antisense polynucleotide agent or antisense polynucleotide agent formulation as described herein.

Subjects can be administered a therapeutic amount of antisense polynucleotide agent, such as about 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.3 mg/kg, 0.35 mg/kg, 0.4 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.65 mg/kg, 0.7 mg/kg, 0.75 mg/kg, 0.8 mg/kg, 0.85 mg/kg, 0.9 mg/kg, 0.95 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1mg/kg, 2.2mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, 3.0 mg/kg, 3.1 mg/kg, 3.2 mg/kg, 3.3 mg/kg, 3.4 mg/kg, 3.5 mg/kg, 3.6 mg/kg, 3.7 mg/kg, 3.8 mg/kg, 3.9 mg/kg, 4.0 mg/kg, 4.1 mg/kg, 4.2 mg/kg, 4.3 mg/kg, 4.4 mg/kg, 4.5 mg/kg, 4.6 mg/kg, 4.7 mg/kg, 4.8 mg/kg, 4.9 mg/kg, 5.0 mg/kg, 5.1 mg/kg, 5.2 mg/kg, 5.3 mg/kg, 5.4 mg/kg, 5.5 mg/kg, 5.6 mg/kg, 5.7 mg/kg, 5.8 mg/kg, 5.9 mg/kg, 6.0 mg/kg, 6.1 mg/kg, 6.2 mg/kg, 6.3 mg/kg, 6.4 mg/kg, 6.5 mg/kg, 6.6 mg/kg, 6.7 mg/kg, 6.8 mg/kg, 6.9 mg/kg, 7.0 mg/kg, 7.1 mg/kg, 7.2 mg/kg, 7.3 mg/kg, 7.4 mg/kg, 7.5 mg/kg, 7.6 mg/kg, 7.7 mg/kg, 7.8 mg/kg, 7.9 mg/kg, 8.0 mg/kg, 8.1 mg/kg, 8.2 mg/kg, 8.3 mg/kg, 8.4 mg/kg, 8.5 mg/kg, 8.6 mg/kg, 8.7 mg/kg, 8.8 mg/kg, 8.9 mg/kg, 9.0 mg/kg, 9.1 mg/kg, 9.2 mg/kg, 9.3 mg/kg, 9.4 mg/kg, 9.5 mg/kg, 9.6 mg/kg, 9.7 mg/kg, 9.8 mg/kg, 9.9 mg/kg, 9.0 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments, for example, when a composition of the invention comprises a antisense polynucleotide agent as described herein and a lipid, subjects can be administered a therapeutic amount of antisense polynucleotide agent, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about

0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8 mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the antisense polynucleotide agent may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In other embodiments, for example, when a composition of the invention comprises a antisense polynucleotide agent as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of antisense polynucleotide agent, such as a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/mg, about 1.5 to about 50 mg/kb, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/mg, about 1.5 to about 45 mg/kb, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/mg, about 1.5 to about 40 mg/kb, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/mg, about 1.5 to about 30 mg/kb, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/mg, about 1.5 to about 20 mg/kb, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. In one embodiment, when a composition of the invention comprises a antisense polynucleotide agent as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of about 10 to about 30 mg/kg of antisense polynucleotide agent. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered a therapeutic amount of antisense polynucleotide agent, such as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

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

Administration of the antisense polynucleotide agent can reduce AGT levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

Before administration of a full dose of the antisense polynucleotide agent, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

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

An antisense polynucleotide agent of the invention may be administered in “naked” form, or as a “free antisense polynucleotide agent.” A naked antisense polynucleotide agent is administered in the absence of a pharmaceutical composition. The naked antisense polynucleotide agent may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the antisense polynucleotide agent can be adjusted such that it is suitable for administering to a subject.

Alternatively, an antisense polynucleotide agent of the invention may be administered as a pharmaceutical composition, such as an antisense polynucleotide agent liposomal formulation.

Subjects that would benefit from a reduction and/or inhibition of AGT gene expression are those having an AGT-associated disease or disorder as described herein. In certain embodiments, a subject having an AGT-associated disease has 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), hypertensive emergency, hypertensive urgency, pregnancy-associated hypertension (*e.g.,* preeclampsia and eclampsia), resistant hypertension, paroxysmal hypertension, renovascular hypertension (also known as renal hypertension), Goldblatt hypertension, ocular hypertension, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension. In certain embodiments, the hypertension is pregnancy-associated hypertension. In certain embodiments, a subject having an AGT-associated disease has hypertensive heart disease. In certain embodiments, a subject having an AGT-associated disease has hypertensive nephropathy. In certain embodiments, a subject having an AGT-associated disease has atherosclerosis. In certain embodiments, a subject having anAGT-associated disease has arteriosclerosis. In certain embodiments, a subject having an AGT-associated disease has chronic kidney disease. In certain embodiments, a subject having an AGT-associated disease has glomerulosclerosis. In certain embodiments, a subject having an AGT disease has coarctation of the aorta . In certain embodiments, a subject having an AGT-associated disease has aortic aneurism In certain embodiments, a subject having an AGT-associated disease has ventricular fibrosis. In certain embodiments, a subject having an AGT-associated disease has Cushing's syndrome. In certain embodiments, a subject having an AGT-associated disease has a glucocorticoid excess state such as chronic steroid therapy, pheochromocytoma, reninoma, primary aldosteronism and other mineralocorticoid excess states. In certain embodiments, a subject having an AGT-associated disease has sleep apnea. In certain embodiments, a subject having an AGT-associated disease has thyroid/parathyroid disease. In certain embodiments, a subject having an AGT-associated disease has heart failure (*e.g.,* left ventricular systolic dysfunction). In certain embodiments, a subject having an AGT-associated disease has had a myocardial infarction. In certain embodiments, a subject having an AGT-associated disease has had a stroke. In certain embodiments, a subject having an AGT-associated disease has diabetes mellitus (*e.g.,* diabetic nephropathy). In certain embodiments, a subject having an AGT-associated disease has diabetes mellitus (*e.g.,* diabetic nephropathy). In certain embodiments, a subject having an AGT-associated disease has renal failure, *e.g.,* chronic renal failure . In certain embodiments, a subject having an AGT-associated disease has systemic sclerosis (*e.g.,* scleroderma renal crisis). In certain embodiments, a subject having an AGT-associated disease has secondary hyptertension. In certain embodiments, a subject having an AGT-associated disease has accelerated hyptertension. In certain embodiments, a subject having an AGT-associated disease has malignant hyptertension. In certain embodiments, a subject having an AGT-associated disease has hyptertensive urgency.

In certain embodiments, has preganancy-associated hyptertension includes 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)). A subject having "chronic hypertension of pregnancy" is one having a blood pressure exceeding 140/90 mm Hg before pregnancy or before 20 weeks' gestation. "Gestational hypertension" or "pregnancy-induced hypertension" refers to hypertension with onset in the latter part of pregnancy (>20 weeks' gestation) without any other features of preeclampsia, and followed by normalization of the blood pressure postpartum. "Mild preeclampsia" is defined as the presence of hypertension (blood pressure ≥140/90 mm Hg) on two occasions, at least six hours apart, but without evidence of end-organ damage, in a woman who was normotensive before 20 weeks' gestation. In a subject with preexisting essential hypertension, preeclampsia is diagnosed if systolic blood pressure has increased by 30 mm Hg or if diastolic blood pressure has increased by 15 mm Hg. "Severe preeclampsia" is defined as the presence of 1 of the following symptoms or signs in the presence of preeclampsia; asystolic blood pressure of 160 mm Hg or higher or diastolic blood pressure of 110 mm Hg or higher on two occasions at least six hours apart; proteinuria of more than 5g in a 24-hour collection or more than 3+ on two random urine samples collected at least four hours apart, pulmonary edema or cyanosis, oliguria (< 400 mL in 24 hours), persistent headaches, epigastric pain and/or impaired liver function, thrombocytopenia, oligohydramnios, decreased fetal growth, or placental abruption. "Eclampsia" is defined as seizures that cannot be attributable to other causes in a woman with preeclampsia. "HELLP syndrome" (also known as edema-proteinuria-hypertension gestosis type B) is Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels in a pregnant subject.

In certain embodiments, an angiotensinogen-associated disease is resistant hypertension. "Resistant hypertension" is blood pressure that remains above goal (*e.g.*, 140/90 mmHg) 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.

Treatment of a subject that would benefit from a reduction and/or inhibition of AGT gene expression includes therapeutic and prophylactic treatment (*e.g.*, the subject has one or more risk factors for pregnancy-associated hypertension).

The invention further provides methods and uses of an antisense polynucleotide agent or a pharmaceutical composition thereof (including methods and uses of an antisense polynucleotide agent or a pharmaceutical composition comprising an antisense polynucleotide agent and an for treatment of the AGT-associated diseases) for treating a subject that would benefit from reduction and/or inhibition of AGT expression, *e.g.*, a subject having an AGT-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an antisense polynucleotide agent targeting AGT is administered in combination with, *e.g.*, an agent useful in treating an AGT-associated disease as described elsewhere herein.

For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reducton in AGT expression, *e.g.,* a subject having an AGT-associated disease, include 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 alpha$_2$-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, steroidal antimineralocorticoid agent, or a combination of any of the foregoing, and a hypertension therapeutic agent formulated as a combination of agents. In certain embodiments, the agent is safe for use in pregnant women.

The antisense polynucleotide agent (and/or agent(s) for treatment of the AGT-associated disease) and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

The present invention also provides methods of using an antisense polynucleotide agent of the invention and/or a composition containing an antisense polynucleotide agent of the invention to reduce and/or inhibit AGT expression in a cell. In other aspects, the present invention provides an antisense polynucleotide agent of the invention and/or a composition comprising an antisense polynucleotide agent of the invention for use in reducing and/or inhibiting AGT expression in a cell. In yet other aspects, use of an antisense polynucleotide agent of the invention and/or a composition comprising an antisense polynucleotide agent of the invention for the manufactuire of a medicament for reducing and/or inhibiting AGT expression in a cell are provided.

The methods and uses include contacting the cell with an antisense polynucleotide agent, *e.g.,* a antisense polynucleotide agent, of the invention and maintaining the cell for a time sufficient to obtain antisense inhibition of a 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. For example, a reduction in the expression of AGT may be determined by determining the mRNA expression level of AGT using methods routine to one of ordinary skill in the art, *e.g.,* northern blotting, qRT-PCR, by determining the protein level of AGT using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques, flow cytometry methods, ELISA, and/or by determining a biological activity of AGT, such as blood pressure monitoring, and/or by determining the biological activity of one or more molecules associated with RAAS system activation or dysregulation.

In the methods and uses of the invention the cell may be contacted *in vitro* or *in vivo, i.e.,* the cell may be within a subject. In embodiments of the invention in which the cell is within a subject, the methods may include further contacting the cell with an agent for treatment of the AGT-associated disease.

A cell suitable for treatment using the methods of the invention may be any cell that expresses an AGT gene. A cell suitable for use in the methods and uses of the invention may be a mammalian cell, *e.g.,* a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (*e.g.*, a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell, *e.g.,* a human liver cell.

AGT expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

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

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

In some embodiments, the administration is *via* a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the antisense polynucleotide agent to the liver.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of an AGT gene in a mammal, *e.g.,* a human. The present invention also provides a composition comprising an antisense polynucleotide agen that targets an AGT gene in a cell of a mammal for use in inhibiting expression of the AGT gene in the mammal. In another aspect, the present invention provides use of an antisense polynucleotide agent that targets an AGT gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the AGT gene in the mammal.

The methods and uses include administering to the mammal, *e.g.,* a human, a composition comprising an antisense polynucleotide agent that targets an AGT gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain antisense inhibition of the mRNA transcript of the AGT gene, thereby inhibiting expression of the AGT gene in the mammal. In some embodiment, the methods further comprise administering an agent for treatment of the AGT-associated disease to the subject.

Reduction in gene expression can be assessed by any methods known it the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.*, ELISA or western blotting, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in AGT gene and/or protein expression. In another embodiment, a blood sample serves as the tissue material for monitoring the reduction in AGT gene and/or protein expression. In other embodiments, inhibition of the expression of an AGT gene is monitored indirectly by, for example, determining the expression and/or activity of a gene in the RAAS pathway (see, *e.g.,* Figure 1). Suitable assays are further described in the Examples section below.

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

EXAMPLES

Example 1. Antisense Synthesis

The antisense polynucleotides targeting AGT were synthesized using standard synthesis methods well known in the art.

A detailed list of antisense molecules targeting AGT is shown in Table 3.

Example 2. *In vitro* screening

Iin vitro scrrening of the antisense polynucleotides was performed by transfecting Huh7 cells with a single 5nM dose of an antisense polynucleotide using methods well known in the art. Table 4 shows the results of single dose transfection screen in cells transfected with the indicated antisense polynucleotide.

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
Af	2’-fluoroadenosine-3’-phosphate
Afs	2’-fluoroadenosine-3’-phosphorothioate
As	adenosine-3’-phosphorothioate
a	2'-O-methyladenosine-3’-phosphate
as	2'-O-methyladenosine-3’- phosphorothioate
C	cytidine-3’-phosphate
dA	2\`-deoxyadenosine-3\`-phosphate
dAs	2\`-deoxyadenosine-3\`-phosphorothioate
Cf	2’-fluorocytidine-3’-phosphate
Cfs	2’-fluorocytidine-3’-phosphorothioate
Cs	cytidine-3’-phosphorothioate
c	2'-O-methylcytidine-3’-phosphate
cs	2'-O-methylcytidine-3’- phosphorothioate
dC	2\`-deoxycytidine-3\`-phosphate
dCs	2\`-deoxycytidine-3\`-phosphorothioate
G	guanosine-3’-phosphate
Gf	2’-fluoroguanosine-3’-phosphate
Gfs	2’-fluoroguanosine-3’-phosphorothioate
Gs	guanosine-3’-phosphorothioate

Abbreviation	Nucleotide(s)
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
dT	2`-deoxythymidine-3`-phosphate
dTs	2`-deoxythymidine-3`-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
dU	2`-deoxyuridine-3'-phosphate
dUs	2`-deoxyuridine-3`-phosphorothioate
s	phosphorothioate linkage
N	any nucleotide (G, A, C, T or U)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(dt)	deoxy-thymine
(5MdC)	5'-methyl-deoxycytidine-3`-phosphate
(5MdC)s	5'-methyl-deoxycytidine-3`-phosphorothioate

Table 3. Antisense polynucleotides targeting AGT. The start and end positions which the antisense polynucleotide agents target NM_000029.3 (SEQ ID NO:1) are provided.

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-134944.1	ascsususcsdTsdGsdAs(5MdC)s(5MdC)s(5MdC)sdTsdGs(5MdC)sdTsgscscscsg	11	30	9
A-134945.1	asascsascsdGsdGsdGsdGsdGs(5MdC)s(5MdC)sdAs(5MdC)sdTsuscsusgsa	23	42	10
A-134946.1	uscsususgs(5MdC)sdTsdTsdAsdGsdGs(5MdC)sdAsdAs(5MdC)sascsgsgsg	35	54	11
A-134947.1	asgsgsgsgsdAsdGsdAsdGsdTs(5MdC)sdTsdTsdGs(5MdC)susSousasgsg	44	63	12
A-134948.1	asgsgsgscsdAsdGsdAsdGsdGsdGs(5MdC)sdAsdGsdGsgsgsasgsa	56	75	13
A-134949.1	cscsgsgsasdGsdGsdTsdGs(5MdC)sdAsdGsdAsdGsdGsgscsasgsa	68	87	14
A-134950.1	ascsasusgs(5MdC)sdAsdGsdGs(5MdC)s(5MdC)sdGsdGsdAsdGsgsusgscsa	77	96	15
A-134951.1	asgsasgsgs(5MdC)s(5MdC)sdAs(5MdC)sdAsdGsdGsdGsdAs(5MdC)sasusgscsa	90	109	16
A-134952.1	usgsusascs(5MdC)s(5MdC)s(5MdC)s(5MdC)sdAsdAsdGsdAsdGsdGscscsascsa	100	119	17
A-134953.1	cscscscsgsdGsdGsdAsdGsdAsdTsdGsdTsdAs(5MdC)scscscscsa	110	129	18
A-134954.1	csususcsusdGsdAs(5MdC)s(5MdC)s(5MdC)sdAsdGs(5MdC)s(5MdC)s(5MdC)scsgsgsgsa	122	141	19
A-134955.1	ascscsascs(5MdC)s(5MdC)sdAsdGsdGs(5MdC)s(5MdC)sdTsdTs(5MdC)susgsascsc	133	152	20
A-134956.1	asgscscsusdGsdAsdGsdGs(5MdC)s(5MdC)sdAsdAs(5MdC)s(5MdC)sascscscsa	145	164	21
A-134957.1	asgsgsusgsdTsdGsdTsdGsdAs(5MdC)sdAsdGs(5MdC)s(5MdC)susgsasgsg	156	175	22
A-134958.1	asgscsasus(5MdC)sdTs(5MdC)s(5MdC)s(5MdC)sdTsdAsdGsdGsdTsgsusgsusg	167	186	23
A-134959.1	asgsasasas(5MdC)sdGsdGsdGsdAsdGs(5MdC)sdAsdTs(5MdC)suscscscsu	176	195	24
A-134960.1	gscscsasasdGsdGsdTsdTs(5MdC)s(5MdC)s(5MdC)sdAsdGsdAsasascsgsg	188	207	25
A-134961.1	csasgsgsasdGsdTs(5MdC)sdGsdGsdGsdGs(5MdC)s(5MdC)sdAsasgsgsusu	199	218	26
A-134962.1	ascscsgsasdAsdGsdTsdTsdTsdGs(5MdC)sdAsdGsdGsasgsuscsg	210	229	27
A-134963.1	gsususascsdAs(5MdC)sdAsdTsdTsdTsdAs(5MdC)s(5MdC)sdGsasasgsusu	221	240	28

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-134964.1	gsusgscsasdGsdGsdGsdTs(5MdC)sdGsdAsdGsdTsdTsascsascsa	233	252	29
A-134965.1	asgsasgsusdGsdAsdGs(5MdC)s(5MdC)sdGsdGsdTsdGs(5MdC)sasgsgsgsu	244	263	30
A-134966.1	csascsusgs(5MdC)sdTsdGsdAsdAs(5MdC)sdAsdGsdAsdGsusgsasgsc	255	274	31
A-134967.1	usgscsasgsdAsdGsdTsdTsdTs(5MdC)sdAs(5MdC)sdTsdGscsusgsasa	265	284	32
A-134968.1	usasgsusgsdAsdTs(5MdC)sdGsdAsdTsdGs(5MdC)sdAsdGsasgsusus u	275	294	33
A-134969.1	cscsasgsgsdAsdAsdGsdTs(5MdC)sdTsdTsdAsdGsdTsgsasuscsg	286	305	34
A-134970.1	gscsusgsgsdGsdAs(5MdC)s(5MdC)sdTs(5MdC)sdTsdTs(5MdC)s(5MdC)sasgsgsasa	299	318	35
A-134971.1	gsascsascsdTs(5MdC)sdAs(5MdC)sdGs(5MdC)sdTsdGsdGsdGsascscsusc	308	327	36
A-134972.1	gsasusgscs(5MdC)sdAsdGsdAsdAsdGs(5MdC)sdGsdAs(5MdC)sascsuscsa	320	339	37
A-134973.1	csasgsasasdGsdGsdAs(5MdC)sdAsdGsdAsdTsdGs(5MdC)scsasgsasa	330	349	38
A-134974.1	ascscsascsdAsdGsdGs(5MdC)sdTsdGsdGs(5MdC)s(5MdC)sdAsgsasasgsg	343	362	39
A-134975.1	ascsususgsdGs(5MdC)s(5MdC)sdAsdGsdAs(5MdC)s(5MdC)sdAs(5MdC)sasgsgscsu	353	372	40
A-134976.1	gsasgsgsgsdTsdTsdAs(5MdC)sdAsdTs(5MdC)sdAs(5MdC)sdTsusgsgscsc	365	384	41
A-134977.1	gsgscsusgsdGsdAsdGsdAsdGsdGsdAsdGsdGsdGsususascsa	375	394	42
A-134978.1	gscscsusgsdTsdGs(5MdC)sdAs(5MdC)sdAsdGsdGs(5MdC)sdTsgsgsasgsa	386	405	43
A-134979.1	gsususcscs(5MdC)sdAsdGsdGs(5MdC)sdTsdGs(5MdC)s(5MdC)sdTsgsusgscsa	397	416	44
A-134980.1	gsgsgsasusdGsdGsdAsdGs(5MdC)sdTsdGsdTsdTs(5MdC)scscsasgsg	408	427	45
A-134981.1	asgscsusgsdAsdGsdGsdGsdGsdTsdGsdGsdGsdGsasusgsgsa	420	439	46
A-134982.1	gscscscsusdAsdTsdTsdTsdAsdTsdAsdGs(5MdC)sdTsgsasgsgsg	431	450	47
A-134983.1	gsgsgsuscsdAs(5MdC)sdGsdAsdTsdGs(5MdC)s(5MdC)s(5MdC)sdTsasususus a	441	460	48
A-134984.1	uscscscscs(5MdC)sdGsdGs(5MdC)s(5MdC)sdGsdGsdGsdTs(5MdC)sascsgsasu	451	470	49
A-134985.1	ascsgsgscsdAsdGs(5MdC)sdTsdTs(5MdC)sdTsdTs(5MdC)s(5MdC)scscscsgsg	463	482	50
A-134986.1	usascscscsdAsdGsdAsdAs(5MdC)sdAsdAs(5MdC)sdGsdGscsasgscsu	474	493	51
A-134987.1	ususcsusgs(5MdC)sdTsdGsdTsdAsdGsdTsdAs(5MdC)s(5MdC)scsasgsasa	485	504	52
A-134988.1	cscsgscsasdTsdAs(5MdC)s(5MdC)s(5MdC)sdTsdTs(5MdC)sdTsdGscsusgsusa	495	514	53

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-134989.1	gsgsgsgsusdGs(5MdC)sdTs(5MdC)sdGs(5MdC)sdTsdTs(5MdC)s(5MdC)sgscsasusa	508	527	54
A-134990.1	asuscsuscsdAsdGsdAs(5MdC)sdTsdGsdGsdGsdGsdTsgscsuscsg	518	537	55
A-134991.1	cscsgsgscsdAsdGsdGsdAsdGs(5MdC)s(5MdC)sdAsdTs(5MdC)suscsasgsa	530	549	56
A-134992.1	csasgsgscsdTs(5MdC)sdAs(5MdC)sdAs(5MdC)s(5MdC)sdGsdGs(5MdC)sasgsgsasg	540	559	57
A-134993.1	usgsgsusgsdGs(5MdC)s(5MdC)s(5MdC)sdTs(5MdC)sdAsdGsdGs(5MdC)suscsascsa	550	569	58
A-134994.1	gsasgsgscsdAsdGsdAsdGsdGsdAsdTsdGsdGsdTsgsgscscsc	561	580	59
A-134995.1	csasgscscs(5MdC)sdAsdGsdGs(5MdC)s(5MdC)sdAsdGsdGsdAsgsgscsasg	574	593	60
A-134996.1	usgscsasgs(5MdC)s(5MdC)sdAsdGsdGs(5MdC)s(5MdC)sdAsdGs(5MdC)scscsasgsg	585	604	61
A-134997.1	cscscsgsgsdTs(5MdC)sdAs(5MdC)s(5MdC)sdTsdGs(5MdC)sdAsdGscscsasgsg	595	614	62
A-134998.1	gsgsusgsusdAsdTsdGsdTsdAs(5MdC)sdAs(5MdC)s(5MdC)s(5MdC)sgsgsuscsa	607	626	63
A-134999.1	asgsgsusgsdGsdAsdAsdGsdGsdGsdGsdTsdGsdTsasusgsusa	617	636	64
A-135000.1	ususgsusgsdGsdAsdTsdGsdAs(5MdC)sdGsdAsdGsdGsusgsgsasa	629	648	65
A-135001.1	gsgsusascsdTs(5MdC)sdTs(5MdC)sdAsdTsdTsdGsdTsdGsgsasusgsa	639	658	66
A-135002.1	csasgscsusdGs(5MdC)sdTs(5MdC)sdAs(5MdC)sdAsdGsdGsdTsascsuscsu	651	670	67
A-135003.1	usgsgscscsdTsdTsdTsdGs(5MdC)s(5MdC)sdAsdGs(5MdC)sdTsgscsuscsa	661	680	68
A-135004.1	gscsususcs(5MdC)s(5MdC)sdGsdGs(5MdC)sdAsdTsdTsdGsdGscscsususu	673	692	69
A-135005.1	gsgsgsgsus(5MdC)sdTsdTsdTsdGsdGsdGs(5MdC)sdTsdTscscscsgsg	684	703	70
A-135006.1	gsgsusasusdGsdAsdAsdGsdGsdTsdGsdGsdGsdGsuscsususu	695	714	71
A-135007.1	asasususgsdGsdAsdGs(5MdC)sdAsdGsdGsdTsdAsdTsgsasasgsg	705	724	72
A-135008.1	usgsuscsusdTsdGsdGs(5MdC)s(5MdC)sdTsdGsdAsdAsdTsusgsgsasg	717	736	73
A-135009.1	csascsasgsdGsdGsdGsdAsdTsdGsdTs(5MdC)sdTsdTsgsgscscsu	726	745	74
A-135010.1	gscscsususdTsdTs(5MdC)sdAsdTs(5MdC)s(5MdC)sdAs(5MdC)sdAsgsgsgsgsa	737	756	75
A-135011.1	csusgsgsus(5MdC)s(5MdC)sdTsdGsdTsdAsdGsdGsdGs(5MdC)scsusususu	750	769	76
A-135012.1	usasgscsas(5MdC)s(5MdC)sdAsdGs(5MdC)sdTsdGsdGsdTs(5MdC)scsusgsusa	759	778	77
A-135013.1	usususgscsdAsdGs(5MdC)sdGsdAs(5MdC)sdTsdAsdGs(5MdC)sascscsasg	770	789	78

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135014.1	uscsgsgsusdGsdTs(5MdC)sdAsdAsdGsdTsdTsdTsdTsgscsasgsc	782	801	79
A-135015.1	csasascsusdTsdGsdTs(5MdC)sdTsdTs(5MdC)sdGsdGsdTsgsuscsasa	792	811	80
A-135016.1	csasususgs(5MdC)sdGsdGs(5MdC)s(5MdC)s(5MdC)sdTs(5MdC)sdAsdAscsususgsu	804	823	81
A-135017.1	asgscsasus(5MdC)s(5MdC)s(5MdC)sdGsdAs(5MdC)s(5MdC)sdAsdTsdTsgscsgsgsc	815	834	82
A-135018.1	asgsasasgsdTsdTsdGsdGs(5MdC)s(5MdC)sdAsdGs(5MdC)sdAsuscscscsg	826	845	83
A-135019.1	usascsgsgsdAsdAsdGs(5MdC)s(5MdC)s(5MdC)sdAsdAsdGsdAsasgsususg	838	857	84
A-135020.1	asusgscscsdAsdTsdAsdTsdAsdTsdAs(5MdC)sdGsdGsasasgscsc	848	867	85
A-135021.1	asgscsuscsdAs(5MdC)sdTsdGsdTsdGs(5MdC)sdAsdTsdGscscsasusa	860	879	86
A-135022.1	csascsgscs(5MdC)s(5MdC)s(5MdC)sdAsdTsdAsdGs(5MdC)sdTs(5MdC)sascsusgsu	870	889	87
A-135023.1	gscscscsdAsdTsdGsdGsdAs(5MdC)s(5MdC)sdAs(5MdC)sdGscscscsa	881	900	88
A-135024.1	gsasgsgsas(5MdC)sdGsdGsdTsdGsdGs(5MdC)s(5MdC)s(5MdC)s(5MdC)sasusgsgsa	891	910	89
A-135025.1	csasgscscsdGsdTsdTsdGsdGsdGsdGsdAsdGsdAsgsgsascsg	904	923	90
A-135026.1	gsusgscscsdAsdAsdAsdGsdAs(5MdC)sdAsdGs(5MdC)s(5MdC)sgsususgsg	914	933	91
A-135027.1	asgsasgsasdGsdGs(5MdC)s(5MdC)sdAsdGsdGsdGsdTsdGscscsasasa	926	945	92
A-135028.1	cscscsasgsdAsdTsdAsdGsdAsdGsdAsdGsdAsdGsgscscsasg	935	954	93
A-135029.1	usgsgsuscs(5MdC)sdAsdAsdGsdGs(5MdC)sdTs(5MdC)s(5MdC)s(5MdC)sasgsasusa	947	966	94
A-135030.1	csusgsuscsdAsdGs(5MdC)sdTsdGsdTsdGsdTsdGsdGsuscscsasa	959	978	95
A-135031.1	gscscsusgsdTsdAsdGs(5MdC)s(5MdC)sdTsdGsdTs(5MdC)sdAsgscsusgsu	968	987	96
A-135032.1	asascsascs(5MdC)s(5MdC)sdAsdGsdGsdAsdTsdTsdGs(5MdC)scsusgsusa	981	1000	97
A-135033.1	csususcscsdAsdAsdGsdGsdAsdAs(5MdC)sdAs(5MdC)s(5MdC)scsasgsgsa	990	1009	98
A-135034.1	usgscsasgsdTsdTs(5MdC)sdTsdTsdGsdTs(5MdC)s(5MdC)sdTsuscscsasa	1003	1022	99
A-135035.1	csasgscscsdGsdGsdGsdAsdGsdGsdTsdGs(5MdC)sdAsgsususcsu	1014	1033	100
A-135036.1	ususgsusgs(5MdC)sdGs(5MdC)sdAsdTs(5MdC)s(5MdC)sdAsdGs(5MdC)scsgsgsgsa	1025	1044	101
A-135037.1	csasgsascsdAsdGsdGsdAs(5MdC)s(5MdC)sdTsdTsdGsdTsgscsgscsa	1036	1055	102
A-135038.1	asgscscsusdGs(5MdC)sdAsdGsdGsdGs(5MdC)sdAsdGsdAscsasgsgsa	1047	1066	103

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135039.1	asgsgscscs(5MdC)sdTsdGsdTsdAs(5MdC)sdAsdGs(5MdC)s(5MdC)susgscsasg	1058	1077	104
A-135040.1	gsgscscsas(5MdC)sdTsdAsdGs(5MdC)sdAsdGsdGs(5MdC)s(5MdC)scsusgsusa	1068	1087	105
A-135041.1	asgscscscsdTsdGs(5MdC)s(5MdC)s(5MdC)sdTsdGsdGsdGs(5MdC)scsascsusa	1080	1099	106
A-135042.1	csusgsgscsdTsdAsdTs(5MdC)sdAsdGs(5MdC)s(5MdC)s(5MdC)sdTsgscscscsu	1089	1108	107
A-135043.1	gscsasgscsdAsdGs(5MdC)sdTsdGsdGsdGs(5MdC)s(5MdC)sdTsgsgscsusa	1102	1121	108
A-135044.1	csascscsgsdTsdGsdGsdAs(5MdC)sdAsdGs(5MdC)sdAsdGscsasgscsu	1113	1132	109
A-135045.1	asascsascsdGs(5MdC)s(5MdC)s(5MdC)sdAs(5MdC)s(5MdC)sdAs(5MdC)s(5MdC)sgsusgsgsa	1124	1143	110
A-135046.1	usgsgsgsgs(5MdC)sdTsdGsdTsdGsdAsdAs(5MdC)sdAs(5MdC)sgscscscsa	1134	1153	111
A-135047.1	gsgsusgscsdAsdGsdGs(5MdC)s(5MdC)sdTsdGsdGsdGsdGscsusgsusg	1144	1163	112
A-135048.1	asascsgsgs(5MdC)sdTsdGs(5MdC)sdTsdTs(5MdC)sdAsdGsdGsusgscsasg	1157	1176	113
A-135049.1	gscscscsusdGs(5MdC)sdAs(5MdC)sdAsdAsdAs(5MdC)sdGsdGscsusgscsu	1167	1186	114
A-135050.1	asusasgsasdGsdAsdGs(5MdC)s(5MdC)sdAsdGsdGs(5MdC)s(5MdC)scsusgscsa	1179	1198	115
A-135051.1	csascsasgsdGsdGsdGsdTsdAsdTsdAsdGsdAsdGsasgscscsa	1188	1207	116
A-135052.1	csgsusgsgsdGsdAsdGsdGsdAs(5MdC)s(5MdC)sdAs(5MdC)sdAsgsgsgsgsu	1199	1218	117
A-135053.1	gsasasgsus(5MdC)s(5MdC)sdAsdGsdAsdGsdAsdGs(5MdC)sdGsusgsgsgsa	1212	1231	118
A-135054.1	uscscsasgsdTsdTs(5MdC)sdTsdGsdTsdGsdAsdAsdGsuscscsasg	1223	1242	119
A-135055.1	csasgscsasdGs(5MdC)sdAsdAs(5MdC)sdAsdTs(5MdC)s(5MdC)sdAsgsususcsu	1234	1253	120
A-135056.1	gsuscsasasdTs(5MdC)sdTsdTs(5MdC)sdTs(5MdC)sdAsdGs(5MdC)sasgscsasa	1245	1264	121
A-135057.1	csasusgsasdAs(5MdC)s(5MdC)sdTsdGsdTs(5MdC)sdAsdAsdTscsususcsu	1254	1273	122
A-135058.1	gsuscsascsdAsdGs(5MdC)s(5MdC)sdTsdGs(5MdC)sdAsdTsdGsasascscsu	1265	1284	123
A-135059.1	gsuscsusus(5MdC)s(5MdC)sdAsdTs(5MdC)s(5MdC)sdTsdGsdTs(5MdC)sascsasgsc	1277	1296	124
A-135060.1	asgsgsgsasdGs(5MdC)sdAsdGs(5MdC)s(5MdC)sdAsdGsdTs(5MdC)sususcscsa	1289	1308	125
A-135061.1	usgsgscsus(5MdC)s(5MdC)s(5MdC)sdAsdTs(5MdC)sdAsdGsdGsdGsasgscsasg	1300	1319	126
A-135062.1	csusgsuscs(5MdC)sdAs(5MdC)sdAs(5MdC)sdTsdGsdGs(5MdC)sdTscscscsasu	1310	1329	127
A-135063.1	asasgscscsdAsdGsdGsdGsdTsdGs(5MdC)sdTsdGsdTscscsascsa	1321	1340	128

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135064.1	csgsusasgsdGsdTsdGsdTsdTsdGsdAsdAsdAsdGscscsasgsg	1333	1352	129
A-135065.1	gsgsasasgsdTsdGsdGsdAs(5MdC)sdGsdTsdAsdGsdGsusgsususg	1342	1361	130
A-135066.1	ususcsasus(5MdC)sdTsdTs(5MdC)s(5MdC)s(5MdC)sdTsdTsdGsdGsasasgsusg	1355	1374	131
A-135067.1	gsasgsasasdGs(5MdC)s(5MdC)s(5MdC)sdTsdTs(5MdC)sdAsdTs(5MdC)sususcscsc	1364	1383	132
A-135068.1	csuscsgsgs(5MdC)s(5MdC)sdAsdGs(5MdC)sdAsdGsdGsdGsdAsgsasasgsc	1377	1396	133
A-135069.1	csuscscsusdGsdGsdGsdGs(5MdC)sdTs(5MdC)sdGsdGs(5MdC)scsasgscsa	1386	1405	134
A-135070.1	usgsuscscsdAs(5MdC)s(5MdC)s(5MdC)sdAsdGsdAsdAs(5MdC)sdTscscsusgsg	1399	1418	135
A-135071.1	gsasgsgsusdGs(5MdC)sdTsdGsdTsdTsdGsdTs(5MdC)s(5MdC)sascscscsa	1409	1428	136
A-135072.1	asascsasgsdAs(5MdC)sdAs(5MdC)sdTsdGsdAsdGsdGsdTsgscsusgsu	1419	1438	137
A-135073.1	asgsasgsasdGs(5MdC)sdAsdTsdGsdGsdGsdAsdAs(5MdC)sasgsascsa	1431	1450	138
A-135074.1	usgscscscsdAsdTsdGs(5MdC)s(5MdC)sdAsdGsdAsdGsdAsgscsasusg	1441	1460	139
A-135075.1	csasgsusgs(5MdC)sdTsdGsdGsdAsdAsdGsdGsdTsdGscscscsasu	1454	1473	140
A-135076.1	asusgsuscsdAs(5MdC)sdTs(5MdC)s(5MdC)sdAsdGsdTsdGs(5MdC)susgsgsasa	1463	1482	141
A-135077.1	asasgsususdGsdTs(5MdC)s(5MdC)sdTsdGsdGsdAsdTsdGsuscsascsu	1475	1494	142
A-135078.1	asgsuscsas(5MdC)s(5MdC)sdGsdAsdGsdAsdAsdGsdTsdTsgsuscscsu	1485	1504	143
A-135079.1	asasgsgsgs(5MdC)sdAs(5MdC)sdTsdTsdGsdAsdGsdTs(5MdC)sascscsgsa	1496	1515	144
A-135080.1	gsgscsgscsdTs(5MdC)sdTs(5MdC)sdAsdGsdTsdGsdAsdAsgsgsgscsa	1509	1528	145
A-135081.1	gscsasgscsdAsdGsdGs(5MdC)sdAsdGsdGs(5MdC)sdGs(5MdC)suscsuscsa	1519	1538	146
A-135082.1	gsasgsgscsdTsdGsdGsdAsdTs(5MdC)sdAsdGs(5MdC)sdAsgscsasgsg	1531	1550	147
A-135083.1	gsasgsgscsdAsdTsdAsdGsdTsdGsdAsdGsdGs(5MdC)susgsgsasu	1541	1560	148
A-135084.1	ususgsuscs(5MdC)sdAsdGsdGsdTs(5MdC)sdAsdGsdAsdGsgscsasusa	1553	1572	149
A-135085.1	ascscscsus(5MdC)s(5MdC)sdAs(5MdC)s(5MdC)sdTsdTsdGsdTs(5MdC)scsasgsgsu	1563	1582	150
A-135086.1	csusgsgsasdAsdAsdGsdTsdGsdAsdGsdAs(5MdC)s(5MdC)scsuscscsa	1575	1594	151
A-135087.1	gsgsgsasgsdTsdTsdTsdTsdGs(5MdC)sdTsdGsdGsdAsasasgsusg	1585	1604	152
A-135088.1	csasuscscsdAsdGsdTsdTsdGsdAsdGsdGsdGsdAsgsususususu	1596	1615	153

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135089.1	gsasusasgsdTsdTsdTs(5MdC)sdTsdTs(5MdC)sdAsdTs(5MdC)scsasgsusu	1607	1626	154
A-135090.1	asusgsgsus(5MdC)s(5MdC)sdGsdGsdGsdGsdAsdGsdAsdTsasgsususu	1619	1638	155
A-135091.1	usgsgsuscsdAsdGsdGsdTsdGsdGsdAsdTsdGsdGsuscscsgsg	1630	1649	156
A-135092.1	csasgsususdGsdGsdGsdGs(5MdC)sdAsdTsdGsdGsdTscsasgsgsu	1641	1660	157
A-135093.1	ususgscsasdGs(5MdC)sdAs(5MdC)s(5MdC)sdAsdGsdTsdTsdGsgsgsgscsa	1650	1669	158
A-135094.1	gsuscsasusdAsdAsdGsdAsdTs(5MdC)s(5MdC)sdTsdTsdGscsasgscsa	1662	1681	159
A-135095.1	asgsgsuscs(5MdC)sdTsdGs(5MdC)sdAsdGsdGsdTs(5MdC)sdAsusasasgsa	1673	1692	160
A-135096.1	csusgsgsgs(5MdC)sdGsdAsdGs(5MdC)sdAsdGsdGsdTs(5MdC)scsusgscsa	1683	1702	161
A-135097.1	gsgsgscsasdGs(5MdC)sdTs(5MdC)sdAsdGs(5MdC)s(5MdC)sdTsdGsgsgscsgsa	1695	1714	162
A-135098.1	gscsasgsasdAsdTsdGsdGs(5MdC)sdGsdGsdGs(5MdC)sdAsgscsuscsa	1705	1724	163
A-135099.1	csasgscsus(5MdC)sdGsdGsdTsdGsdTsdGs(5MdC)sdAsdGsasasusgsg	1716	1735	164
A-135100.1	usususgs(5MdC)sdAsdGsdGsdTsdTs(5MdC)sdAsdGs(5MdC)suscsgsgsu	1727	1746	165
A-135101.1	gsuscsasusdTsdGs(5MdC)sdTs(5MdC)sdAsdAsdTsdTsdTsususgscsa	1740	1759	166
A-135102.1	ascscscsusdGsdAsdTsdGs(5MdC)sdGsdGsdTs(5MdC)sdAsususgscsu	1751	1770	167
A-135103.1	gscsascscsdTs(5MdC)s(5MdC)s(5MdC)s(5MdC)s(5MdC)sdAs(5MdC)s(5MdC)s(5MdC)susgsasusg	1762	1781	168
A-135104.1	asusgscsusdGsdTsdTs(5MdC)sdAsdGs(5MdC)sdAs(5MdC)s(5MdC)suscscscsc	1772	1791	169
A-135105.1	asgscsuscsdAsdAsdAsdAsdAsdAsdAsdAsdTsdGscsusgsusu	1784	1803	170
A-135106.1	asuscscsgs(5MdC)sdTsdTs(5MdC)sdAsdAsdGs(5MdC)sdTs(5MdC)sasasasasa	1794	1813	171
A-135107.1	gsgsgscsus(5MdC)sdTs(5MdC)sdTs(5MdC)sdTs(5MdC)sdAsdTs(5MdC)scsgscsusu	1806	1825	172
A-135108.1	asgsascsus(5MdC)sdTsdGsdTsdGsdGsdGs(5MdC)sdTs(5MdC)suscsuscsu	1815	1834	173
A-135109.1	usasasgscsdTsdGsdTsdTsdGsdGsdGsdTsdAsdGsascsuscsu	1828	1847	174
A-135110.1	uscsasgsgs(5MdC)sdTsdTsdGsdTsdTsdAsdAsdGs(5MdC)susgsususg	1838	1857	175
A-135111.1	cscsuscscsdAsdAsdGsdAs(5MdC)s(5MdC)sdTs(5MdC)sdAsdGsgscsususg	1849	1868	176
A-135112.1	gsgsususcsdAsdGsdGsdGsdTs(5MdC)sdAs(5MdC)s(5MdC)sdTscscsasasg	1861	1880	177
A-135113.1	asgsgsasasdTsdGsdGsdGs(5MdC)sdGsdGsdTsdTs(5MdC)sasgsgsgsu	1871	1890	178

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135114.1	csascsasgs(5MdC)sdAsdAsdAs(5MdC)sdAsdGsdGsdAsdAsusgsgsgsc	1881	1900	179
A-135115.1	gscsususudGsdAsdTs(5MdC)sdAsdTsdAs(5MdC)sdAs(5MdC)sasgscsasa	1893	1912	180
A-135116.1	gsgsgscsasdGsdTsdGsdGs(5MdC)sdGs(5MdC)sdTsdTsdTsgsasuscsa	1903	1922	181
A-135117.1	cscsasgsgsdAsdAsdGsdTsdGs(5MdC)sdAsdGsdGsdGscsasgsusg	1915	1934	182
A-135118.1	gscscsascsdGs(5MdC)sdGsdGs(5MdC)s(5MdC)s(5MdC)sdAsdGsdGsasasgsusg	1925	1944	183
A-135119.1	csuscsasgs(5MdC)sdGsdGsdGsdTsdTsdGsdGs(5MdC)s(5MdC)sascsgscsg	1937	1956	184
A-135120.1	uscsasusgs(5MdC)sdTsdGsdTsdGs(5MdC)sdTs(5MdC)sdAsdGscsgsgsgsu	1947	1966	185
A-135121.1	gsgsgscscs(5MdC)sdTsdGsdGs(5MdC)s(5MdC)sdTs(5MdC)sdAsdTsgscsusgsu	1958	1977	186
A-135122.1	csascsusgsdTsdGsdTsdTs(5MdC)sdTsdGsdGsdGsdGscscscsusg	1970	1989	187
A-135123.1	gscscsususdGs(5MdC)s(5MdC)sdAsdGsdGs(5MdC)sdAs(5MdC)sdTsgsusgsusu	1981	2000	188
A-135124.1	csasgsgsgsdGs(5MdC)sdAsdGsdAsdGsdGs(5MdC)s(5MdC)sdTsusgscscsa	1992	2011	189
A-135125.1	cscsuscsasdAsdAsdGsdGs(5MdC)s(5MdC)sdAsdGsdGsdGsgscsasgsa	2002	2021	190
A-135126.1	csusgscsusdGsdGs(5MdC)s(5MdC)sdTsdTsdTsdGs(5MdC)s(5MdC)suscsasasa	2015	2034	191
A-135127.1	ususgsususdAsdTs(5MdC)sdTsdGs(5MdC)sdTsdGs(5MdC)sdTsgsgscscsu	2025	2044	192
A-135128.1	ususgsus(5MdC)s(5MdC)sdGsdGsdGsdGsdTsdTsdGsdTsusasuscsu	2036	2055	193
A-135129.1	csascsasus(5MdC)sdGs(5MdC)sdTsdGsdAsdTsdTsdTsdGsuscscsgsg	2047	2066	194
A-135130.1	ascsusgsgsdGsdGsdGsdTsdGsdAs(5MdC)sdAs(5MdC)sdAsuscsgscsu	2058	2077	195
A-135131.1	asasasgsgsdTsdGsdGsdGsdAsdGsdAs(5MdC)sdTsdGsgsgsgsgsu	2069	2088	196
A-135132.1	csasususasdGsdAsdAsdGsdAsdAsdAsdAsdGsdGsusgsgsgsa	2079	2098	197
A-135133.1	uscsasasasdGsdTs(5MdC)sdGsdAs(5MdC)sdTs(5MdC)sdAsdTsusasgsasa	2091	2110	198
A-135134.1	gscsususus(5MdC)s(5MdC)sdAsdGs(5MdC)sdTs(5MdC)sdAsdAsdAsgsuscsgsa	2101	2120	199
A-135135.1	gsasgsasasdAs(5MdC)sdGsdGs(5MdC)sdTsdGs(5MdC)sdTsdTsuscscsasg	2112	2131	200
A-135136.1	csascsususdAsdGsdAs(5MdC)s(5MdC)sdAsdAsdGsdGsdAsgsasasasc	2125	2144	201
A-135137.1	csasusgscsdAsdGs(5MdC)sdAs(5MdC)sdAs(5MdC)sdTsdTsdAsgsascscsa	2134	2153	202
A-135138.1	usascsusgs(5MdC)sdTs(5MdC)sdAs(5MdC)sdTs(5MdC)s(5MdC)sdAsdTsgscsasgsc	2146	2165	203

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135139.1	csusgcsasdGsdGs(5MdC)sdTsdTs(5MdC)sdTsdAs(5MdC)sdTsgcsuscsa	2157	2176	204
A-135140.1	csasusususdGsdTsdGs(5MdC)s(5MdC)sdGs(5MdC)sdTsdGs(5MdC)sasgsgscsu	2168	2187	205
A-135141.1	asascsusgsdGsdGsdAsdGsdGsdTsdGs(5MdC)sdAsdTsususgsusg	2180	2199	206
A-135142.1	asascscscsdAsdGs(5MdC)sdAsdAsdAs(5MdC)sdTsdGsdGsgsasgsgsu	2189	2208	207
A-135143.1	uscsuscsusdAsdAsdAsdAsdTsdAsdAsdAs(5MdC)s(5MdC)scsasgscsa	2200	2219	208
A-135144.1	uscscscscsdAs(5MdC)s(5MdC)s(5MdC)s(5MdC)s(5MdC)sdAsdTsdTs(5MdC)suscsusasa	2213	2232	209
A-135145.1	usgsgsusus(5MdC)sdTsdTsdGs(5MdC)s(5MdC)sdTs(5MdC)s(5MdC)s(5MdC)scsascscsc	2224	2243	210
A-135146.1	gscsgscsusdAsdAsdAs(5MdC)sdAs(5MdC)sdTsdGsdGsdTsuscsususg	2235	2254	211
A-135147.1	csasgsusasdGsdTs(5MdC)s(5MdC)s(5MdC)sdGs(5MdC)sdGs(5MdC)sdTsasasascsa	2245	2264	212
A-135148.1	uscsusususdTsdTsdGsdGsdAsdAs(5MdC)sdAsdGsdTsasgsuscsc	2256	2275	213
A-135149.1	csgsgsususdGsdGsdAsdAsdTsdTs(5MdC)sdTsdTsdTsususgsgsa	2266	2285	214
A-135150.1	asascsasasdGs(5MdC)sdTsdGsdGsdTs(5MdC)sdGsdGsdTsusgsgsasa	2277	2296	215
A-135151.1	ususususgsdTsdTsdTs(5MdC)sdAs(5MdC)sdAsdAsdAs(5MdC)sasasgscsu	2289	2308	216
A-135152.1	asasgsgsgsdAsdAs(5MdC)sdAs(5MdC)sdTsdTsdTsdTsdTsusgsususu	2301	2320	217
A-135153.1	csasascsusdTsdGsdAsdAsdAsdAsdGsdGsdGsdAsascsascsu	2310	2329	218
A-135154.1	asususususdTsdGsdTsdTs(5MdC)sdTs(5MdC)sdAsdAs(5MdC)sususgsasa	2321	2340	219
A-135155.1	asususususdAsdAsdAsdAs(5MdC)s(5MdC)s(5MdC)sdAsdAsdTsusususug	2334	2353	220
A-135156.1	usgsusasusdAs(5MdC)sdTsdTsdTsdAsdAsdTsdTsdTsusasasasa	2345	2364	221
A-135157.1	asasusgscsdAsdAsdAsdAsdAsdTsdGsdTsdAsdTsascsususu	2355	2374	222
A-135158.1	asasascscsdGsdAsdAsdGsdGs(5MdC)sdAsdAsdTsdGscsasasasa	2366	2385	223
A-135159.1	gsascsascsdTsdAsdAsdAsdTsdAs(5MdC)sdAsdAsdAscscsgsasa	2378	2397	224
A-135160.1	csususascsdAsdTsdTs(5MdC)sdAsdAsdGsdAs(5MdC)sdAscsusasasa	2389	2408	225
A-135161.1	gsasgsgsus(5MdC)sdAsdTsdGsdTsdTs(5MdC)sdTsdTsdAscsasususc	2400	2419	226
A-135162.1	csascsusas(5MdC)sdAs(5MdC)sdGsdGsdAsdGsdGsdTs(5MdC)sasusgsusu	2409	2428	227
A-135163.1	asasgsgsusdAsdTsdTsdAs(5MdC)sdAsdGsdAs(5MdC)sdAscsusascsa	2422	2441	228

Antisense Polynucleotide Name	Modified sequence	Start	End	SEQ ID NO.
A-135164.1	gsasasasasdAsdAs(5MdC)sdTsdAsdAsdGsdGsdTsdAsususascsa	2431	2450	229
A-135165.1	ascsasasgs(5MdC)sdAsdTs(5MdC)sdTsdGsdTsdGsdGsdAsasasasasa	2444	2463	230
A-135166.1	uscsasasasdAsdAsdTs(5MdC)sdAs(5MdC)sdAsdAsdGs(5MdC)sasuscsusg	2453	2472	231
A-135167.1	usususcsas(5MdC)sdGsdTsdAsdTsdTsdGsdTsdTs(5MdC)sasasasasa	2466	2485	232
A-135168.1	gsusgscsusdTsdGs(5MdC)sdAsdTs(5MdC)sdTsdTsdTs(5MdC)sascsgsusa	2477	2496	233
A-135169.1	csasgsasasdAsdTsdTs(5MdC)sdAsdGsdGsdTsdGs(5MdC)sususgscsa	2488	2507	234
A-135170.1	cscsgscsasdTsdTs(5MdC)sdAsdAsdAs(5MdC)sdAsdGsdAsasasususc	2499	2518	235
A-135171.1	gscsusasusdGsdGsdTsdTs(5MdC)s(5MdC)sdGs(5MdC)sdAsdTsuscsasasa	2508	2527	236
A-135172.1	gsasgsasasdAsdTsdAsdAs(5MdC)s(5MdC)sdAsdGs(5MdC)sdTsasusgsgsu	2520	2539	237
A-135173.1	csusasascsdAs(5MdC)sdAsdAsdGsdGsdGsdAsdGsdAsasasusasa	2531	2550	238
A-135174.1	gsascsgsusdTsdTsdAsdTsdTsdAs(5MdC)sdTsdAsdAscsascsasa	2542	2561	239
A-135175.1	usasususgsdTsdGsdGs(5MdC)sdAsdAsdGsdAs(5MdC)sdGsusususasu	2553	2572	240
A-135176.1	usususgsgsdAsdGsdGs(5MdC)sdTsdTsdAsdTsdTsdGsusgsgscsa	2563	2582	241

Table 4. AGT Single Dose Screen in Huh7 cells.

Antisense polynucleotide agent name	KD	SD
A-134944.1	80.3	14.7
A-134945.1	95.3	8.3
A-134946.1	102.4	7.3
A-134947.1	93.0	5.0
A-134948.1	102.7	7.9
A-134949.1	103.4	5.8
A-134950.1	98.9	13.0
A-134951.1	108.8	9.4
A-134952.1	130.4	10.3
A-134953.1	111.7	4.5
A-134954.1	88.4	2.8
A-134955.1	89.2	11.2
A-134956.1	105.4	12.8
A-134957.1	90.0	16.8
A-134958.1	103.8	14.9
A-134959.1	103.7	9.9
A-134960.1	89.3	16.5
A-134961.1	92.9	2.4
A-134962.1	109.3	15.9
A-134963.1	137.7	14.9
A-134964.1	101.0	7.7
A-134965.1	87.7	6.5
A-134966.1	97.8	11.5
A-134967.1	119.9	7.5
A-134968.1	107.0	11.9
A-134969.1	97.6	4.9
A-134970.1	99.9	7.8
A-134971.1	106.6	5.2
A-134972.1	120.9	4.9
A-134973.1	119.8	4.4
A-134974.1	94.4	4.7
A-134975.1	94.7	10.4
A-134976.1	104.2	13.2
A-134977.1	112.4	17.5
A-134978.1	104.0	12.1
A-134979.1	91.3	9.4
A-134980.1	88.9	10.9
A-134981.1	94.5	4.7

Antisense polynucleotide agent name	KD	SD
A-134982.1	96.0	4.3
A-134983.1	106.5	4.9
A-134984.1	64.6	5.5
A-134985.1	58.9	6.5
A-134986.1	35.5	5.1
A-134987.1	56.7	15.1
A-134988.1	67.4	13.6
A-134989.1	58.8	2.2
A-134990.1	60.6	27.9
A-134991.1	76.4	28.6
A-134992.1	64.1	7.2
A-134993.1	53.9	4.0
A-134994.1	105.1	10.8
A-134995.1	94.9	4.5
A-134996.1	109.5	5.0
A-134997.1	121.2	25.9
A-134998.1	112.4	24.0
A-134999.1	97.4	36.0
A-135000.1	127.5	13.6
A-135001.1	117.9	21.8
A-135002.1	100.3	3.5
A-135003.1	81.0	16.3
A-135004.1	68.1	15.5
A-135005.1	76.0	25.0
A-135006.1	83.7	4.4
A-135007.1	93.6	22.7
A-135008.1	80.9	3.9
A-135009.1	92.4	7.4
A-135010.1	99.0	2.9
A-135011.1	81.1	11.8
A-135012.1	66.8	1.9
A-135013.1	75.1	4.6
A-135014.1	77.7	4.2
A-135015.1	89.5	8.3
A-135016.1	102.1	13.1
A-135017.1	99.1	11.9
A-135018.1	84.5	22.5
A-135019.1	94.8	23.6
A-135020.1	63.4	10.0
A-135021.1	64.8	6.9

Antisense polynucleotide agent name	KD	SD
A-135022.1	66.2	4.6
A-135023.1	97.2	8.3
A-135024.1	80.6	7.6
A-135025.1	74.1	11.4
A-135026.1	87.4	5.4
A-135027.1	74.0	9.0
A-135028.1	65.3	8.0
A-135029.1	81.0	11.5
A-135030.1	73.5	4.4
A-135031.1	88.4	6.5
A-135032.1	46.7	3.7
A-135033.1	65.3	4.4
A-135034.1	47.5	3.0
A-135035.1	84.3	15.5
A-135036.1	102.2	14.3
A-135037.1	111.7	12.2
A-135038.1	116.9	8.5
A-135039.1	98.9	14.6
A-135040.1	111.8	8.3
A-135041.1	77.8	10.3
A-135042.1	85.2	15.2
A-135043.1	104.0	23.9
A-135044.1	62.7	10.7
A-135045.1	70.7	9.1
A-135046.1	74.7	5.4
A-135047.1	90.9	17.3
A-135048.1	84.1	7.3
A-135049.1	48.4	6.4
A-135050.1	90.2	5.2
A-135051.1	97.0	1.5
A-135052.1	98.8	5.9
A-135053.1	113.1	3.7
A-135054.1	47.9	5.1
A-135055.1	37.2	4.8
A-135056.1	34.0	3.8
A-135057.1	44.7	5.8
A-135058.1	61.6	1.9
A-135059.1	30.2	4.2
A-135060.1	70.0	9.1
A-135061.1	80.4	6.5

Antisense polynucleotide agent name	**KD**	**SD**
A-135062.1	81.5	11.1
A-135063.1	63.4	4.0
A-135064.1	84.5	12.6
A-135065.1	85.6	7.4
A-135066.1	91.2	4.9
A-135067.1	63.4	3.9
A-135068.1	92.3	5.7
A-135069.1	66.4	3.3
A-135070.1	52.6	5.2
A-135071.1	87.5	15.6
A-135072.1	68.2	5.1
A-135073.1	105.9	9.3
A-135074.1	74.1	11.8
A-135075.1	81.3	9.7
A-135076.1	91.1	13.2
A-135077.1	85.4	7.9
A-135078.1	80.3	6.3
A-135079.1	89.7	7.4
A-135080.1	91.0	4.6
A-135081.1	79.0	6.8
A-135082.1	90.4	6.3
A-135083.1	96.5	7.2
A-135084.1	65.5	5.6
A-135085.1	52.4	3.7
A-135086.1	86.0	5.5
A-135087.1	103.5	4.5
A-135088.1	46.6	5.3
A-135089.1	42.5	6.7
A-135090.1	98.3	2.6
A-135091.1	94.3	3.3
A-135092.1	96.9	6.7
A-135093.1	93.2	4.6
A-135094.1	82.9	8.3
A-135095.1	105.2	6.6
A-135096.1	103.0	14.5
A-135097.1	104.1	10.0
A-135098.1	95.9	7.7
A-135099.1	95.3	6.0
A-135100.1	48.5	3.1
A-135101.1	37.1	3.6

Antisense polynucleotide agent name	KD	SD
A-135102.1	47.0	2.7
A-135103.1	72.3	2.2
A-135104.1	26.5	14.0
A-135105.1	21.8	8.0
A-135106.1	28.7	6.7
A-135107.1	69.0	6.2
A-135108.1	55.7	5.6
A-135109.1	51.8	6.5
A-135110.1	49.9	2.5
A-135111.1	30.2	4.2
A-135112.1	86.4	11.9
A-135113.1	104.9	8.1
A-135114.1	59.7	15.3
A-135115.1	29.6	15.0
A-135116.1	110.1	46.8
A-135117.1	85.2	32.9
A-135118.1	103.0	35.6
A-135119.1	79.7	10.4
A-135120.1	59.6	9.2
A-135121.1	85.6	8.7
A-135122.1	95.5	9.1
A-135123.1	53.2	6.0
A-135124.1	103.4	12.8
A-135125.1	123.4	6.8
A-135126.1	82.6	6.6
A-135127.1	94.6	6.8
A-135128.1	54.4	3.1
A-135129.1	71.3	3.2
A-135130.1	60.3	4.0
A-135131.1	109.4	16.5
A-135132.1	96.4	7.2
A-135133.1	47.9	3.2
A-135134.1	44.4	7.6
A-135135.1	73.8	5.6
A-135136.1	31.7	5.8
A-135137.1	50.5	17.5
A-135138.1	41.8	5.2
A-135139.1	73.0	16.4
A-135140.1	85.9	17.2
A-135141.1	108.2	28.6

Antisense polynucleotide agent name	**KD**	**SD**
A-135142.1	109.1	37.9
A-135143.1	36.0	2.1
A-135144.1	18.8	4.0
A-135145.1	31.3	3.5
A-135146.1	61.7	5.8
A-135147.1	73.3	3.6
A-135148.1	33.5	3.9
A-135149.1	91.5	2.5
A-135150.1	96.6	16.5
A-135151.1	91.0	2.1
A-135152.1	56.7	2.8
A-135153.1	24.3	2.0
A-135154.1	38.7	5.7
A-135155.1	83.8	7.3
A-135156.1	121.4	14.0
A-135157.1	74.0	10.3
A-135158.1	111.4	16.8
A-135159.1	96.6	6.7
A-135160.1	114.3	7.5
A-135161.1	114.6	16.2
A-135162.1	109.0	11.7
A-135163.1	99.4	8.6
A-135164.1	133.9	5.3
A-135165.1	153.6	9.7
A-135166.1	115.6	2.7
A-135167.1	142.6	5.9
A-135168.1	101.3	8.5
A-135169.1	123.3	6.4
A-135170.1	110.7	2.8
A-135171.1	99.1	2.5
A-135172.1	111.4	2.3
A-135173.1	127.8	3.9
A-135174.1	99.7	4.6
A-135175.1	127.0	11.4
A-135176.1	101.2	5.1

Example 3: ***In Vivo*** **AGT Silencing in a mouse expressing hAGT**

Experiments are performed to assess hAGT target knockdown in a mouse expressing hAGT from an AAV8 expression vector which has a strong liver tropism. Briefly, mice are infected with 1 x 10^{11} AAV8 particles containing an expression construct for human AAV under the control of a constitutive promoter. Two weeks after infection with the AAV8 virus, mice are administered a single dose of various amounts of a polynucleotide agent targeting AGT or PBS as a control. The level of expression of hAGT is assessed by qPCR and ELISA of liver tissue at predetermined time points after administration of the polynucleotide agent. Polynucleotide agents with the most potent hAGT knockdown activity are selected for further testing.

Example 4. ***In Vivo*** **AGT Silencing - Amelioration of Preeclamptic Sequelae**

Transgenic female Sprague-Dawley rats harboring the complete genomic human AGT gene (*e.g.,* [TGR(hAGT)L1623] (see, *e.g.,* Bohlender J, , *et al.* (1996) *Hypertension* 27: 535–540 and Bohlender J, , *et al.* (2000) *J Am Soc Nephrol* 11: 2056–2061)) are surgically implanted with a device for measuring blood pressure by telemetry. Subsequent to recovery from the procedure, these rats are mated with transgenic male Sprague-Dawley rats harboring the entire genomic human REN gene (*e.g.,* [TGR(hREN)L10J] (see, *e.g.*, Bohlender J, *et al.* (1997) *Hypertension* 29: 428–434 and Bohlender J, , *et al.* (2000) *J Am Soc Nephrol* 11: 2056–2061)). The female progeny of this cross (referred to herein as “PE rats”) are a model of preeclampsia and develop albuminuria and intrauterine growth restriction (IUGR), and have a blood pressure spike beginning around gestation day 13.

Beginning on day 3 of gestation, a subset of pregnant PE rats are administered an appropriate dose of a polynucleotide agent provided herein targeting AGT. A second subset of pregnant PE rats are left untreated as a control. Dosing is continued to gestation day 15. Rats are sacrificed on about day 19 of gestation, and blood and tissue samples are collected from the mothers and the fetuses.

Maternal blood pressure is monitored throughout the experiment *via* the surgically implanted devices for measuring blood pressure by telemetry. Treatment with the polynucleotide agent results in significantly lowered mean arterial blood pressure. In addition, as determined by ELISA analysis of serum albumin, maternal albuminuria is significantly reduced following administration of the polynucleotide agent targeting AGT. These two conditions are the hallmarks of preeclampsia. The reduced blood pressure reduces cardiovascular events associated with the disease, while reduced albuminuria is indicative of improved renal function.

Preeclampsia is also associated with reduced placental size, possibly related to poor perfusion. As a result of the condition, fetal growth is impaired. Following maternal administration of the polynucleotide agent targeting AGT, however, uteroplacental unit weight and fetal weight are increased, and there is normalization of the fetal brain:liver ratio, all indicative of a more normal placental and fetal development.

As determined by RT-qPCR analysis, mRNA expression of hAGT in maternal liver and placenta, it is shown that, while there is substantial silencing of hAGT in the maternal liver, there was no significant silencing in the placenta. This indicates a lack of penetration of the polynucleotide agent into the placenta.

In addition, analysis of maternal liver samples, placenta samples, and fetal liver samples demonstrates that the exposure of the fetal liver to the iRNA is more than 100-fold lower than the exposure to the maternal liver. Tissue exposure can be determined, for example, by stem-loop qPCR, as previously described (see, *e.g.,* Landesman, Y., *et al.* (2010) *Silence* 1:16).

Thus, maternal treatment with an antisense polynucleotide agent targeting hAGT is superior to maternal treatment with small-molecule renin-angiotensin inhibitors, such as ACE inhibitors, and angiotensin receptor blockers, as these compounds cross the placenta and are fetotoxic.

As the placenta acts as the conduit for nutrients to the fetus, and waste removal from the fetus, its composition is critical. Accordingly, placental pathology is evaluated (*i.e.,* by measuring the area of tissue slices immunohistochemically stained for cytokeratin). While the maternal portion of the placenta (the mesometrial triangle) and the trophospongium (a uniform layer of cells which are precursors of differentiated trophoblasts) are unchanged by administration of the polynucleotide agent targeted to AGT, overall placental size is increased. In particular, the size of the villous placenta, which is the site of nutritional exchange between fetal and maternal blood, is increased by maternal treatment with the polynucleotide agent targeted to AGT.

Regulators of angiotensins, such as the anti-angiogenic soluble fms-like tyrosine kinase-1 (sFLT1) and the angiogenic placental growth factor (PLGF), play a key role in preeclampsia, being released from the placenta into the maternal blood stream and causing endothelial dysfunction. sFlt-1, is largely released from the placenta into the maternal blood stream and causes endothelial dysfunction. PLGF is placental derived, and promotes angiogenesis. The ratio of sFlt-1:PLGF is diagnostic, with a higher sFlt-1:PLGF associated with more severe disease.

Evaluation of the mRNA expression of sFLT1 and PLGF by RT-qPCR analysis demonstrates that the levels of both sFLT1 and PLGF are significantly reduced in the maternal kidney, but remain unchanged in the placenta following maternal administration of the polynucleotide agent targeted to AGT. However, as sFlt-1 is reduced to a greater extent than PLGF in the maternal kidney, the serum sFlt-1:PLGF ratio may be improved.

Agonistic autoantibodies to the angiotensin II receptor type I (AT1) have been identified in preeclamptic women. When pregnant rodents are exposed to AT1 autoantibodies, a preeclampsia-like syndrome develops. As activation of AT1 is associated with vasopressor effects as well as aldosterone secretion and sFlt-1 production, reduction of AT1 autoantibodies would be expected to reduce the preeclamptic phenotype. Accordingly, the level of production of agonistic autoantibodies to the AT1 receptor (AT1-AA) may also be evaluated by biochromatography and subsequent bioassay of isolated antibodies. AT1-AA levels are measured as has been described (see, *e.g.,* Dechend, *et al.* (2005) *Hypertension* 45:742–746) by assessing the impact of isolated, purified AT1-AA antibodies on the spontaneous beating rate of neonatal rat cardiomyocytes. A significant reduction in the level of AT1-AA following administration of the polynucleotide agent targeting AGT to pregnant PE rats is observed.

Example 5. *In Vivo* AGT Silencing in a Transgenic Rat Model of Preeclampsia

Polynucleotide agents targeting AGT are tested for the ability to silence hAGT expression in pregnant transgenic female Sprague-Dawley rats harboring the complete genomic human AGT gene (*e.g.,* [TGR(hAGT)L1623]) described above. Beginning on day 3 of gestation, a subset of pregnant PE rats are administered appropriate doses of the polynucleotide agent targeted to AGT. Liver and blood are collected at gestation day 21 and levels of hAGT and rAGT protein and mRNA are assayed and compared to untreated, pregnant transgenic rats, and both pregnant and non-pregnant Sprague-Dawley rats. A significant reduction of circulating AT2 and hAT1 are observed in the pregnant PE rats treated with the polynucleotide agent targeting to AGT as compared to control pregnant PE rats. A significant reduction of rat AT2, although less than the reduction observed for human AGT, is also observed in the pregnant PE rats treated with the polynucleotide agent targeting AGT as compared to control pregnant PE rats. Significant silencing of hepatic AGT mRNA is observed in the pregnant PE rats treated with the polynucleotide agent targeting AGT as compared to control pregnant PE rats. A significant reduction in the serum level of AT1 and AT2 following administration of the polynucleotide agent targeting AGT to pregnant PE rats.

We claim:

1. An antisense polynucleotide agent for inhibiting expression of angiotensinogen (AGT), wherein the agent comprises about 4 to about 50 contiguous nucleotides, wherein at least one of the contiguous nucleotides is a modified nucleotide, and wherein the nucleotide sequence of the agent is about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1-4.

2. The agent of claim 1, wherein the equivalent region is any one of the target regions of SEQ ID NO:1 provided in Table 3.

3. The agent of claim 1, wherein the equivalent region is selected from the group consisting of nucleotides 1223-1296, 1596-1626, 1727-1813, 1727-1770, 1772-1813, 1838-1868, 2091-2120, 2200-2243, 2321-2340, 474-493, 981-1000, 1003-1022, 1167-1186, 1223-1242, 1234-1253, 1245-1264, 1254-1273, 1277-1296, 1596-1615, 1607-1626, 1727-1746, 1740-1759, 1751-1770, 1772-1791, 1784-1803, 1794-1813, 1838-1857, 1849-1868, 1893-1912, 2091-2110, 2101-2120, 2125-2144, 2146-2165, 2200-2219, 2213-2232, 2224-2243, 2256-2275, 2310-2329, and 2321-2340 of SEQ ID NO:1.

4. An antisense polynucleotide agent for inhibiting expression of angiotensinogen (AGT), wherein the agent comprises at least 8 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences listed in Table 3, and wherein the agent is about 8 to about 50 nucleotides in length.

5. An antisense polynucleotide for inhibiting expression of angiotensinogen (AGT), wherein the agent comprises at least 8 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences selected from the group consisting of nucleotides 1223-1296, 1596-1626, 1727-1813, 1727-1770, 1772-1813, 1838-1868, 2091-2120, 2200-2243, 2321-2340, 474-493, 981-1000, 1003-1022, 1167-1186, 1223-1242, 1234-1253, 1245-1264, 1254-1273, 1277-1296, 1596-1615, 1607-1626, 1727-1746, 1740-1759, 1751-1770, 1772-1791, 1784-1803, 1794-1813, 1838-1857, 1849-1868, 1893-1912, 2091-2110, 2101-2120, 2125-2144, 2146-2165, 2200-2219, 2213-2232, 2224-2243, 2256-2275, 2310-2329, or 2321-2340 of SEQ ID NO:1.

6. The agent of any one of claims 1-5, wherein substantially all of the nucleotides of the antisense polynucleotide agent are modified nucleotides.

7. The agent of any one of claims 1-5, wherein all of the nucleotides of the antisense polynucleotide agent are modified nucleotides.

8. The agent of any one of claims 1-7, which is 10 to 40 nucleotides in length.

9. The agent of any one of claims 1-7, which is 10 to 30 nucleotides in length.

10. The agent of any one of claims 1-7, which is 18 to 30 nucleotides in length.

11. The agent of any one of claims 1-7, which is 10 to 24 nucleotides in length.

12. The agent of any one of claims 1-7, which is 18 to 24 nucleotides in length.

13. The agent of any one of claims 1-7, which is 20 nucleotides in length.

14. The agent of any one of claims 1-7, which is 14 nucleotides in length.

15. The agent of any one of claims 1-3 and 6-7, wherein the modified nucleotide comprises a modified sugar moiety selected from the group consisting of: a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

16. The agent of claim 15, wherein the bicyclic sugar moiety has a (—CH2—)n group forming a bridge between the 2′ oxygen and the 4′ carbon atoms of the sugar ring, wherein n is 1 or 2.

17. The agent of any one of claims 1-3 and 6-7, wherein the modified nucleotide is a 5-methylcytosine.

18. The agent of any one of claims 1-3 and 6-7, wherein the modified nucleotide comprises a modified internucleoside linkage.

19. The agent of claim 18, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

20. The agent of any one of claims 1-3 and 6-7, comprising a plurality of 2′-deoxynucleotides flanked on each side by at least one nucleotide having a modified sugar moiety.

21. The agent of claim 20, wherein the agent is a gapmer comprising a gap segment comprised of linked 2′-deoxynucleotides positioned between a 5′ and a 3′ wing segment.

22. The agent of claim 20, wherein the modified sugar moiety is selected from the group consisting of a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

23. The agent of claim 21, wherein the 5'-wing segment is 1 to 6 nucleotides in length.

24. The agent of claim 21, wherein the 3'-wing segment is 1 to 6 nucleotides in length.

25. The agent of claim 21, wherein the gap segment is 5 to 14 nucleotides in length.

26. The agent of claim 21, wherein the 5'-wing segment is 2 nucleotides in length.

27. The agent of claim 21, wherein the 3'-wing segment is 2 nucleotides in length.

28. The agent of claim 21, wherein the 5'-wing segment is 3 nucleotides in length.

29. The agent of claim 21, wherein the 3'-wing segment is 3 nucleotides in length.

30. The agent of claim 21, wherein the 5'-wing segment is 4 nucleotides in length.

31. The agent of claim 21, wherein the 3'-wing segment is 4 nucleotides in length.

32. The agent of claim 21, wherein the 5'-wing segment is 5 nucleotides in length.

33. The agent of claim 21, wherein the 3'-wing segment is 5 nucleotides in length.

34. The agent of claim 21, wherein gap segment is 10 nucleotides in length.

35. An antisense polynucleotide agent for inhibiting angiotensinogen (AGT) expression, comprising

a gap segment consisting of linked deoxynucleotides;

a 5'-wing segment consisting of linked nucleotides;

a 3'-wing segment consisting of linked nucleotides;

wherein the gap segment is positioned between the 5'-wing segment and the 3'-wing segment and wherein each nucleotide of each wing segment comprises a modified sugar.

36. The agent of claim 35, wherein the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is five nucleotides in length.

37. The agent of claim 35, wherein the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is four nucleotides in length.

38. The agent of claim 35, wherein the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is three nucleotides in length.

39. The agent of claim 35, wherein the gap segment is ten 2′-deoxynucleotides in length and each of the wing segments is two nucleotides in length.

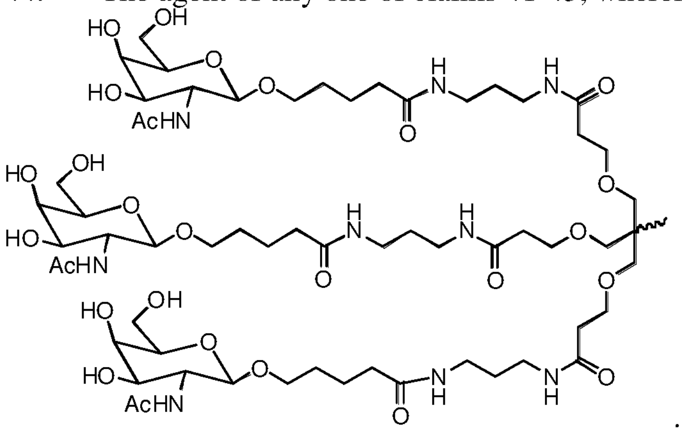
40. The agent of claim 35, wherein the modified sugar moiety is selected from the group consisting of a 2′-O-methoxyethyl modified sugar moiety, a 2′-methoxy modified sugar moiety, a 2′-O-alkyl modified sugar moiety, and a bicyclic sugar moiety.

41. The agent of any one of claims 1-35, wherein the agent further comprises a ligand.

42. The agent of claim 41, wherein the antisense polynucleotide agent is conjugated to the ligand at the 3'-terminus.

43. The agent of claim 41, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

44. The agent of any one of claims 41-43, wherein the ligand is



45. A pharmaceutical composition for inhibiting expression of an angiotensinogen (AGT) gene comprising the agent of any one of claims 1-44.

46. The pharmaceutical composition of claim 45, wherein agent is present in an unbuffered solution.

47. The pharmaceutical composition of claim 46, wherein the unbuffered solution is saline or water.

48. The pharmaceutical composition of claim 46, wherein the agent is present in a buffer solution.

49. The pharmaceutical composition of claim 48, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

50. The pharmaceutical composition of claim 48, wherein the buffer solution is phosphate buffered saline (PBS).

51. A pharmaceutical composition comprising the agent of any one of claims 1-44, and a lipid formulation.

52. The pharmaceutical composition of claim 51, wherein the lipid formulation comprises a LNP.

53. The pharmaceutical composition of claim 51, wherein the lipid formulation comprises a MC3.

54. A method of inhibiting angiotensinogen (AGT) expression in a cell, the method comprising contacting the cell with the agent of any one of claims 1-44 or a pharmaceutical composition of any one of claims 45-53, thereby inhibiting expression of the AGT gene in the cell.

55. The method of claim 54, wherein the cell is within a subject.

56. The method of claim 55, wherein the subject is a human.

57. The method of claim 56, wherein the AGT expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

58. A method of treating a subject having a disease or disorder that would benefit from reduction in angiotensinogen (AGT) expression, the method comprising administering to the subject a therapeutically effective amount of the agent of any one of claims 1-44 or a pharmaceutical composition of any one of claims 45-53, thereby treating the subject.

59. A method of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in angiotensinogen (AGT) expression, the method comprising administering to the subject a prophylactically effective amount of the agent of any one of claims 1-44 or a pharmaceutical composition of any one of claims 45-53, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in AGT expression.

60. The method of claim 58 or 59, wherein the administration of the antisense polynucleotide agent to the subject causes a decrease in intravascular hemolysis, a stabilization of hemoglobin levels and/or a decrease in AGT protein levels.

61. The method of claim 58 or 59, wherein the disorder is an angiotensinogen (AGT)-associated disease.

62. The method of claim 61, wherein the AGT-associated disease is selected from the group consisting of hypertension, borderline hypertension, primary hypertension, secondary hypertension, hypertensive emergency, a hypertensive urgency, pregnancy-associated hypertension, resistant hypertension, paroxysmal hypertension, renovascular hypertension, Goldblatt hypertension, ocular hypertension, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension, essential edema-proteinuria-hypertension (EPH) gestosis, hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, chronic kidney disease, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, Cushing's syndrome, and other glucocorticoid excess states including chronic steroid therapy, pheochromocytoma, primary aldosteronism and other mineralocorticoid excess states, sleep apnea, thyroid/parathyroid disease, heart failure, myocardial infarction, stroke, diabetes mellitus, renal failure, and systemic sclerosis.

63. The method of claim 62, wherein the AGT-associated disease is hypertension.

64. The method of claim 62, wherein the AGT-associated disease pregnancy-associated hypertension.

65. The method of claim 58 or 59, wherein the subject is human.

66. The method of any one of claims 58-65, further comprising administering an additional agent for treatment of the AGT-associated disease to the subject.

67. The method of any one of claims 58-66, wherein the antisense polynucleotide agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

68. The method of claim 67, wherein the antisense polynucleotide agent is administered at a dose of about 10 mg/kg to about 30 mg/kg.

69. The method of claim 67, wherein the antisense polynucleotide agent is administered to the subject once a week.

70. The method of claim 67, wherein the antisense polynucleotide agent is administered to the subject twice a week.

71. The method of claim 67, wherein the antisense polynucleotide agent is administered to the subject twice a month.

72. The method of any one of claims 58-71, wherein the antisense polynucleotide agent is administered to the subject subcutaneously.

73. The method of claim 58 or 59, further comprising measuring a blood pressure level in the subject.

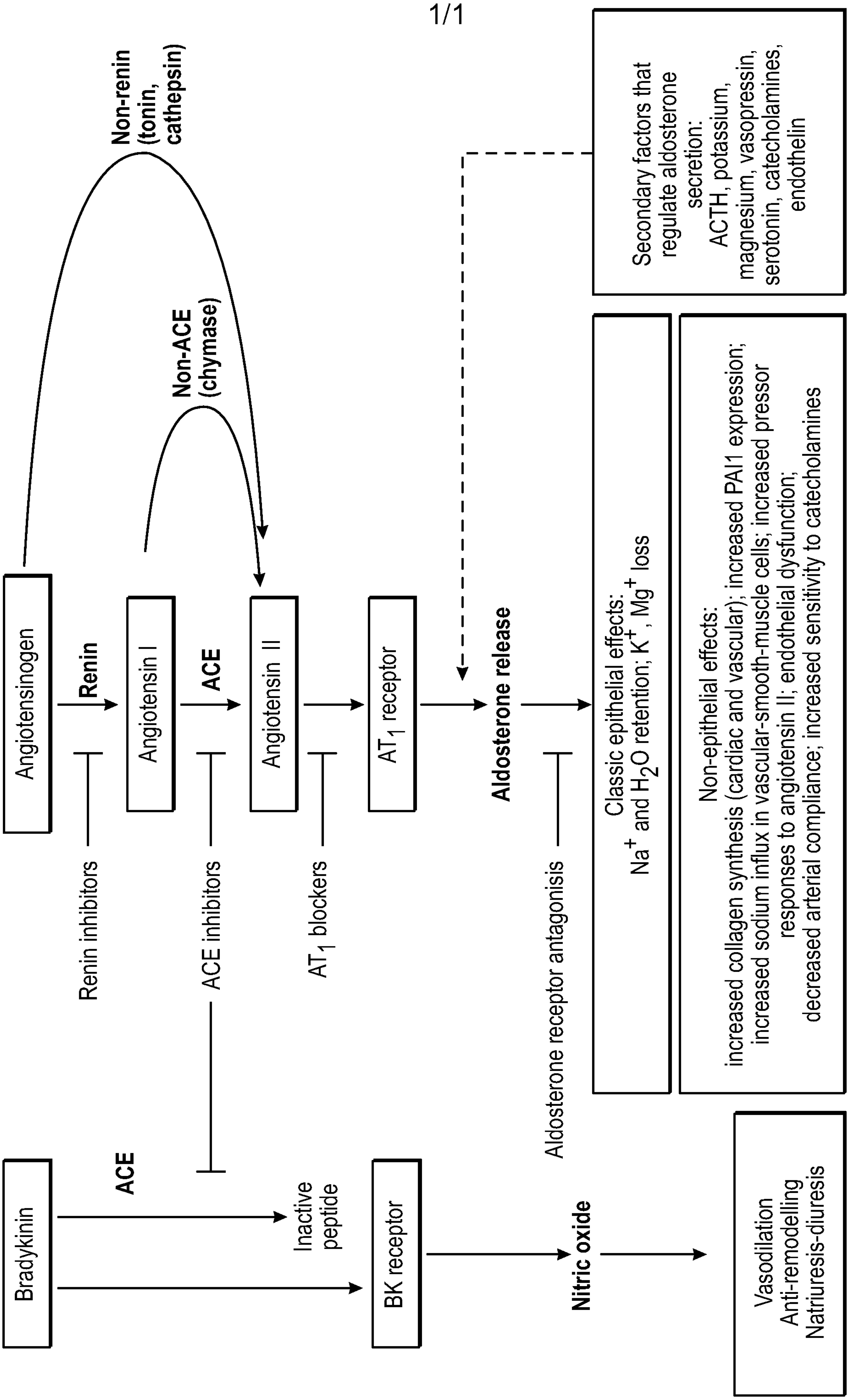

Angiotensinogen
Renin
Angiotensin I
ACE
Angiotensin II
AT1 receptor
Aldosterone release
Non-renin (tonin, cathepsin)
Non-ACE (chymase)
Renin inhibitors
ACE inhibitors
AT1 blockers
Aldosterone receptor antagonisis
Secondary factors that regulate aldosterone secretion: ACTH, potassium, magnesium, vasopressin, serotonin, catecholamines, endothelin
Classic epithelial effects: Na+ and H2O retention; K+, Mg+ loss
Non-epithelial effects: increased collagen synthesis (cardiac and vascular); increased PAI1 expression; increased sodium influx in vascular-smooth-muscle cells; increased pressor responses to angiotensin II; endothelial dysfunction; decreased arterial compliance; increased sensitivity to catecholamines
Bradykinin
ACE
Inactive peptide
BK receptor
Nitric oxide
Vasodilation
Anti-remodelling
Natriuresis-diuresis

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/034062

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2016.01) C12N 15/113, A61K 31/712500, A61K 31/712, A61P 9/10

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2016.01) C12N 15/113, A61K 31/712500, A61K 31/712, A61P 9/10

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: NCBI, Google Patents, CAPLUS, BIOSIS, EMBASE, MEDLINE, Google Scholar
Search terms used: (antisense oligonucleotide or iRNA) and (angiotensinogen or AGT) and modified sugar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014018930 A1 ISIS PHARMACEUTICALS. INC, CROOKE, ROSANNE M, ; GRAHAM, MARK J 30 Jan 2014 (2014/01/30) claims 1-38; description page 14 (lines 25-27), page 17 (lines 24-31), page 18 (lines 20-31), page 38 (line 32) – page 39 (line 22), pages 45 (line 1) - page 48 (line 31), page 49 (lines 6-30), page 53 (lines 9-23); examples 1-3.	1-42,45-51,53-63, 65-73
Y	claims 1-38; description page 14 (lines 25-27), page 17 (lines 24-31), page 18 (lines 20-31), page 38 (line 32) – page 39 (line 22), pages 45 (line 1) - page 48 (line 31), page 49 (lines 6-30), page 53 (lines 9-23); examples 1-3.	52,64
Y	Tomita, N., Morishita, R., Higaki, J., Aoki, M., Nakamura, Y., Mikami, H., & Ogihara, T. (1995). Transient decrease in high blood pressure by in vivo transfer of antisense oligodeoxynucleotides against rat angiotensinogen. Hypertension, Vol. 26, no.1, pages 131-136. 31 Dec 1995 (1995/12/31) section "methods"	52

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 Jul 2016	31 Jul 2016
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer MAZEL Alexander Telephone No. 972-2-5651716

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Choi, Y. S., Kwon, H., Kim, J. H., Shin, J. E., Choi, Y., Yoon, T. K., & Kim, N. K. (1 June 2011). Haplotype-based association of ACE I/D, AT1R 1166A> C, and AGT M235T polymorphisms in renin–angiotensin–aldosterone system genes in Korean women with idiopathic recurrent spontaneous abortions. European Journal of Obstetrics & Gynecology and Reproductive Biology, Vol. 158, no.2, pages 225-228. 01 Jun 2011 (2011/06/01) page 226, second paragraph	64
E	WO 2015179724 A1 ALNYLAM PHARMACEUTICALS, INC 26 Nov 2015 (2015/11/26) whole document, especially claims 1-101	1-73

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2016/034062

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2014018930 A1	30 Jan 2014	WO 2014018930 A1	30 Jan 2014
		EP 2877579 A1	03 Jun 2015
		EP 2877579 A4	16 Mar 2016
		US 2015297629 A1	22 Oct 2015
WO 2015179724 A1	26 Nov 2015	WO 2015179724 A1	26 Nov 2015
		WO 2015179724 A8	18 Feb 2016
		WO 2015179724 A9	10 Mar 2016